Traditional Herbal Medicines for Modern Times

Sho-Saiko-To

Scientific Evaluation and Clinical Applications

Edited by Yukio Ogihara and Masaki Aburada

Sho-Saiko-To

Traditional herbal medicines for modern times
Series Editor Dr Roland Hardman

Each volume in this series provides academia, health sciences and the herbal medicines industry with in-depth coverage of the herbal remedies for infectious diseases, certain medical conditions or the plant medicines of a particular country.

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Preface

The headwaters of Han's medicine in Japan ascends to the era when Han doctors came over to Japan from Korea Peninsula in the fifth century at the request of Yamato Imperial Court (described in KOJIKI, a historical book written in the early eighth century). That is, Kampo medicine is a traditional medicine that underlies the classic medicine founded more than 2000 years ago in China and independently developed in Japan after introduction into Japan about 1500 years ago, and it is developing as a medicine peculiar to Japan.

On the other hand, it is a fact agreed by all people that Western medicine markedly progressed in the recent times, that is, only about hundred years and greatly contributes to treatment of diseases, but the nature of life has not been elucidated yet, and the type of diseases is surely increasing with the coming of ageing society and stressful society. In such a medical situation, the Kampo medicine that has been brought down by physicians and pharmacists specializing in Kampo medicine begins to be recognized as an important therapeutic method in the medical practice of Western medicine, and at the present time, it is no exaggeration to say that there are almost no principal hospital in Japan not using Kampo formulae. In addition, more than 100 Kampo formulae are applied in health insurance in Japan and prescribed for patients in combination with Western drugs or alone at low prices.

In this book, we took Sho-saiko-to as a representative one of many Kampo formulae and could complete editorial works to introduce its utility to many people in the world in cooperation with the experts of basic medicine and clinical medicine. With this book, I want many people in the world to recognize that Kampo preparations for ethical use, which are used in Japan, maintain high quality, and that the active ingredients of the respective Kampo formulae and the efficacy of them as mixtures of many compounds were scientifically elucidated in detail and that clinical evaluation are steadily progressing.

Nowadays, a term, 'EBM', has been taken actively in evaluation of drugs, but the authors consider that Kampo formulae which have been brought down for more than 1000 years and accumulated clinical studies do not take kindly to evaluation of the EBM of Western drugs which have accumulated clinical studies for less than 100 years only and that the traditional drugs emphasizing individual medicine may require a separate EBM. The authors want readers to understand that there are many problems in evaluation of Kampo medicine and then consider that it will be fortunate, if advice is given.

Finally, at completion of this manuscript, the authors acknowledge Dr Roland Hardman, the series editor, and the collaborative authors in charge of each chapter. In addition, we acknowledge Taylor & Francis Ltd. for cooperating to publish this book and Jemma Nissel for cooperating in editorial works.

Yukio Ogihara, Ph.D.
Nagoya City University, Meijo University
May 2002

1 Introduction

Yukio Ogihara

Today, as we feel the limits of Western medicine, there is a trend throughout the world to reconsider traditional medicine. In addition to traditional medicine, there are new terms, such as complementary medicine in Europe and alternative medicine or integrative medicine in the US. These have attracted a great deal of attention in the medical field. This main pillar of traditional medicine, which we have nurtured in eastern Asia for several thousands of years, was widely disseminated as Kampo medicine by the Han[1] during the Han Dynasty (206 BC–AD 220). I believe that this form of medical therapy can be considered to be preventive medicine that can treat pre-illness. This field of medicine is not well addressed by the Western medicine, which rather has absolute strength in specific therapy; Kampo medicine also has great potential in geriatric medicine, where potent medicines are not desirable. There is no doubt that its importance in medicine will be re-established in the twenty-first century.

Short history of Kampo medicine

There is a saying that one can gain new knowledge from the old. I would like to first review the old, and begin by reviewing the cultures common to China and Japan, the Chinese character-based culture that originated in the great Yellow River, and the Kampo medicine that developed in this setting. The 'Records of the Historian' by Sima Qian states that the first dynasty of China was the Xia dynasty (2205–1766 BC), but the details are not known. Currently the oldest recognized dynasty is the Shang dynasty (1766–1122 BC). In 1899, Wang Yirong, a government official in the late Qing dynasty (AD 1644–1911), noted the hieroglyphics carved in the long gu (dragon bone: FOSSILIA OSSIS MASTODI) purchased for use in the treatment of a certain illness. This led the discovery of ideographs, the oldest written form of the Yellow River civilization. Based on the findings of crude drugs and long gu, an excavation of the Yinxu (ancient capital city of the Shang) was started in Anyang county, Henan Province in 1928 and clearly demonstrated the existence of the Shang dynasty.

As indicated earlier, the popular use of herbs and functional foods has become a social phenomenon in the Western world mainly as a result of marketing by the health industry in the recent years. To be sure, from the standpoint of preventive medicine, it is certainly appropriate to consider diet as an important first measure in disease prevention. During the middle of the eleventh century BC, in the Yellow River basin surrounded by the Wei River and the Jing River King Wu of the Zhou, a tribe living primarily as grain farmers, defeated King Zhou of the Shang, and ended the Shang dynasty. The brother of King Wu, the Zhougong Dan, was a great figure who solidified the Zhou dynasty, and was said to have been involved in the establishment of many standards. Among these, the 'Zhouli', which was the first law and established the governmental structure of the Zhou dynasty, describes four specialized positions for physicians

in the form of dietary physician, internal physician, surgical physician and veterinary physician. The important dogma of Kampo medicine is to 'treat the pre-illness', and this viewpoint dates back 3000 years. In China where preventive medicine is emphasized, dietary physicians were given the highest status. It is also of interest to note that during the Hellenism era of fourth century BC to first century AD, where there is a close relationship to European cultures, there also existed three types of physicians, 'Diaitetike' (dietician), 'Pharmakeutike' (pharmacist) and 'Cheirourgia' (surgeon). History truly repeats itself.

A copy of documents on 'Zhouli' from the Ming era, whose original is not available, places importance on wu wei, the 'five tastes of foods'. Furthermore, in the introduction to 'Shennong Bencao Jing', the Materia Medica of Shennong, which can be said to be the oldest known Pharmacopoeia, it is stated that there are five tastes of foods, sour (suan), salty (xian), sweet (gan), bitter (ku) and pungent (xin). Following this other medical texts also have notations on the five tastes. These emphasize that the diet in daily life is the most important determinant of health. More detail of how the four types of physicians may handle patients, will be given. The dietary physician (dietary therapist) would suggest that a patient consume large quantities of sour in the spring, bitter in the summer, pungent in the fall, and salt in the winter, and that these be flavoured skilfully using the sweet taste. The secret to maintaining health is to eat the seasonal foods appropriate for the season, indicating the divine Providence of Nature. The internal physician (internist) treats those patients in whom the illness already has manifested itself. Thus, they aimed at promoting body strength through eating the five grains (hemp, bean, wheat, broomcorn millet and millet) and using judiciously the five drugs depending on the medical condition and physical condition. The five drugs are grass, tree, insect, mineral and grain and are, thus, the Shennong Bencao Jing, itself. Of course, one also took into account the five tastes. The surgical physician (surgeon) handles patients with severe illnesses and, thus, must use potent drugs, the Five Poisons (HgS, As_4S_4, $CuSO_4 \cdot 5H_2O$, Fe_3O_4 and $FeAsS + FeAs_2$) with an emphasis on the use of aggressive therapy. Anyway the medical knowledge disseminated during the Han period by the Han constituted the main form of medical treatment of Japan until 1895 during the Meiji Restoration, when the traditional medicine was prohibited and Western medicine was recognized as mainstream medicine. Among the history of medicine, the texts most valued by the Japanese were Huangdi Neijing (the Yellow Emperor's Canon of Internal medicine), Shennong Bencao Jing and Shanghan Zabing Lun (text book on clinical treatment with Kampo medicine written by Zhang Zhong Jing).

Table 1.1 summarizes in brief the history of medicine in Japan. In the fifth century, at the request of the Yamato Imperial court, a Han physician[2] visited Japan from the Korean peninsula, and this is recorded in the official history of Japan in the Kojiki and Nippon Shoki texts.

Table 1.1 Short history of Japanese Kampo medicine

		Notes
AD 413	Kon Bu from Korea	Kojiki (711)
AD 459	Toku Rai from Korea	Nihonsyoki (720)
AD 581	Ti Sou from China	First foreign book
AD 603	Eniti and Fukuin to China	Kenzuisi
AD 701	'Taihouritsuryou'	First Code
AD 754	Gan Jin	Many Chinese drugs
AD 927	'Engisiki'	Selection of text books
AD 984	'Isinpo'	Japanese (oldest)

Note
Kampo medicine vs Rampo medicine (from Holland).

Thereafter, in the sixth through ninth centuries, Japan aggressively imported Han medicine through dispatching of envoys and receipt of Buddhist monks from China. At the end of the tenth century, Tamba Yasuyori compiled Japan's first medical text, 'Ishinpo'. Thereafter, traditional medicine in Japan came to be called a 'Hougi (medical skill)' or a 'Houjyutsu (medical art)' and after Dutch medicine was imported in the seventeenth century, and called 'Rampo', it came to be called 'Kampo'. Han medicine,[3] which originated in China and was introduced to Japan 1500 years ago, was imported as the knowledge of China into the bodies of Japanese and came to become the medicine of experience of today.

What is Kampo medicine?

Nowadays, in Japan 147 kinds of Kampo medical prescriptions have been approved by the Ministry of Health and Welfare for clinical application. Then, about 80% of clinical doctors trained in Western medicine are experienced in their use, but the scientific foundation for Kampo medicines is still terribly poor. So, clear scientific evaluation to prove the efficacy of Kampo medicines is really required. Now, I would like to refer to a few experiments conducted using Kampo medicines, a field which is often left ignored by modern medicine, which itself has a history of only 200 years, because Kampo cannot be explained by scientific theories. The following experiments were carried out to get some idea on Kampo medicine from the materialistic point of view.

The herbal medicines, which constitute Kampo prescriptions are based primarily on plants, so we will initially consider the components that are generally found in plants, followed by touching on the specific constituents in medicinal plants to explain the material properties of Kampo prescriptions.

*Gallic acid obtained from Fufang muji (**Inoue** et al., 1994, 1995; **Koide** et al., 1998; **Nose** et al., 1998a; **Sakaguchi** et al., 1998)*

This Chinese medicine is used in the treatment of chronic hepatitis in the northeastern region of China. Thus, an analytical study of the constituent components of this medicine was undertaken in the usual way. First, among the four herbal constituents of Fufang muji, Sophora root, Cuscuta semen, Catalpa bark (Juglans manshurica) and Ganoderma polysaccharide, the Catalpa bark (bark from a walnut tree) was selected for the analysis and identification of the constituents. Based on the general principle that Kampo medicines can be extracted in hot water, a hot water extract was prepared, and by ethanol precipitation was divided into supernatant and precipitate. It is common knowledge that precipitate contains the high-molecular-weight polysaccharides, while the supernatant contains low-molecular-weight material that is easy to study from the point of view of plant chemistry. The supernatant was partitioned as usual, shown in Figure 1.1. Separation and purification of the ether fraction by chromatography revealed a small amount of a previously known compound, gallic acid and syringic acid (Figure 1.1).

From the standpoint of plant chemistry, the results were extremely disappointing. Gallic acid is widely distributed in plants as a major component of the aqueous tannins. In plant chemistry, it is a disappointment if a new, structurally interesting compound cannot be purified. Figure 1.2 shows the results of an assessment of effects of gallic acid on six types of cultured cancer cell lines. Gallic acid, which is a compound that exists widely in nature, induced deaths of the cancer cells at a low concentration. At this point, a not-so-interesting compound had given a not-so-interesting result. However, since gallic acid is non-toxic with LD50 of 4 g/kg, its effects on normal cells was tested. As shown in this figure, it had no toxicity in normal hepatic

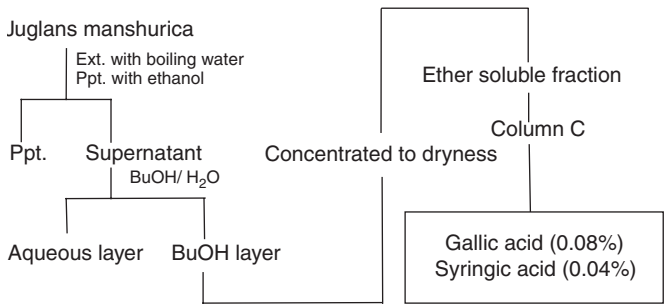


Figure 1.1 Separation and purification of the ether fraction by chromatography.

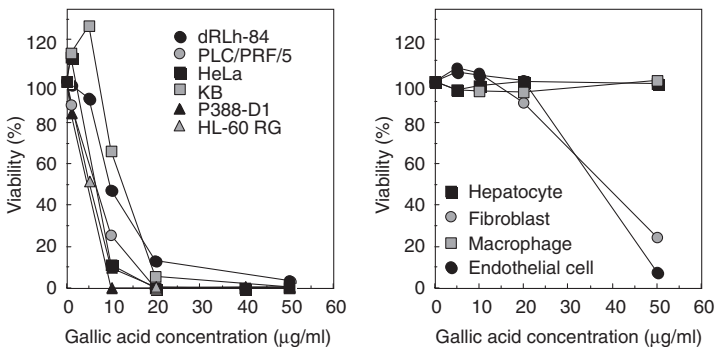


Figure 1.2 Selective cytotoxicity of gallic acid.

parenchymal cells and peritoneal macrophages, while toxicity was seen at high doses in cells with a high rate of cell proliferation, such as vascular endothelial cells and fibroblasts. Therefore, a very common component that is widely distributed in plants was presumed to play a role in identifying the difference between normal cells and abnormal cells, a very interesting experimental result.

In general Kampo medicines are orally administered. Thus, it is ultimately necessary to conduct an evaluation by *in vivo* experiments in which a disease model is established in animals, the drug is administered, and the response in the animals is investigated. *In vitro* experiments in which drugs are added to isolated organs or cultured cells are only adjuncts. In addition, natural products are different from synthetic medical products, that often undergo structural changes in the intestinal tract as a result of the actions of gastric acid, digestive enzymes and intestinal flora. So the compound that is absorbed and flowing through the blood stream to reach the active site is unlikely to have the same structure as the one isolated from plants.

Figure 1.3 shows the experimental results obtained by using liver tumour induced by an intravenous injection of P815 cells to mice, which causes death in the animals. There was a clear prolongation of survival when the gallic acid was administered intraperitoneally. Thus, a compound that is widely found in plants as a fundamental component of water soluble tannin, could promote the elimination of abnormal products such as cancer cells and play a role in the maintenance of homoeostasis.

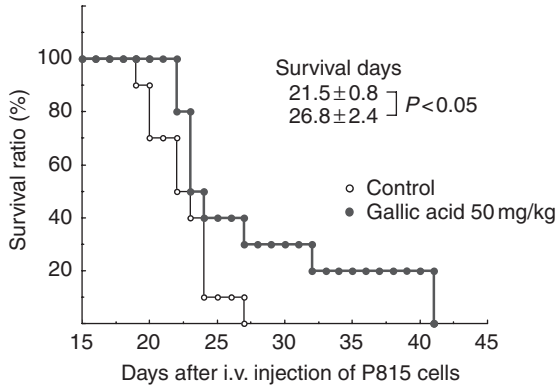
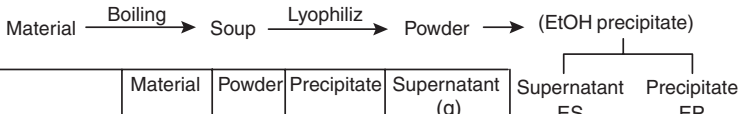


Figure 1.3 Effect of gallic acid on survival ratio.



	Material	Powder	Precipitate	Supernatant (g)
Sho-saiko-to	24.0	8.3	1.9	6.4
Dai-saiko-to	23.0	8.3	1.6	6.7
Hochu-ekki-to	24.5	9.0	1.6	7.3
Hachimi-jio-gan	22.0	6.9	0.7	6.2
Vegetable soup*	300.0	10.8	1.6	8.8

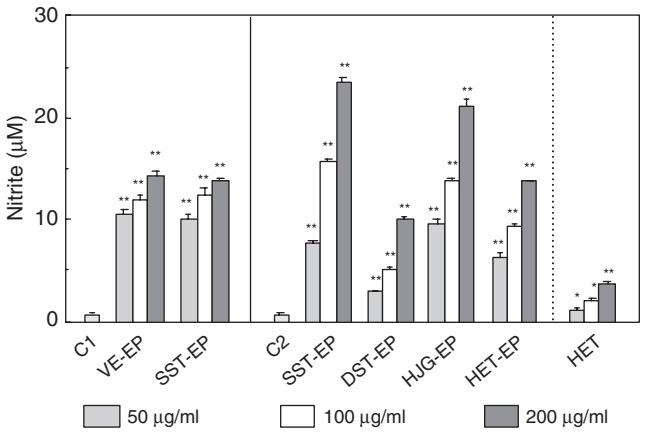
* 10 kinds of vegetables (30 g each): onion, gobo root, carrot Daikon, tomato, eggplant, cucumber, cabbage, spinach, soyabean spruce

Figure 1.4 Preparation of samples.

Polysaccharides from several Kampo prescriptions and vegetable soup (Terawaki* et al., *1997*, *1998*; *Nose* et al., *1997*, *1998)

Let us consider the polysaccharides, a high-molecular-weight component that is also widely found in plants. Four representative Kampo prescriptions used widely in Japan and the combination of 10 vegetables from a normal diet were processed to obtain polysaccharide fractions (Figure 1.4).

The amount of Kampo prescription used was equivalent to one day's dose of Japanese Kampo and to one day's intake of vegetables recommended as the standard nutritional intake as established by the Ministry of Health and Welfare. The following two experiments were conducted. The polysaccharide fraction was used to treat mouse peritoneal macrophages, and Nitric oxide (NO) produced was measured to study the activation of the macrophages, which are involved in immunomodulation. As shown in Figure 1.5, there are some differences in the activity of the polysaccharide fractions (EP), depending on the different source, but in all cases the macrophage



C: Control, VE: Vegetable extracts, SST: Sho-saiko-to, DST: Dai-saiko-to, HJG: Hachimi-jio-gan, HET: Hochu-ekki-to
* $P < 0.05$,
** $P < 0.01$ vs Control

Figure 1.5 Effects of polysaccharide-rich fractions (EP fractions) on NO production by murine peritoneal macrophages.

activity was stimulated in a dose-dependent manner. The polysaccharide obtained from vegetables showed a similar degree of immune stimulation as the polysaccharide fraction derived from Sho-saiko-to.

The previous experiment compared the activity of various polysaccharides in an *in vitro* experimental model. In the next experiment, the changes in the peritoneal macrophages were studied after administration of polysaccharides. After administration of EP fraction to mice for week, the peritoneal macrophages were collected, and reactivity to lipopolysaccharide (LPS) (B cell mitogen) was studied through NO production. Although there are variations depending on the type of Kampo medicine used, all polysaccharides found in these medicines were stimulatory (Figure 1.6).

Table 1.2 describes Sho-saiko-to, which I have studied for 25 years. It is made of seven herbal drugs, Bupleurum root (saiko; chai hu), Ginseng (ninjin; ren shen), Pinellia tuber (hange; ban xia), Ginger (syoukyou; sheng jiang), Glycyrrhiza liquorice root (kanzou; gan cao), Jujube (taisou; da zao) and Scutellaria root (ougon; huang qin).

Table 1.3 summarizes the specific components isolated from the Sho-saiko-to that has been structurally analysed and evaluated pharmacologically. According to Table 1.3, one can say that these individual components have many pharmacological properties when evaluated from the standpoint of Western medicine. For example, saikosaponin d, which was isolated from Bupleurum root, had pharmacological efficacy comparable to prednisolone, a steroid-type anti-inflammatory agent, in studies of anti-granulation effects using the pledget assay. The route of administration was intra-muscular in this study, and oral administration essentially had no effect. It has been demonstrated that intra-intestinal metabolism (see Chapter 3III) converts this drug into an inactive form. Furthermore, the amount of saikosaponin d contained in one day's dose of Sho-saiko-to is only 0.08 mg. Thus, it would take a great deal of courage to explain the efficacy of Kampo medicines on the basis of isolated few active ingredients.

Table 1.4 presents the composition of Kampo medicines. In all plants, some components, such as polysaccharide, tannin, carbohydrate and lipid etc. are found. It has been reported by

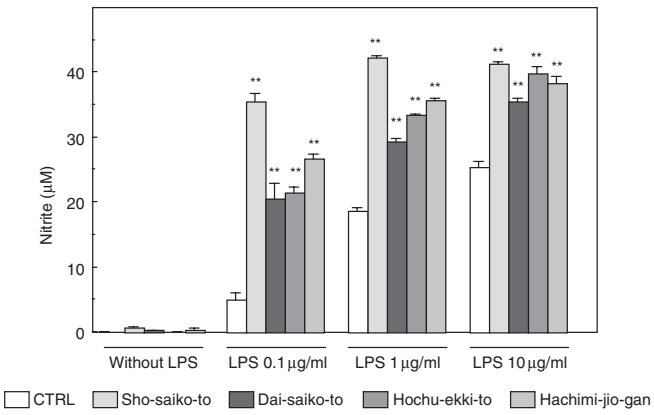


Figure 1.6 Effects of polysaccharide-rich fractions of Kampo medicines on NO production in murine macrophages.

Table 1.2 Example of Kampo medicine

Ingredients	*Efficacy*
Bupleuri radix (7 g)	Various febrile diseases,
Scutellariae radix (3 g)	Pneumonia, Bronchitis,
Zingiberis rhizoma (1 g)	Colds, Pleurisy, Tuberculosis,
Glycyrrhizae radix (2 g)	Liver complaints, Lymphangitis,
Pinelliae tuber (5 g)	incomplete recovery from
Ginseng radix (3 g)	child-birth, chronic complaints of
Zizyphi fructus (3 g)	the digestive system

many investigators that high-molecular-weight compounds contain polysaccharides, protein, tannin, lignin and cellulose, and that these compounds modulate the immune system or metabolism and alter the activity of intestinal micro-organisms. It is also well known that low-molecular-weight compounds, such as carbohydrates, lipids, amino acids and vitamins are involved as nutritional compounds, and as endocrine and immune modulating agents.

Our gallic acid also eliminates abnormal products. These components found in common support to play an important role in early therapy. In addition, the specialized components found in individual herbs, chosen to fit the symptom or condition of the patient, act against diseases as medicines, that is to say, reagents, in a manner similar to Western medicine. For example, berberine has antibiotic activity, while ephedrine has anti-tussive activity. Therefore, Kampo medicines can fulfill many needs and many uses and can provide the necessary elements to fit the needs of the patient. Because Kampo medicines are composed of multiple components with multiple effects, they differ fundamentally from synthetic drugs made from a single component. This is Kampo medicine. I am reluctant to place Kampo medicines in the same category as the synthetic medical products. It is out of the question to consider Kampo medicines, which have been chosen through several thousand years of medical history of trial and error, in the same category as the recently popularized functional foods and health foods. I would rather say that Kampo medicine should be placed between drugs and food or any other position.

Table 1.3 Pharmacological effects of identified ingredients in Sho-saiko-to

	Ginseng R.			*Zig. R.*		*Pinel. T.*			*Bupleuri R.*				*Zi. F.*		*Glycyrrh. R.*				*Sc. R.*		*No. of*
	Rb_1	*Rg_1*	*Ps*	*sol*	*gol*	*ep*	*sP*	*Ps*	*sa*	*sd*	*Ps*	*sP*	*ef*	*Ps*	*gl*	*ga*	*il*	*Ps*	*bi*	*be*	●
Inflammatory				●	●				●	●					●		●		●	●	8
Allergy					●				●	●			●		●				●	●	7
Dyshepatia									●	●					●	●				●	5
Immunity	●		●				●				●	●		●				●			7
Arterosclerosis										●					●		●				3
Glycometabolism		●								●											2
CNS	●	●		●	●				●							●			●	●	8
Anti-emetic								●													1
Anti-tussive				●		●									●						3
Anargesic				●	●																2
Anti-spasmodic				●	●											●					3
Anti-pyretic				●	●																2
Cholagogic																			●		1
Stomachic				●	●					●										●	4
Anti-ulcerative	●	●	●								●					●					5
Sthenia		●													●				●		3

Notes
Rb_1: ginsenoside Rb_1, Rg_1: ginsenoside Rg_1, Ps: Polysaccharide, sp: soluble protein, sol: shogaol, gol: gingerol, ep: ephedrine, sa: saikosaponin a, sd: saikosaponin d ef: ethyl- α-D-fructofranoside, gl: glycyrrhizin, ga: glycyrrhetinic acid, il: isoliquirigenin, bi: baicalin, be: baicalein.

Table 1.4 Composition of Kampo medicines

Composition	*Biological activity*
Common (primary physiological modulator)	*Homoeostatic effect*
Macromolecule	Regulation
Polysaccharide, lignin, protein, tannin (amall), Fibre etc.	Immunity, metabolism, intestinal flora etc.
Small molecule	Effects on system
Carbohydrate, lipid, amino acid, vitamin, peptide, metal, GA etc.	Endocrine, autacoid, immune, enzyme etc.
Characteristic ingredient	*Medicinal effect*
Sennoside, berberine, ephedrine baicalin, glycyrrhizin, saikosaponin	Analgesic, chlagogic, anti-pyretic etc.

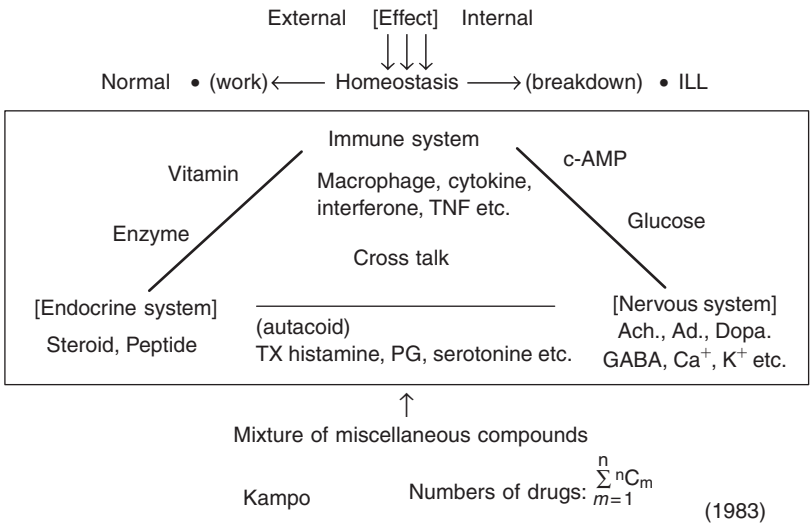
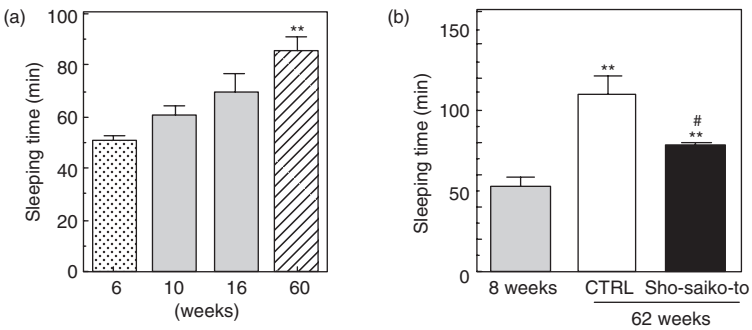


Figure 1.7 The trinity that maintains homoeostasis.

I will now discuss the characteristics of Kampo drugs from a mechanistic standpoint. Human beings maintain health through normal operation of a mechanism that maintains constant homoeostasis; when this mechanism fails, illness occurs. As indicated in Figure 1.7, the immune system, endocrine system and neurological system form the trinity that maintains homoeostasis.

Thus, various numerous compounds in Kampo medicines are thought to work together at different sites and guide the physiological activity towards a direction most suited to the individual. In contrast to Western medicine, which considers pure compounds as drugs, Kampo medicines are mixtures of numerous compounds.

Here, we present data from a recent experiment designed to evaluate the drug efficacy of a Kampo medicine. This study is based on an experiment conducted 13 years ago by Amagaya *et al*. (1988). Rat hepatic injury is induced using carbon tetrachloride or dimethyl nitrosamine.



Each column represents the mean ± S.E.M. of 4-5 mice.

**$P<0.01$ vs 8 weeks,
# $P<0.1$ vs CTRL.

Figure 1.8 Effect of Sho-saiko-to on Pentobarbital-Na induced sleeping time in aged female ICR mice.

The sGOT and sGPT in the blood increases, and the P450 in the liver microsomes decreases. When Sho-saiko-to was administered, there was a clear improvement. This suggested that Sho-saiko-to influenced the liver metabolic enzyme activity.

We decided to use a hypnotic agent to study the relationship between liver metabolic function and Kampo medicine (Tamura, 1999). To mice of 6, 10, 16 and 60 weeks of age, 60 mg/kg of the hypnotic agent pentobarbital was administered intraperitoneally, and the sleep time, as measured from loss of the righting reflex to recovery, was measured. As indicated in Figure 1.8, elder mice had longer sleeping time, indicating a slower drug metabolism. However, the animals in the Sho-saiko-to-administered group showed a decrease in the sleep time. This result suggests that Sho-saiko-to improve the liver's metabolic function.

What genes are expressed that code metabolic enzymes? To compare the mRNA expression levels, the drug was administered for two weeks or four weeks, and the liver was isolated and analysed by competitive reverse transcription-polymerase chain reaction (RT-PCR). The expression of P450 mRNAs for seven P450 isozymes after Sho-saiko-to treatment was determined by agarose gel electrophoresis. The results indicate that for 2B, the lane showing a 1 : 1 signal changed from A to B as a result of Sho-saiko-to administration, indicating a four-fold increase of mRNA expression, and 3A1 also increased four-fold. Since phenobarbital administration induces CYP2B and 3A1 and decreases the pentobarbital induced sleep time, it is possible to explain how Sho-saiko-to decreases the sleep time. Table 1.5 summarizes the results of the studies on seven genes. Sho-saiko-to also dramatically increased the expression of CYP4A1, but fortunately, it did not affect 1A1 and 1A2, which are involved in chemical carcinogenesis. CYP3A constitutes about 30% of the P450 isoenzymes present in human-liver microsomes and is known to be involved in the metabolism of many drugs. We will in the future need to determine whether these CYPs are induced at the protein level.

Thus, I expect that Kampo medicines will have extremely interesting possibilities for clinical treatment in the twenty-first century. Currently in Japan, under the approval of the Ministry of Health and Welfare, there are 146 types of Kampo prescriptions used in the national heath programme. About one-half of these are listed in the Shanghan Zabing Lu. I hope this historic treasure accumulated by Japan and China will be appreciated all over the world during the coming new century.

Table 1.5 Effect of Sho-saiko-to on the expression of Cytochrome P450 mRNA in female SD rats

P-450 isozyme	*mRNA expression amount (copies/ng total RNA)*					
	1–2 weeks			*1–4 weeks*		
	CTRL	*Sho-saiko-to*	*Inductive rate*	*CTRL*	*Sho-saiko-to*	*Inductive rate*
CYP 1A1	N.D.	N.D.	±	N.D.	N.D.	±
CYP 1A2	N.D.	N.D.	±	N.D.	N.D.	±
CYP 2B	1.6 × 105	6.4 × 105	× 4	1.6 × 105	1.6 × 105	±
CYP 2E1	5.2 × 106	1.1 × 107	× 2	2.6 × 106	1.1 × 107	× 4
CYP 3A1	6.4 × 105	2.6 × 106	× 4	1.3 × 106	2.6 × 106	× 2
CYP 3A2	N.D.	N.D.	±	N.D.	N.D.	±
CYP 4A1	1.6 × 105	1.3 × 106	× 8	6.4 × 105	1.3 × 106	× 2

Note
N.D.: Not detected.

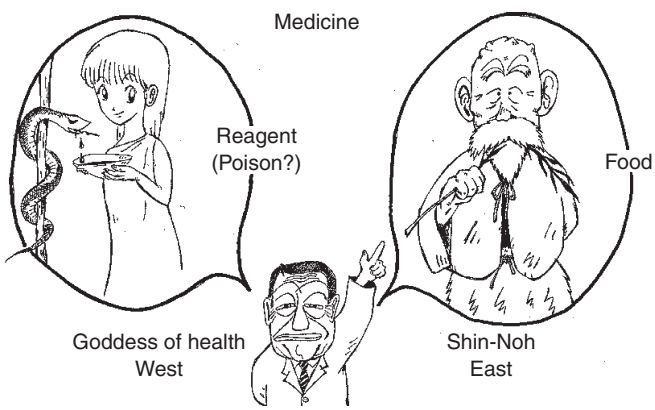


Figure 1.9 The fundamental difference between Eastern and Western medicine.

Finally, the cartoon in Figure 1.9 indicates the fundamental difference between Eastern medicine and Western medicine. Western medicine is an offensive medicine in which various means are used to attack the illness externally. While Eastern medicine could be considered to be a defensive medicine, in which the alleviative means such as acupuncture and Kampo medicine are used to induce the power in the patient to eliminate the illness. The twenty-first century is said to be the era of self-medication. There is no doubt that Kampo medicines will be essential in 'preventive medicine' to 'treat pre-illness' and in geriatric medicine, where we should not administer potent medicines unnecessarily.

Purpose of medical treatment is to relieve patients from fear or pain of disease. To achieve this, we should try every possible means, according to the condition or syndrome of patients. Whatever means it is, modern medicine (scientific Western one) or traditional medicine (Kampo)! Evidence, which is required for the base of medicine, should be established by the scientific methods that have been developed during only the last 100 years. Is it enough? Is the science Almighty?

Notes

1 The Han here was different from the present Chinese, which is composed of 56 races, that is 94.5% of Han and 5.5% of 55 minority nationalities, and covering southeastern part of Chinese continent as shown in the right-hand side orange part on the map.
2 Physician educated by Han medicine. At that time, physicians of Korea were very much influenced by Han medicine.
3 This medicine was created in China, and brought to Japan *via* Korean peninsula at fifth century and has followed an independent course of development as the medicine of experience, which is different from present Chinese medicine and called Kampo medicine today.

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2 Traditional usage and therapeutic indication

Masaki Aburada and Sen Shinohara

Composition of Sho-saiko-to formulation

Approach to drug formulation according to Kampo medicine

'Treatise on Febrile Diseases Caused by Cold (Shanghan Lun)' describes the prescription names and composition of the drugs, as is described for Sho-saiko-to in Figure 2.1, as well as the dosages and methods of decoction, but does not describe the activity of individual drug components in the formulation nor the significance of the combination of the drugs. Thus, to understand the significance of the composition of the formulation, it is necessary to understand the

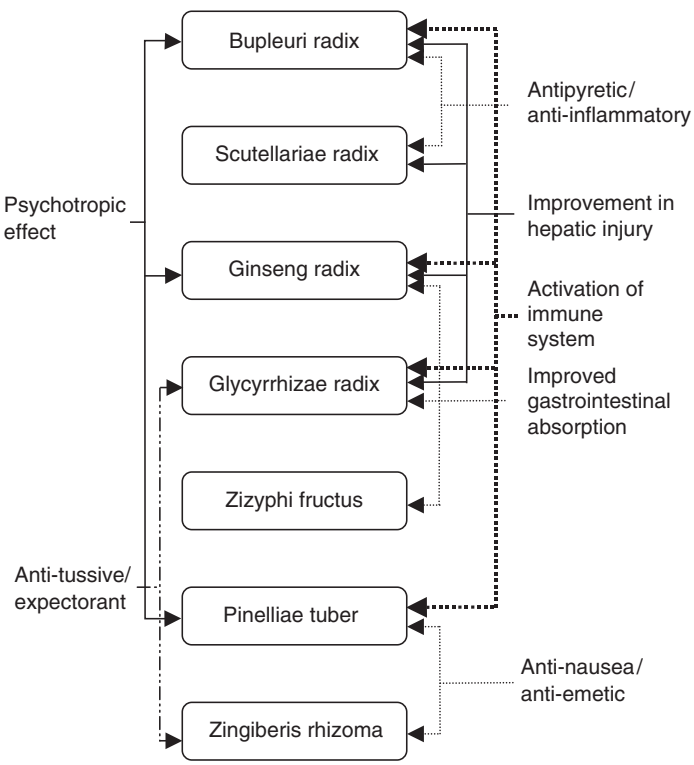


Figure 2.1 Structure of Sho-saiko-to formulation.

basis for drug formulations used in Kampo medicine and based on such an understanding that determine the significance of the composition of Sho-saiko-to formulation. Comparison of Kampo prescription suggests the following principles:

1 Patients generally have multiple disease-causing factors and many complaints, and it is impossible for a single drug component to affect all factors and improve all complaints. Multiple drug components are needed. Because drugs possess multiple effects, it is important to select those drugs that are most appropriate for the various complaints of the patient.
2 When considering drug efficacy, it is often possible to obtain synergistic effects and improve the drug efficacy by using specific drug combinations rather than simply increasing the dose of one drug component. The composition of drug formulations should be based on such combinations. Such combinations reduce the dosage needed of a given drug component and improve the safety margin.
3 If the use of a drug component is expected to induce an adverse effect, it is necessary to include in the composition another drug that suppresses the adverse effect.
4 The changes in composition and the synergistic drug effects achieved through the use of drug combinations must not result in adverse effects.
5 The drug formulation should satisfy the requirements described above and must also be a rational composition that uses as few numbers of drugs as possible at the lowest possible doses and in the most compatible combination.

Significance of the Sho-saiko-to formulation

Effects of drug components in Sho-saiko-to. In order to develop a formulation based on the principles described in the first section, it is necessary to know the effects of each of the constituent drugs. The old medical text, Pen Ts'ao Shiyi (*Compendium of Materia Medica*), describes the effects of many historically known drug components. However, these effects are not the same as the modern day pharmacological effects but rather have been handed down based on experience; these are thus termed pharmacological function. Table 2.1 lists the pharmacological functions of drugs in Sho-saiko-to in modern terminology and compares them to the currently known pharmacological effects.

Two types of effective combinations seen in Sho-saiko-to formulation. Pen Ts'ao Shiyi (*Compendium of Materia Medica*) also describes the following with regard to the effective use of combinations of two different drugs in creating a formulation. In Kampo medicine, there are seven different patterns of drug combinations. Among these, the most important are xiang xu, or potentiation, in which two drugs of similar properties potentiate each other, resulting in a synergistic effect, and xiang shi, or enhancement, in which the therapeutic effects of the main drug are enhanced by the addition of other drugs. The drug combination used in Sho-saiko-to is significant from this standpoint (Table 2.2).

Overall formulation of Sho-saiko-to. Figure 2.1 summarizes the relationship among the drugs based on the drug functions and the two types of combinations. According to the *Treatise on Febrile Diseases Caused by Cold* (Shanghan Lun), Sho-saiko-to is useful in the treatment of subacute respiratory tract infections and viral infections such as hepatitis, urinary tract infections, peripartum infections and other chronic inflammatory diseases. According to Figure 2.1, for respiratory tract infections, Sho-saiko-to has anti-pyretic/anti-inflammatory effects and anti-tussive/expectorant effects; improves the gastrointestinal symptoms that develop with progression of disease; and aids in maintaining the resistance of the body against diseases. Although not previously

Table 2.1 Comparison of pharmacological functions and effects of component drugs in Sho-saiko-to

Drug component	*Summary of drug function*	*Portion of elucidated pharmacological effects*
Bupleuri radix	Anti-pyretic, sedative effects, improves smooth muscle tension	Anti-inflammatory, CNS inhibition, activation of immune system, anti-allergic effects, improves injured liver, etc.
Scutellariae radix	Anti-pyretic, anti-inflammatory, detoxification, haemostatic, prevents miscarriage	Anti-pyretic, anti-inflammatory, capillary vessel reinforcement, prevents liver injury, anti-hypertensive, etc.
Ginseng radix	Functional rehabilitation, improves shock, improves gastrointestinal absorption, tranquilizer, etc.	CNS stimulant, stress relief, reduces fatigue, tonic, promotes metabolism, circulatory improvement, improves injured liver, activates immune system, etc.
Pinelliae tuber	Improves water metabolism, anti-emetic, improves abnormal laryngeal in larynx	Stress relief, anti-emetic, activates immune system, anti-allergic effects, etc.
Glycyrrhizae radix	Improves gastrointestinal absorption, expectorant, anti-tussive, analgesic, anti-inflammatory, detoxification, etc.	Sedative effects, anti-spasmodic, anti-tussive, prevents gastrointestinal ulcer, cholagogue, prevents liver injury, activates immune system, anti-allergic effects, etc.
Zingiberis rhizoma	Anti-pyretic, anti-emetic, anti-tussive, expectorant, detoxification	Anti-pyretic, analgesic, anti-convulsant, anti-tussive, anti-emetic, promotion of peristalsis, etc.
Zizyphi fructus	Improves appetite, tranquilizer	Anti-allergic effects, prevents gastrointestinal ulcer, stress relief, sedative effects, etc.

Sources: Pharmacological function is based on *Chinese Medicine for Chinese Medicine Practitioners*, Kobe Chinese Medicine Research Center, Ishiyaku Shuppan Publishing Co.; Pharmacological effects are based on the *Tsumura Drug Handbook,* 4th edition (Tsumura & Co.), Yamada Mitsutane and Tei Sotetsu, eds.

Table 2.2 Cooperating effects of the drug formulation in Sho-saiko-to

Bupleuri radix + Scutellariae radix
→ anti-pyretic/anti-inflammatory
Bupleuri radix + Ginseng radix
→ improvement in smooth muscle tension
(useful in gastroptosia, nephroptosia, rectal prolapse, etc.)
Scutellariae radix + Pinelliae tuber/Zingiberis rhizoma
→ anti-nausea/anti-emetic
Ginseng radix + Glycyrrhizae radix
→ improved gastrointestinal absorption
Ginseng radix + Zingiberis rhizoma/Zizyphi fructus
→ improved gastrointestinal absorption
Pinelliae tuber + Zingiberis rhizoma
→ anti-nausea/anti-emetic

Source: Based on *Chinese Medicine for Chinese Medicine Practitioners*, Kobe Chinese Medicine Research Center, Ishiyaku Shuppan Publishing Co.

Figure 2.2 The explanatory books of *Sho-Kan-Ron* written by Japanese.

known, recent pharmacological studies have also shown that immunologic resistance is improved, thus conferring efficacy in viral infections. For patients with chronic liver diseases, Sho-saiko-to suppresses the inflammation in hepatitis and activates the immune system to eradicate the virus, thereby improving the liver injury and improving the associated symptoms of malaise, loss of appetite, nausea and irritability, so that the formulation is extremely suitable in this setting.

Cho Chukei (Zhang Zhongjing), a governor in the later Han dynasty of ancient China, compiled two practical medical books named, *Sho-Kan-Ron* (shang-han-lun) (Figure 2.2) and *Kinki-Yoryaku* (jin-kui-yao-lue) (Figure 2.3) about 1800 years ago. Sho-saiko-to (xiao-chai-hu-tang) is one of the traditional Chinese herbal medicines described in these books.

The *Sho-Kan-Ron* (shang-han-lun) is a treatise on Sho-kan meaning acute infectious disease (it likely corresponds to typhoid fever), where the pathology of the disease is classified into stages, and Chinese herbal medicines to be used in each stage and their usage are described (Figure 2.4).

Figure 2.3 *Kinki-Yoryaku* written by Japanese.

Bupleuri radix	10.0 g
Scutellariae radix	4.0 g
Ginseng radix	4.0 g
Glycyrrhizae radix	4.0 g
Pinelliae tuber	2.5 g
Zingiberis rhizoma	4.0 g
Zizyphi fructus	4.0 g

小柴胡湯方
柴胡 半斤○十金 翼作二八兩
黃芩 三兩
人參 三兩
半夏 半升 洗
甘草 炙
生薑 各三兩 切
大棗 十二枚 擘○金 書十三枚
右七味以水一斗二升煮取六升去滓再煎取三升溫服
一升日三服

The seven raw herbs described are extracted with 2400 ml of boiling water and the decoction is concentrated to 1200 ml. Herbal residue is removed and the 1200 ml of the decoction is further boiled down to 600 ml. Take 200 ml of the warm concentrated decoction three times a day.

Figure 2.4 Preparation of Sho-saiko-to decoction.

Namely 102 traditional Chinese herbal medicines were described in the 'Sho-Kan-Ron', and it can be decided easily according to four techniques of diagnosis called 'shi-shin' (four diagnostic procedures) what medicine should be suitable to what patients.

The *Kinki-Yoryaku* (Jin-kui-yao-lue), which is a companion volume to the *Sho-Kan-Ron*, is a treatise on nomenclature, symptoms and therapeutic regimens concerning diseases (miscellaneous diseases) other than acute febrile disease. The *Kinki-Yoryaku* covers almost all diseases including cardiovascular disorder, respiratory disorder, urinary system disorder, gastrointestinal disorder, skin disease, gynaecological disease and psychiatric disease, and furthermore, emergency care and prohibited foods.

Diagnostic procedures

Shi-shin (four techniques of diagnosis) includes 'bo-shin' (wang-zhen), 'bun-shin' (wen-zhen), 'mon-shin' (wen-zhen) and 'ses-shin' (qie-zhen), each of which is a unique technique of diagnosis specified in the next paragraph.

(1) 'Bo-shin' (wang-zhen) is visual observation, where appearance of a patient is examined, for instance, whether he/she is fat or thin, he/she looks healthy or pale, his/her expression is lively or lifeless, the skin is moist or dry, and how is the condition of the tongue (called 'zetsu-shin'; she-zhen; tongue diagnosis). (2) 'Bun-shin' (wen-zhen) is examination of listening for sounds or sniffing at something given by the patient. (3) 'Mon-shin' (wen-zhen) is verbal examination by questioning of the patient, for instance, by asking the patient about complaints and conditions to obtain general information on the illness. (4) 'Ses-shin' (qie-zhen) is equivalent to palpation. It is divided into 'myaku-shin' (mai zhen) and 'fuku-shin' (fu-zhen). 'Myaku-shin' is examination by touch to take the pulse ('myaku') and 'fuku-shin' is to examine conditions of the abdomen ('fuku') by touch. The 'Sho-Kan-Ron' established these unique procedures for diagnosis according to the nature of disease.

Course of disease and its stage category in *Sho-Kan-Ron*

Pathological stages according to the course of disease described in *Sho-Kan-Ron* (shang-han-lun)' are briefly explained here. In the *Sho-Kan-Ron*, diseases are categorized into six stages called 'san-in-san-yo (san-yin-san-yang; three Yin stages and three Yang stages)' according to the course of disease. San-yang stage diseases comprise 'tai-yo' disease (tai-yang-bing), 'Sho-yo' disease (shao-yang-bing) and 'yomei' disease (yang-ming-bing). San-yin stage diseases comprise 'tai-in' disease (tai-yin-bing), 'Sho-in' disease (shao-yin-bing) and 'kech-in' disease (jue-yin-bing). The tai-yo disease (tai-yang-bing) is the initial stage of disease. The focus of disease is regarded to be in the ectodermal area. The pulse can be felt superficially. Furthermore, it is characterized by headache, stiffness of the nape (an area from the neck to the shoulder) and chillness. The 'Sho-yo' disease (shao-yang-bing) is the second stage of disease where the focus is regarded to be in the mesodermal area. Patients complain of bitter taste in the mouth, dry throat, dizziness, etc. In addition, patients feel stiff and heavy in the area from the chest to the side. In palpation, stiffness (increased tension of the muscle) and tenderness around the upper and lower areas of the costal arch or stiffness with choked feeling in the epigastric area are observed. The yomei disease (yang-ming-bing) is the advanced stage of the disease. Patients in this stage have constipation and distention of the abdomen. If the disease progresses further without any treatment, the focus will invade into the deeper area, and the disease will proceed to the tai-in disease (tai-yin-bing), Sho-in disease (shao-yin-bing) and finally kech-in disease (jue-yin-bing).

Description of Sho-saiko-to in *Sho-Kan-Ron* and its interpretation

The text in the *Sho-Kan-Ron* (shang-han-lun) where therapeutic effects of Sho-saiko-to (xiao-chai-hu-tang) are discussed is as follows (Figure 2.5):

The following is the translation or interpretation into plain text from the above original text.

1 Five or six days after suffering from acute infectious disease (acute febrile disease or inflammatory disease), chills and fever that are the main symptoms of the disease may change to intermittent chills and fever, that is, whenever chills leave, fever rises, on the contrary, whenever fever leaves, chills develop, and these episodes alternate with each other. The patient may have fullness distress and tenderness of the hypochondrium; he/she may feel restless,

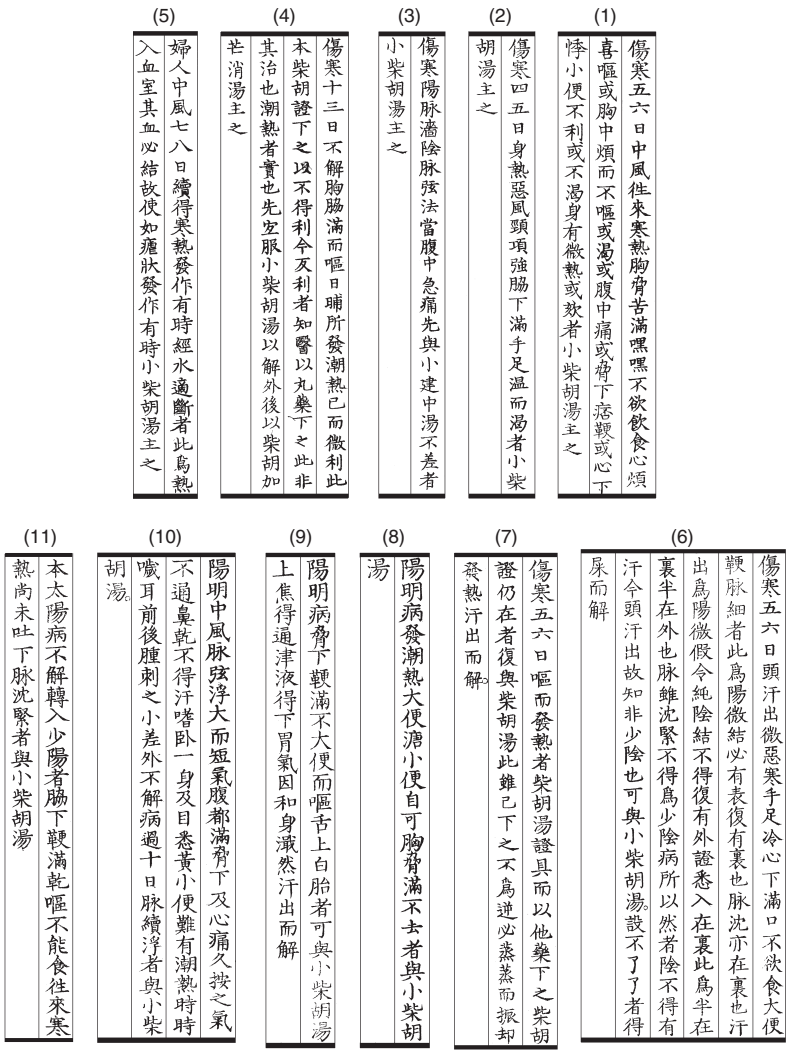


Figure 2.5 The text in the *Sho-Kan-Ron*.

keeps silent and loses appetite; and he/she may have discomfort of the chest and frequent vomiting. Sho-saiko-to is effective in patients who have these symptoms or conditions.

In addition to the above main symptoms, occasionally, the patient may have discomfort of the chest but no vomiting, dry mouth, abdominal pain, fullness in the inferior margin of the costal arch, epigastric throbbing pulsation, a difficulty in urination, no dry mouth, slight fever or a cough. Patients who have these symptoms or conditions should be also treated with Sho-saiko-to.

2 Four or five days after suffering from acute infectious disease, the patient may have endodermal fever (feverish feeling in the deep area of the body) and chills, and have stiffness of

the shoulder and neck. In addition, he/she may have fullness of the hypochondrium, hot feeling in the extremities and thirst. Patients who have these symptoms or conditions should be treated with Sho-saiko-to.

3 In a patient suffering from acute infectious disease, the pulse may falter when slightly touched and it may be felt like a touch to a bowstring when strongly touched, and the patient naturally complains of abdominal pain. Sho-kenchu-to (xiao-jian-zhong-tang) should be administered to patients with these symptoms or conditions at first. If the symptoms are not improved by Sho-kenchu-to, Sho-saiko-to should be used.

4 Thirteen days after suffering from acute infectious disease, the disease is not improved yet. The patient has fullness of the hypochondrium and vomit. A passing fever appears in the late afternoon as though a tide rises and fills the whole body. In addition, the patient has loose stool or tendency towards diarrhoea. Patients who have these symptoms or conditions should be treated with Sho-saiko-to at first.

5 Seven or eight days after a woman suffers from acute moderate infectious disease (equivalent to a disease like a common cold), chills and fever appear spasmodically like malarial fever. She may have no menstruation as scheduled due to the disease (or if she is during menstruation, the menstruation is halted earlier than scheduled). Sho-saiko-to should be used in women with these symptoms or conditions.

6 Five or six days after suffering from acute infectious disease (acute febrile disease or inflammatory disease) when the disease reaches the stage of Sho-yo disease (shao-yang-bing), the patient may have sweating on the head, slight chills and coldness of the extremities, and fullness and distress of the epigastrium. He/she may lose appetite, and have hard stool and thready pulse (feeble pulse barely touched). Sho-saiko-to is effective in patients who have these symptoms or conditions. In a patient who does not feel relieved after taking Sho-saiko-to, when evacuating the bowels (if spontaneous bowel movement is not expected, use a purgative formulation), he/she will start feeling well and recover. [This description is not included in the original text of the *Sho-Kan-Ron*, and is likely added by a medical expert of a later generation.]

7 Five or six days after suffering from acute infectious disease, if a patient starts vomiting and has fever, Sho-saiko-to will be indicated for the treatment. Even if the patient has constipation, Sho-saiko-to should be used. If the patient has diarrhoea induced by a purgative formulation, Sho-saiko-to can be used as far as symptoms (sho, zheng) indicate treatment with Sho-saiko-to. In this case, the patient may have severe chills and shivering after taking Sho-saiko-to, and thereafter have a fever. However, the disease is resolved with sweating.

8 In a patient suffering from acute infectious disease, the disease may not show a tendency to be resolved, and progress to the stage of yomei disease (yang-ming-bing). Such a patient has a regularly recurring fever as though the tide rises and the fever fills the whole body. If stool does not become hard and remains soft, urination is normal, and fullness of the hypochondrium is not resolved, Sho-saiko-to should be administered.

9 In a patient suffering from acute infectious disease, the disease may not show a tendency to be resolved, and progress to the stage of yomei disease (yang-ming-bing). If the patient has rigidity and fullness of the hypochondrium, no bowel movement, nausea and white coating of the tongue, Sho-saiko-to should be used. The disease will be resolved with improvement of fullness of the hypochondrium, bowel movement, relieving nausea and profuse sweating.

10 In a patient suffering from moderate acute febrile disease in the stage of yomei disease (yang-ming-bing), the pulse may be full and rapid with a feeling of pounding when slightly touched. The patient gasps for breath, has fullness of the abdomen and has tenderness of the hypochondrium and chest. When the abdomen is pressed for massage for a while,

the patient has more rapid breathing and dried nose but no sweating. The patient prefers lying in the bed to leaving it. He/she has jaundice in the whole body including the face and eyes, difficult urination, and a high fever recurring regularly as though the tide rises and falls (the difference between the maximum and minimum temperature is small). The patient has hiccups occasionally. The areas around the ears swell up, which is slightly improved by phlebotomy. If the superficial symptoms (gai-sho, wai zheng) are not improved even after 10 days have passed since onset of the disease, and if the pulse is still clear, Sho-saiko-to should be administered to the patient. [This description is not included in the original text of the *Sho-Kan-Ron*, and is likely added by a medical expert of a later generation.]

11 In a patient suffering from mild acute febrile disease in the stage of taiyo disease (tai-yang-bing), the disease may not show a tendency to be resolved, and progress to the stage of shoyo disease (shao-yang-bing) specified as the second stage of yang disease in the *Sho-Kan-Ron*. The patient has rigidity and fullness of the hypochondrium, and cannot eat anything due to dry retching. Chills and fever appear alternately, that is, whenever chills leave, fever rises, on the contrary, whenever fever leaves, chills develop. While the patient is not treated by emesis and purgation, he/she has a deep pulse, which can be barely touched by heavily pressing with fingertips and is felt like a fine string. When pressed more strongly, the pulse swings. Sho-saiko-to should be administered to patients with these symptoms or conditions.

Indications of Sho-saiko-to (xiao-chai-hu-tang) in modern medicine

Based on the above description in the *Sho-Kan-Ron* (shang-han-lun), pathology of disease for which Sho-saiko-to is effective and therapeutic effects of Sho-saiko-to have been investigated from aspects of the modern medicine. Currently, the following indications are established for Sho-saiko-to in Japan:

1 In patients with moderate constitution who have fullness and distress of the upper abdomen, coating of the tongue and discomfort in the mouth, anorexia and occasionally have a low grade fever, nausea, etc., Sho-saiko-to is indicated for treatment of the following diseases or conditions: acute febrile diseases, pneumonia, bronchitis, common cold, supportive therapy of tuberculous diseases such as pleuritis and pulmonary tuberculosis, lymphaedenitis, chronic gastrointestinal disorder and postpartum disorder.
2 Improvement of hepatic function in patients with chronic hepatitis.

The validity of these indications have been proved by many clinical studies including pharmacological studies and double-blind studies as described later.

3 Crude drugs I

Taxonomical items, collection and cultivation, production etc.

Masami Higuchi and Susumu Terabayashi

Bupleurum root

The Bupleurum root was recorded in the Chinese oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933a). It has anti-pyretic effects, detoxifying effects, analgesic effects and anti-inflammatory effects and has been noted as an important crude drug successively in classical pharmacopoeia in China and Japan. It is a component of such Kampo prescription as Sho-saiko-to (Xiachaihutang), Dai-saiko-to (Dachaihutang), Sairei-to (Chailingtang), Shigyaku-san (Sinisan) and so on. *The Pharmacopoeia of Japan*, 13th edition, *Supplement I* (Committee for editing of the commentaries on Japan Pharmacopoeia, 1998a) specifies that the Bupleurum root is the root of *Bupleurum falcatum* L. (Umbelliferae).

The properties of the Bupleurum root in the market are as follows. The roots are made of a main root and thin lateral roots. The root is 10–20 cm in length, and 0.5–1.5 cm in diameter at the upper portion and 0.4–0.6 cm at the middle portion. Externally, the root is pale brown to brown in colour, with longitudinal wrinkles. It is easily broken, and the fractured surface is somewhat fibrous. It is particularly fibrous in the upper portion. The Bupleurum root has a characteristic odour, and has a slightly bitter taste.

The following features are observed in a transverse section of the Bupleurum root under a microscope. The outermost part is of cork layers, surrounding the cortex composed of parenchymatous cells. The cortex constitutes 1/3 to 1/2 of the radius of the root and contains several oil canals of 10–40 μm in diameter. The cortex and the xylem are separated by a cambium. The vessels in xylem are arranged in radiating direction, often accompanied by fibre bundles. In the parenchymatous cells of the cortex and the xylem starch grains and oil drops occur. The starch grains are simple grains of 2–10 μm in diameter or compound grains.

The history, plant origin, producer region and selection of the Bupleurum root are now discussed briefly (Figures 3.1 and 3.2).

History: The Bupleurum root is designated in *Materia Medica of Shen Nong*. The plant origin of the Bupleurum root is thought to have been several plants based on the descriptions and figures in the classical herbal texts. In the current Chinese market, products carrying the Bupleurum root name include the plants of the genus *Bupleurum* (Umbelliferae), as well as those of the families, Compositae, Caryophyllaceae and Rosaceae. The authentic product is considered to be root of the Bupleurum species.

In Japan, *Bupleurum falcatum* growing in the wild in the Mishima region of Shizuoka Prefecture and Kyushu have been used since the Edo period (1603–1867). The root of this species from the Mishima region were called 'Mishima-saiko' and those from Kyushu were called 'Kamakura-saiko' or 'Kyushu-saiko'. The annual production of several metric tons continued until about the 1970s.

Figure 3.1 *Bupleurum falcatum* L.

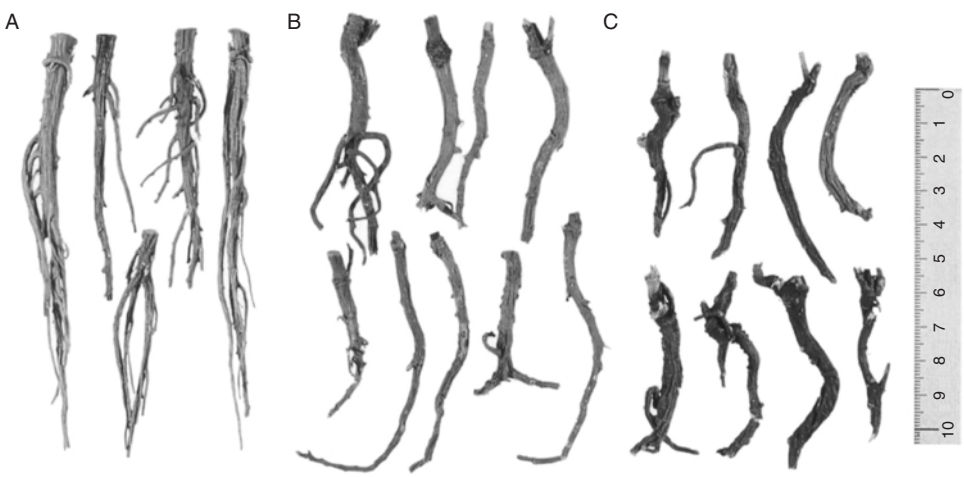


Figure 3.2 Bupleurum root. A: Bupleurum Root from Japan. B: Bupleurum Root from Korea (Shoku-saiko: *B. falcatum*). C: Bupleurum Root from China (Kita-saiko: *B. chinese*).

In recent years, however, with the demand of Kampo extract formulae for ethical use, sufficient quantities cannot be obtained from the wild, and cultivated products are used.

Plant origin: Bupleurum falcatum L. (Umbelliferae) is a perennial herb of 40–70 cm in height, with an erect stem branched at the upper part. The leaves are alternate, linear to linear-lanceolate in shape, and with parallel veins. The inflorescence is terminal and a compound umbell. The umbell is composed of 5–10 yellow small flowers. Each flower consists of 5 petals curved inwardly, 5 stamens and a terminal pistil. The fruit is ellipsoid in shape and about 3 mm in length. This species is distributed in Honshu, Shikoku and Kyushu in Japan. Three cyto-types; the chromosome numbers 2n = 26, 2n = 20 and 2n = 32, are reported for this species in Japan (Ohta *et al*., 1986). The three cyto-types have different distributions.

Producer regions: The plants cultivated in the areas west of the Kanto region have a chromosome number of 2n = 26. In the recent years, the Bupleurum root prepared from *B. falcatum* has been produced in China. The Bupleurum roots used in China are prepared from *B. chinense* DC. called Kita-saiko on the market, and *B. scorzornerifolium* Willd. called Nan-saiko, as well as from other species of Bupleurum growing in the wild, such as *B. marginatum* Wall. ex DC., *B. yinchowense* Shan et Y. Li, *B. bicaule* Helm., *B. smithii* Wolff var. *parrifolia* Shan et Y. Li, *B. microcephalum* Diels, *B. sibiricum* De Vest, and *B. komarovianum* Lincz (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1982a). In some regions in China, the whole plants of Bupleurum are used. Among the Bupleurum root derived from various species of Bupleurum in China, the roots of *B. chinense* are mainly exported to Japan. During 1980s, the Bupluerum root based on *B. falcatum* called Shoku-saiko was produced in Korea and exported to Japan in large quantities, but in recent years, very little has been seen.

Production: In fall-winter, the aerial parts are cut, and the roots are dug up. The roots are washed with water and dried in the sun. When partially dried, the rootlets are removed by hand, and after shaping the root, they are further dried (Pharmaceutical Bureau, Ministry of health and welfare, ed. 1992). At one time, the domestic production peaked to 400 metric tons annually, but in the recent several years, the domestic production has declined to about 150 tons caused by the sift of cultivation from Japan to China. On the other hand, the amount of the Bupleurum roots imported from China is about 600 metric tons in 1997.

Selection: Those considered to be of high quality have a reddish external colour, a soft pliable root, high oil content, moist texture and a strong fragrance.

Pinellia tuber

The Pinellia tuber was recorded in the Chinese oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933c) and it has been noted as an important crude drug, recorded in successive pharmacopoeia in both China and Japan. It has anti-nausea, anti-emetic, sedative, antitussive and expectorant effects and is a necessary Kampo component in numerous Kampo prescriptions such as Sho-saiko-to (Xiachaihutang), Sho-seiryu-to (Xioquinglongtang), Hange-koboku-to (Banxiahoupotang) and Choto-san (Goutengsan). *The Pharmacopoeia of Japan*, 13th edition (Committee for editing of the commentaries on Japan Pharmacopoeia, 1996a) specifies that the Pinellia tuber is the tuber of *Pinellia ternata* Breitenbach (Araceae), from which the cork layer has been removed.

This raw herb has a somewhat compressed spherical shape, of 0.7–2.5 cm in diameter, 0.7–1.5 cm in height, and white-grey to white-yellow colour, with a dimple in the upper portion which represents the stem, and surrounding tiny dimples, which are the remnants of roots. The texture is firm hard and the cut surface is white and powdery. It essentially has no odour. It initially has no taste but has a slight mucous texture and later leaves a strong acrid taste.

Most of the Pinellia tubers seen commercially are produced in China, and they are graded on the basis of size, from the largest to smallest, into five grades, Tokkyu, Kohkyu, Otsukyu, Heikyu, Chinjukyu. Higher grade commands higher price. One kilogram of each grade corresponds respectively to about less than 800 granules, 900–1000 granules, 1700–1800 granules and 3000 granules or greater.

Microscopic observation of the transverse section of the Pinellia tuber reveals that it is made of parenchyma filled with starch grains, and scattered with a few mucilage cells containing raphides of calcium oxalate of 25–150 μm in length and a few closed vascular bundle. The starch grains are simple grains measuring 5–12 μm in diameter and 2–4 compound grains measuring 10–32 μm in diameter (Figures 3.3 and 3.4).

The history, plant origin, producer region and selection of the Pinellia tuber are now discussed briefly.

History: The Pinellia tuber has been noted in *Materia Medica of Shen Nong* and has long been used as an essential component in anti-emetics and anti-tussives. The crowdipper, *Pinellia ternata* Breitenbach, which is the plant origin, was imported from China. It is unknown when it was introduced in Japan, and so it is considered to have been a pre-historic naturalized plant. It has

Figure 3.3 *Pinellia ternata* Breitenbach.

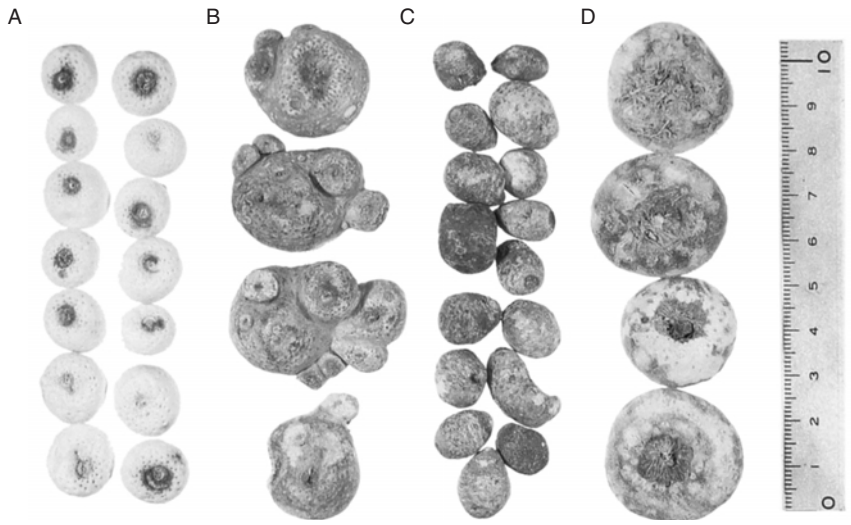


Figure 3.4 Pinellia tuber and related raw herbs. A: Pinellia tuber (*P. ternata*). B: Shoyo-hange (*P. pedatisecta*). C: Sui-hange (*Typhonium flagelliforme*). D: Arisaema tuber (*Arisaema* sp.).

been propagated in various regions in Japan, and at one point was produced in Japan. In the Miura Peninsula region of Kanagawa Prefecture, the Pinellia tuber was called 'secret savings'. This name comes from the local farmers who, during breaks from the farming work, would collect the tubers of the Pinellia plant on the farm, remove the external layer on the beach, rinse the tubers with water, and then dry them in the sun. The dried product was then sold to brokers to earn extra income.

Plant origin: *Pinellia ternata* Breitenbach is distributed widely in China, the Korean peninsula and Japan and is a perennial herb found wild in the plains and farm fields. The tubers are spherical–oval in shape. The leaves are radical. The petiole reaching 25 cm in length bears bulbils of 2–7 mm in diameter at the lower portion and at the uppermost portion. When the plants are young, the leaves are simple and ovate, 2–5 cm in lengths. The leaves of a mature plant are three parted. The lobes are narrowly ovate to lanceolate in shape, with the median lobe being 5–15 cm in length and the laterals being somewhat shorter. The flowers are unisexual, monoecious and open in the early part of the summer. The spathe is green or variable, 6–7 cm in length, and the tubular part 1.5–2 cm in length. The inflorescence is a yellow-green spadix, with the male flowers at the upper part and female flowers at the lower part. The spadix-appendage is filiform, 6–11 cm in length, growing outward. The berry is ovoid and greenish in colour.

Producer region: In the past, the Pinellia tuber was collected in the areas from the Kanto region to the Kyushu region in Japan, among which the ones from the Osaka area and the Yamaguchi Prefecture were valued for their white skin. Currently, because of production costs, only a small quantity is being produced in the Iwate and Kumamoto Prefectures.

The Pinellia tubers in the Japanese market are currently imported from China, some from Korean peninsula were available until about 1985. In China, the major producer regions are the Sichuan Province followed by Hubei, Henan, Guizhou, Yunnan and Anhui Provinces. In 1995 and 1996, 900 metric tons were imported annually into Japan.

Production: The enlarged tubers are harvested during the summer, and after being washed of dirt, the root hairs and the cork layer are removed, followed by drying in the sun or by heat. In China, a roasting step is performed to remove the acrid taste. Examples include the Kyo-hange Pinellia tuber processed using ginger. The efficacy of these processed Pinellia tubers also changes so that the Pinellia tuber is said to be effective as an expectorant, and the Kyo-hange Pinellia tuber effective as an anti-emetic. These processed products are in general use in China, while they are not used in Japanese Kampo medicines.

Selection: Those considered to be of high quality are large granules with white external colour.

Related raw herbs: Perhaps because the Pinellia tuber is produced widely, there are a number of known counterfeit products. In China there are seven known species in the Pinellia genus. Among these, the tuber of the *P. pedatisecta* is commonly called Shoyo-hange or Kosho. In China, it is commonly used as the raw herb for Arisaema species, but it can also be found being sold under the name of Pinellia tuber. In addition to plants of Pinellia and Arisaema, the tuber of *Typhonium flagelliforme* are also occasionally sold as Pinellia tuber named Sui-hange, thus caution is required. (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1982b.) It has been reported that one can make a distinction between these related raw herbs and the Pinellia tubers (*P. ternata*) on the basis of morphology and the sequence of *rbcL* genes in the chloroplast. (Higuchi and Okada, 1980; Okada, 1995; Kondo *et al*., 1998).

Scutellaria root

The Scutellaria root was recorded in Chinese oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933b) and it has been noted as an important crude drug, recorded in successive pharmacopoeia in China and Japan. It has anti-pyretic, anti-inflammatory, gastric promotion and detoxification effects and is often included in many Kampo prescriptions such as Sho-saiko-to (Xiachaihutang), Saiko-keishi-to (Chaihuguizhitang), Sairei-to (Chailingtang) and Oren-gedoku-to (Huanglingjiedutang). *The Pharmacopoeia of Japan*, 13th edition (Committee for editing of the commentaries on Japan Pharmacopoeia, 1996b) specifies that the Scutellaria root is the root of *Scutellaria baicalensis* Georgi (Labiatae), from which the periderm has been removed.

The properties of the Scutellaria root distributed commercially are cone shaped, semi-tubular or flat and are twisted, with a length of 5–20 cm and diameter of 0.5–3 cm. The external appearance is yellow-brown in colour, with coarse and marked longitudinal wrinkles, and with scattered scars of lateral root and remains of brown periderm. The upper part has the scars of stem or remains of stem. In old roots the central portion of xylem is rotten and black-brown in colour, or may be hollow. The texure is hard but easily breakable. The fractured surface is fibrous and yellow in colour. There is essentially no odour, and the taste is slightly bitter (Figures 3.5 and 3.6).

Almost all commercial products are from China, and most are from the wild, although some cultivated products have been seen recently. Cultivated products have a surface that is relatively smooth with firm and heavy texture, and it shows little rotting. On the other hand, those from the wild with the tip of the root removed or the periderm of the young roots removed are called 'Sengon', 'Shigon' or 'Jogon'. Those with a rotted, black-brown xylem or those where hollows are formed in the middle parts of root caused by a falling of a rotted xylem are called 'Kogon'. Those that have broken due to processing into semi-tubular or flat shapes are called 'Hengon'.[1]

Microscopic observation of the transverse section of the Scutellaria root reveals that the outermost periderm is composed of cork cells reaching twenty layers, and the external layer is often completely denuded. The primary cortex is made of relatively thin layer of the parenchyma. The stone cells and fibres, alone or in groups were scattered in the parenchyma. The secondary cortex is composed of phloem and ray, and the border is indistinct. The stone cell and fibres also

Figure 3.5 *Scutellaria baicalensis* Georgi.

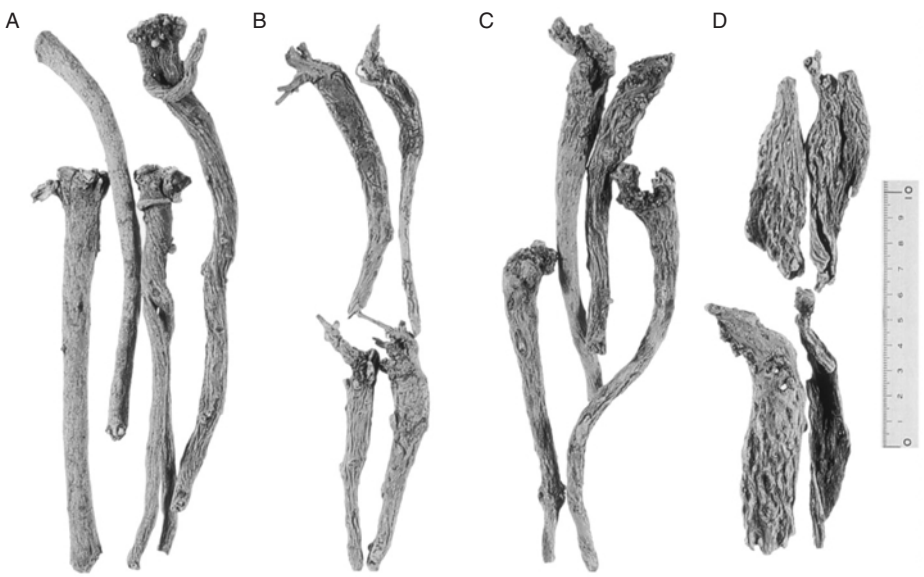


Figure 3.6 Scutellaria root. A: Cultivated product. B: Wild product (Sengon). C: Wild product (Shigon). Wild product (Hengon).

exist in the phloem parenchyma. The cambium is relatively distinct. The xylem is composed of vessels, xylem fibres and xylem parenchyma. In old roots, cork layers appear in the central portion of xylem. The pith is absent. Starch grains exist in the cortical parenchyma, phloem parenchyma and xylem parenchyma. They are mainly simple grains, spherical and 4–10 μm in diameter.

The history, plant origin, producer region and selection of the Scutellaria root are now discussed briefly.

History: Scutellaria root is a herb that is recorded in *Materia Medica of Shen Nong* and is a raw herb that was imported into Japan. There is a record that the seeds were imported from the Korean peninsula in 1726 (Edo period) and cultivated in the Koishikawa Herb Garden. Currently, cultivation is possible in Japan also.

Plant origin: Scutellaria baicalensis Georgi (Labiatae) is a perennial herb and distributed in the north-eastern China, Siberia and the Korean peninsula. The habitats of this species are grass lands and waste lands that receive a lot of sun. It is also widely cultivated. The plant is about 25–60 cm in height, and the main root is cone shaped, with the periderm being yellow-brown and the inner portion being yellow. The stems are square in transverse sections and branch from base. The basal parts of the stems are creeping but the upper parts are erect and branched. The plant is sparsely pilose. The leaves are opposite, lanceolate in shape and 1.5–4.5 cm in length. In July and August, a spike appears at the top of the stem, and numerous flowers with lips of purple to indigo-purple colour open. The corolla is about 2.5 cm in length. The nutlets black in colour are small, and mature in August–September.

Producer regions: Hebei, Neimenggu, Shanxi, Shaanxi, Liaoning, Heilongjiang Provinces in China are the major producer regions (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1979a). Among these, the Hebei and Shanxi Provinces are the largest producers, and the Chengde region in Hebei Province is considered to produce the highest quality product.

There are some Japanese products available commercially, but most are imported from China. In the recent years, cultivation has progressed, but the majority are still from the wild. In 1994 and 1995, about 750 metric tons annually were imported into Japan.

Production: In the fall, after the aerial parts have turned yellow, the plants are dug up, and after being semi-dried in the sun, the periderm is removed and then completely dried.

The cultivated products in Japan are harvested in the fall of the second year when the aerial parts have turned yellow. The aerial parts are cut and then the plants are dug up taking care not to damage the root. After removing the stem at the top and the fine roots and washing with water, the periderm is removed using a bamboo spatula, and then the roots are immediately dried in the sun or by heat.

Selection: Depending on the differences in the shape, the Scutellaria roots are classified as Sengon, Shigon, Jogon, Kogon and Hengon. Those currently available in the market are largely classified as Sengon and Hengon, and the Sengon is considered to be of high quality. Desirable features are those with minimal hollowness or rotting in the xylem, hard and firm in texture, yellow colour and a bitter taste.

Jujube

The Jujube was recorded in the Chinese oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933a) and it has been noted as an important crude drug, recorded in successive pharmacopoeia in China and Japan. It has relaxant, sedative, analeptic, diuretic and haematogenic effects, and is often included in many Kampo prescriptions, such as Sho-saiko-to (Xiachaihutang), Kakkon-to (Gegentang) and Sairei-to (Chailingtang).

The Pharmacopoeia of Japan, 13th edition, *Supplement I* (Committee for editing of the commentaries on Japan Pharmacopoeia, 1998b) specifies that the Jujube is the fruit of *Zizyphus jujuba* Miller var. *inermis* Rehder (Rhamnaceae).

The Jujube fruit distributed commercially as a raw herb is ellipsoid or widely ovoid and is 2–3 cm in length and 1–2 cm in diameter. Its outer appearance shows a red-brown colour with rough wrinkles, or a dark-grey red colour with fine wrinkles, with a glossy surface. The both ends slightly dented, with a scar of style on one end and a scar of peduncle on the other. The fruit is a drupe, with the epicarp being thin and leathery, while the mesocarp is thick and dark greyish brown in colour, spongy, soft and sticky. The endocarp is extremely hard, fusiform, and has two locules. These occasionally contain seeds. Seeds are flat and ovoid. It has a faint characteristic odour and a sweet taste (Figures 3.7 and 3.8).

The history, plant origin, producer region and selection of Jujube are now discussed briefly.

History: The genus of *Zizyphus* originated in the northern Africa, western Europe, and the fruits historically have been widely used as a drug or nutritional support in various regions of the world, such as India, Europe, Africa, America and Australia (Grieve, 1971; Cribb and Cribb, 1981). Jujube has been used in China for a long time, and the palaeograph, 'Jiga' (1000 BC) notes eleven different cultivars. It is an important fruit not only for food and drug use, but also for ceremonial use (Editorial committee of the Modern Horticultural Encyclopedia, 1944). Uses similar to those in China food, drug and ceremonial were introduced from China to Korea. It is likely that it was also introduced to Japan from China. The 'Manyoshu' of the Nara period notes songs written about the Jujube, indicating that it had already been introduced to Japan by this period. After the Nara period, it continued to be cultivated for use as a food and as a drug. During the Edo period (1603–1867), with the dissemination of Kampo medicines, the Jujube

Figure 3.7 *Zizyphus jujuba* Miller var. *inermis* Rehder.

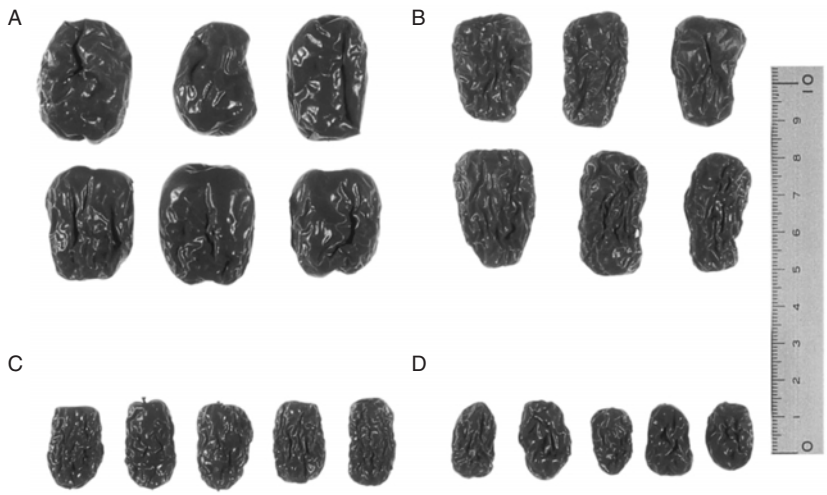


Figure 3.8 Cultivars of Jujube. A: Taihoso. B: Taikaiso. C: Koso. D: Shoso.

came to be cultivated widely, and the Osaka region was particularly known for fruits of high quality (Editorial committee of the Modern Horticultural Encyclopedia, 1944). Production declined thereafter, and currently, it is cultivated in small amounts in the Chubu, Tohoku and Setouchi regions of Japan.

Plant origin: The plants of *Z. jujuba* Mill. nearly thorn-less are classified as *Z. jujuba* var. *inermis* Rehder and is often cultivated in homes. It is a deciduous tree of 10 m in height, and occasionally, new growths can appear from the extended root systems. The thorns may appear at the base of the branches. The leaves are alternate, ovate to narrowly ovate, 2–6 cm in length, and 1–2 cm in width. The apex is obtuse or sometimes acute, while the base is obtuse, and the margin is obtusely serrate. The upper surface is lustrous, and the three main veins are prominent. The leaves have very short petioles, and in the late fall, they fall with the small branches. In June–July, small flowers of faint yellow-green colour of 5 mm diameter appear on the leaf axil or the branch tip. The fruits ellipsoid or ovoid in shape, 2–3 cm in length develop in September–October, and yellow-brown to dark red when mature.

Producer regions: This fruit is cultivated in all parts of China, particularly in Shandong, Hebei, Henan, Shanxi, Shaanxi, Sichuan and Guizhou Provinces (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1984). It is also produced in the Korean peninsula. Small amounts, of no commercial significance, are also produced in Japan.

The Jujube fruits produced in China come in many cultivars, and approximately 400 cultivars are known, named for their shape or producer region (Qu, 1943).They are used as food and drug. Examples are given below. Taihoso, Taikaiso, Shinso, Koso, Keishinso, Riso, Sankoteitaiso, Shoso, Soso, Gijosenso, Baso and Gichoso. Most of the raw herbs distributed commercially in Japan are produced in China, and large-sized products of Taihoso and Taikaiso, and small-sized products of Koso and Shoso are seen. Most of the commercial products in use are the large-sized Taihoso and Taikaiso. In 1995 and 1996, about 800 metric tons annually were imported to Japan.

Production: Red ripened fruits are harvested in the fall, and after a brief boiling are dried. It has a high sugar content, with a risk of insect and mould contamination, thus requiring careful storage.

Selection: Those considered to be of high quality are large, red and moist, with a strong sweet taste and no acidity, and have an external luster, thick flesh and small endocarp.

Ginseng

The Ginseng was recorded in the Chinese oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933a) and it has been considered to be an important crude drug, recorded in successive pharmacopoeia in China and Japan. It has gastro-protective, analeptic, and tonic effects, and is often included in many Kampo prescriptions, such as Sho-saiko-to (Xiachaihutang), Juzen-taiho-to (Shiquandabutang), Ninjin-yoei-to (Renshenyangrongtang) and Dai-kenchu-to (Dajianzhongtang). *The Pharmacopoeia of Japan*, 13th edition (Committee for editing of the commentaries on Japan Pharmacopoeia, 1996c) specifies that the Ginseng is the root of *Panax ginseng* C. A. Meyer (Araliaceae), from which rootlets have been removed, or the root has been quickly passed through hot water.

The Ginseng available commercially are thin and long cylindrical to fusiform root, often with 2–5 lateral root branches from the middle and are 5–20 cm long, main root 0.5–3 cm in diameter. The outer colour is faint yellow to brown, and on the surface, one can see longitudinal wrinkles and scars of the rootlets. At the tip, one can often see short and somewhat constricted rhizome of 1–4 cm in length and 0.3–0.5 cm in diameter. The fractured surface is almost flat, light yellow-brown in colour, and brown in the neighbourhood of the cambium. There is a characteristic odour, and the taste is initially slightly sweet and then somewhat bitter thereafter.

Observation of the transverse section of the main root using a microscope reveals that the outermost periderm is often denuded, although some have 4–6 layers. Inside of the periderm is the collenchyma, which is contact with the parenchyma. In the parenchyma which is made of relatively large parenchymatous cells, one can see large gaps. The inner cells become smaller and are arranged in a regular pattern along the ray, and the phloem made of small cells have a radiating pattern. The cambium is made of small thin-walled cells of 2–4 layers and can be clearly seen. In the xylem, the ray has a radiating pattern and this xylem portion is composed of the vessels and the xylem parenchyma. The vessel is arranged in a radiating pattern singly or in groups of 2–3. The resin canal exists in the collenchyma, parenchyma, phloem and ray. Throughout the collenchyma and the parenchyma, there exist starch grains, solitary and clustered crystals of calcium oxalate 30–60 μm in diameter, and orange-coloured resin in the resin canal (Figures 3.9 and 3.10).

The history, plant origin, producer region and selection of Ginseng are now discussed briefly.

History: The history of the use of Ginseng for medicinal purposes dates back to 196–220 AD in China. There is a record in old palaeograph of the use of Ginseng for medicinal purposes in Japan in 739 AD, and it came to be widely used for medicinal purposes in the Edo period. In 1728, the Shogunate government designated an official project of growing Ginseng seeds in Nikko and was successful in cultivation of this plant. Thereafter, the Shogunate government distributed the cultivated seeds, called 'Otane-ninjin (in Japanese)', to the feudal lords and encouraged their cultivation. The feudal lords competed aggressively in the cultivation, and it is said that many feudal clans profited greatly through this activity. In the Meiji era, which was the beginning of the Western medicine, Ginseng production decreased, but even today Ginseng cultivation continues in the regions of Shinshu, Aizu and Unshu (eastern part of Shimane Prefecture).

Plant origin: *Panax ginseng* C.A. Meyer (Araliaceae) is a perennial herb of which make the Primorskii Krai, northeastern parts of China and northern parts of Korean peninsula to be an

Figure 3.9 *Panax ginseng* C. A. Meyer.

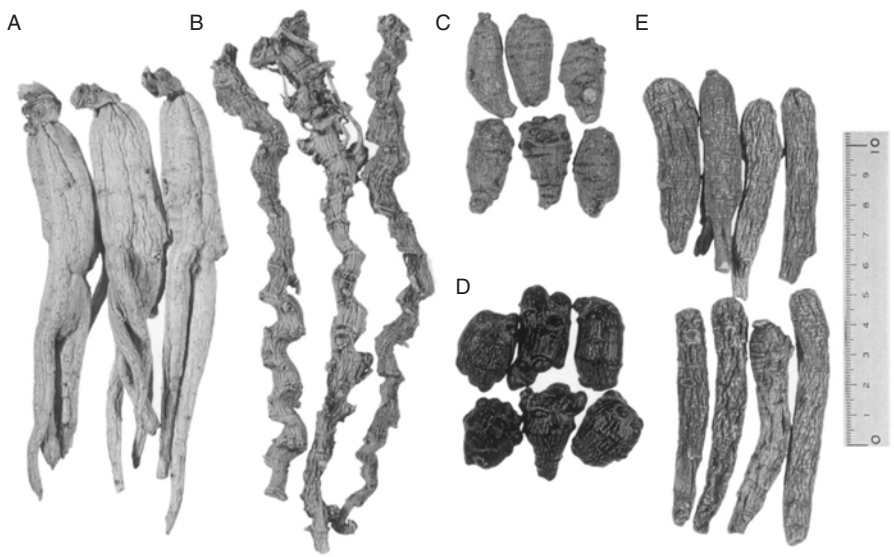


Figure 3.10 Ginseng and related raw herbs. A: Ginseng (*Panax ginseng*). B: Panax rhizome (*P. japonicus*). C: Notoginseng root (*P. notoginseng*). D: Notoginseng root, treated with beeswax (*P. notoginseng*). E: American ginseng (*P. quinquefolium*).

origin. The stem is erect with no hair and produces one palmately compound leaf per year. The length of the petiole is about the same length as the leaf-blade, and the leaflets usually are 5–7 in number. Each leaflet is obovate, with an acute apex, and is somewhat membranaceous. The flower stalk appears from the shoot apex, and are terminated by an umbel. The flowers are

light yellow-green. The drupe is about 5–9 mm in diameter, and when mature, is salmon pink in colour, and inside are two stones which are semi-circular. The flowering period is April–July.

Producer regions: Ginseng is cultivated in Japan in the Saku region of Nagano Prefecture, Aizu region of Fukushima Prefecture and Daikon Island of Shimane Prefecture. In other countries, cultivation is being conducted in China (Jilin and Heilongjiang Provinces) and Korea (Kyonggi-do, Chungchengpuk-do, Chungchengnam-do, Chollapuk-do regions).

When one compares the domestic production with imports, the amounts were about 50 and 980 tons, respectively, in 1995 and about 30 and 730 tons in 1996, so that a greater quantity was imported.

Production: Those cultivated for 4–6 years, after the leaves have fallen, are harvested by gently digging them up. Based on differences in the processing method, they are classified as White Ginseng (Ginseng) and Red Ginseng. For White Ginseng, the fresh root is washed with water, peeled with a bamboo spatula and dried in the sun to a white colour. There is a 'Otane-ninjin' in which the fresh root has been quickly passed through hot water; in this product, the outer peel is not removed, while the rootlets and root branches are removed, and the main root has been quickly passed through hot water and then dried by heat or in the sun. For Red Ginseng, the fresh root after washing in water is steamed for several hours and then dried at a high temperature.

Selection: Those considered to be of high quality are thick, heavy and moist, with a sweet taste and slight bitterness. Those harvested at 5–6 years are considered to be the best.

In the genus of Panax, there are other species, such as *P. japonicus, P. notoginseng* and *P. quinquefolius*, all with the name Ginseng. These Ginseng-type herbs are clearly different from the authentic Ginseng in the botanical origin and the quality (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1979b).

Glycyrrhiza root

The Glycyrrhiza root was recorded in the Chinese oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933a). It has detoxifying and analgesic effects and has been noted as an important crude drug successively in classical pharmacopoeia in China and Japan. It is an important component of such Kampo prescriptions as Sho-saiko-to (Xiachaihutang), Daio-kanzo-to (Dahuanggancaotang), Shakuyaku-kanzo-to (Shaoyaogancaotang), Kakkon-to (Gegentang) and so on. *The Pharmacopoeia of Japan*, 13th edition (Committee for editing of the commentaries on Japan Pharmacopoeia, 1996d) specifies that the Glycyrrhiza root is the root or stolon of *Glycyrrhiza uralensis* Fischer, *G. glabra* L. or their allied species (Leguminosae).

The properties of the Glycyrrhia root found on the market are as follows. The root is stick-like in shape, and 0.5–3.0 cm in diameter. The surface is brown to reddish brown in colour, and lenticels are present. At the head of the root is a knot-like bud. The cut surface of the root shows that the cortex and the xylem are distinct, and occasionally, there are radiating splits in the cortrex. The stolon has a pith in the centre. The Glycyrrhiza root has a characteristic faint odour and has a very sweet taste (Figures 3.11 and 3.12).

The following features are observed in a transverse section of the Glycyrrhiza root under a microscope. The outermost part is made up of cork layers, surrounding the cork cortex of 2–3 cells thick. The cortex is composed mainly of parenchyma. The rays and obliterated sieve portion are arranged alternately. In the phloem fibre bundles are surrounded by rows of cells in which crystals of calcium oxalate occur. In xylem the parenchyma and vessels are arranged alternately. The vessels are associated with xylem fibres and xylem parenchyma surrounded by rows of cells containing crystals

Figure 3.11 *Glycyrrhiza uralensis* Fischer.

Figure 3.12 Glycyrrhiza root. A: North-eastern Glycyrrhiza root. B: North-western Glycyrrhiza root. C: Xinjiang Glycyrrhiza root.

of calcium oxalate. The stolon has a pith composed of parenchymatous cells. The parenchyma of the cortex and the xylem contains starch grains, and often solitary crystals of calcium oxalate.

The history, plant origin, producer region and selection of the Glycyrrhiza root are now discussed briefly.

History: The Glycyrrhiza root is described in *Materia Medica of Shen Nong* and also noted in the text, *Enanji* of China published in the BC period. It appears to have been brought into Japan during the Nara Period and is included among the more than 50 kinds of raw herbs deposited in the Shosoin Treasure House in Nara. There exists a village in Yamanashi Prefecture that produced the Glycyrrhiza root and sent it to the Shogunate government during the Edo period; this village today is known as 'The mansion of Glycyrrhiza root' and is preserved as an important national cultural asset. Glycyrrhiza plants are still cultivated there, but only a little.

Plant origin: *Glycyrrhiza uralensis* is a perennial herb of 30–80 cm in height. The leaves are alternate and pinnately compound. The leaflets are 5–17 in number and are ovate to elliptical in shape. The inflorescence is an axillary raceme. The flowers open in June–July. The calyx is toothed. The corolla is whitish-violet. The stamens are 10 in number. The fruit is a twisted legume and covered with numerous glandular hairs. The root grows deeply in the vertical direction, while the stolon spreads horizontally. *G. uralensis* is distributed throughout the northeastern to northwestern parts of China, Siberia and Mongolia, and grows in semiarid areas, grasslands and river beds (Lin and Tung, 1977). *G. glabra* is similar to *G. uralensis* in morphology, but the fruit has no glandular hair on the surface. *G. glabra* is distributed from central Asia to Europe (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1979c). The habitats of this species are drier than those of *G. uralensis*.

Producer regions: China produces three kinds of commercial Glycyrrhiza root: Northeastern Glycyrrhiza root (Tohoku-kanzo in Japanese), Northwestern Glycyrrhiza root (Seihoku-kanzo), and Xinjiang Glycyrrhiza root (Shinkyo-kanzo).

The Northeastern Glycyrrhiza roots are made of *G. uralensis* and are produced in the northeastern China and Inner Mongolia. The Northwestern Glycyrrhiza roots are produced from *G. glabra* and *G. uralensis*, and are made in northwestern China and Inner Mongolia. The Xinjiang Glycyrrhiza roots are produced in Xinjiang and are produced primarily from *G. inflata* but can also be produced from *G. uralensis* or *G. glabra* (Kondo, 1999). The commercial products are mainly from the wild, but recently cultivated ones are increasing. In addition to the products from China, Russian, Spanish and Persian Glycyrrhiza roots appear in the market. The Glycyrrhiza root is imported as a sweetening agent and as raw materials for foods, in addition to herbal use. The current annual quantity of the Glycyrrhiza root imported is 3000 metric tons (1995).

Production: The aerial parts are cut in the fall–winter, and the roots and stolon are dug up. After removing the dirt, buds and rootlets, they are dried in the sun. One can remove the periderm prior to the drying to produce the 'the Glycyrrhiza root without periderm'.

Selection: The commercial products are graded as number 1, number 2 and number 3. Those of desirable quality are reddish brown on the outside, strongly yellow at the cut surfaces, and have a very sweet but no bitter taste. The Northeastern Glycyrrhiza roots have a very sweet taste and are favoured for Kampo medicines .

Ginger

The Ginger was recorded in the oldest existing pharmacopoeia, *Materia Medica of Shen Nong* (Mori, ed. 1933b) under the name of dried ginger. It has a gastrointestinal promoting effect, an anti-pyretic effect, analgesic and anti-spasmodic effects, and has been included in such Kampo prescriptions as Keishi-to (Guizhitang), Kakkon-to (Gegentang), Rikkunshi-to (Liujunzitang) and so on. It is cultivated world-wide for use as a medicine, food and spice. *The Pharmacopoeia of Japan*, 13th edition (Committee for editing of the commentaries on Japan Pharmacopoeia, 1996e) specifies that the Ginger is the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae) (Figures 3.13 and 3.14).

Figure 3.13 *Zingiber officinale* Roscoe.

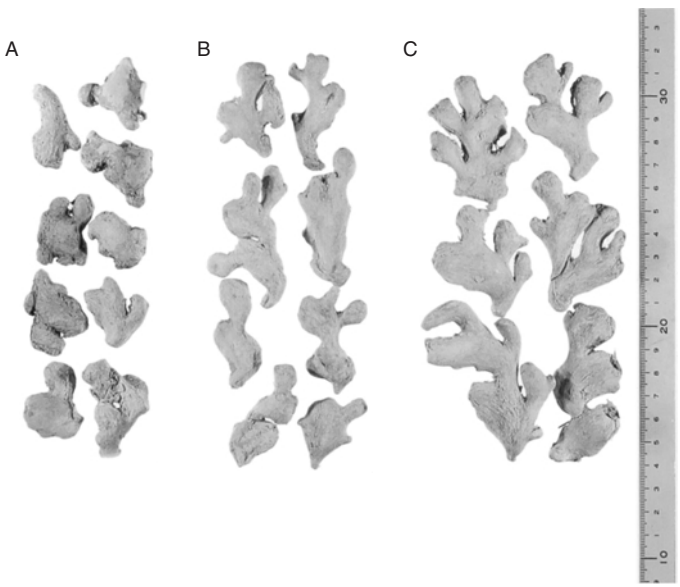


Figure 3.14 Ginger. A: Small Ginger. B: Medium Ginger. C: Large Ginger.

The Ginger seen commercially has properties as follows. The rhizome is somewhat flat and branched. The branch portions are ovate to oblong – ovate in shape, 2–4 cm in length and 1–2 cm in width. The external surface of the rhizome is greyish white to faint greyish brown in colour, and occasionally is covered in a white powder. The cut surface of the rhizome is powdery, somewhat fibrous, and faint yellow to yellow-white in colour.

In a transverse section, one can see a ring of endodermis and numerous vascular bundles. Fine brown dots (depending on secretion) are also recognized. The Ginger has a characteristic odour and has a very spicy taste.

Microscopic observation of the transverse section of the Ginger reveals that the cortex and the stele are separated by endodermis. Numerous vascular bundles are scattered both in the cortex and the stele. In the parenchyma of the cortex and the stele secretory cells and cells filled with resin-like substances are observed. In the parenchyma starch grains and crystals of calcium oxalate occur. The starch grains may be simple, compound or semi-compound, and the largest ones may reach 60 μm in diameter.

The history, plant origin, producer region and selection of the Ginger are now discussed briefly.

History: The Ginger was recorded in *Materia Medica of Shen Nong*. During the ancient Greek period, the Ginger is thought to have been imported from India and used as a spice and for medicinal purposes. The Ginger was also noted in the classic European herbal text, *Materia Medica* by Dioscorides.

In Japan, the Ginger is thought to have been imported from China or from South Asia before the third century AD. It is noted under the name of 'Kurenohajikami' in the texts 'Shinsenjikyo' of the Nara period and 'Honzowamei' of the Heian period. The major types imported initially were small-sized (Small Ginger) or medium-sized (Medium Ginger). During the Edo period, large-sized products (Large Ginger) were imported, but Large Ginger took time to become accepted in Japan and was imported several times before becoming commonly available.

Plant origin: *Zingiber officinale* Roscoe (Zingiberaceae) is a perennial herb of tropical Asian origin. The rhizome proliferates along the ground. The leafy stem 50–100 cm in height develops from the node of the rhizome. The leaves are alternate and linear – lanceolate. In warm climates, it bears a flower stalk of 20–30 cm in length, with a terminal spike of ellipsoid in shape and about 5 cm in length. The flower is zygomorphic and subtended by green bract. The calyx is tubular. The corolla is yellowish green, lateral lobes ovate and the lips obovate. The stamen is single. The ovary is inferior and tri-locular. The fruit is a capsule, globose to oblong in shape.

There are many cultivars generally grouped into three types: Large Ginger, Medium Ginger and Small Ginger. 'Otafuku' and 'Tosa' are the Large Gingers, 'Makino' and 'Settu' are Medium Ginger, and 'Kintoki' and 'Yanaka' are Small Gingers. In general, Small Gingers are spicy and suited for medicinal use, while Large Gingers are less spicy and suited for food use.

Producer region: The Ginger is cultivated in various parts of the world: China (Sichuan, Guizhou, Guangdong and Zhejiang Provinces), (Institute of Materia Medica, Chinese Academy of Medical Sciences *et al*., eds, 1982c) southeast Asia, western India and Africa. In Japan, it is cultivated in Shizuoka, Aichi, Kanagawa and Okayama Prefectures, and other areas west of the Kanto region. The domestic production is 5 metric tons, while 6500 tons are imported annually (in 1995).

Production: The rhizome stored from the prior year is planted in mid April and harvested in October–December of that year. Those harvested in the fall–winter are called 'Shinshoga', and those stored over the winter are called 'Hineshoga'.[2] The ginger used as crude drugs is the 'Hineshoga'. The Ginger can be used to prepare three types of crude drugs:

1 Fresh Ginger: the rhizome is stored raw and used in that state.
2 Ginger: the periderm is peeled and dried as is or after covering with quicklime.
3 Dried Ginger: the rhizome is steamed or passed through hot water, and then dried.

Selection: Those considered to be of high quality have a prominent hypertrophy, a fibrous cut surface, faint yellow-white to white colour, with a characteristic spicy taste.

Notes

1 'Sengon', 'Shigon', 'Jogon' and 'Hengon' are named based on the shape of the material. 'Sen', 'Shi', 'Jo' means acute, branch, string respectively, and 'Gon' means Scutellaria Root. 'Sengon', 'Shigon', and 'Jogon' are prepared from the relatively young roots, not broken and stick like in shape. 'Hen' means flat. 'Hengon' is prepared from basal part of old root and broken and flat in shape. 'Kogon' is also prepared from old root. 'Ko' means matured. Differing from 'Hengon', 'Kogon' is not broken.
2 'Shinshoga' and 'Hineshoga' are distinguished by whether or not rhizomes are stocked over the winter. 'Shin' and 'Hine' mean new and old respectively, and 'Shoga' means Ginger. Compared with 'Hineshoga', 'Shinshoga' is new or fresh, because 'Shinshoga' is not stored over the winter after harvested. 'Shinshoga' is usually used for food, and 'Hineshoga' is for crude drug.

Crude drugs II

Phytochemical studies of ingredients and analytical evaluation

Hiromi Sasaki

Introduction

Sho-saiko-to consists of seven crude drugs and its plant sources are shown in *The Japanese Pharmacopoeia, Thirteenth Edition (1996)*. See Table 3.1.

Chemical constituents contained in the above crude drugs, or their metabolites, affect disease processes directly or *via* reactions with chemical mediators causing disease. Alternatively, they stimulate or inhibit receptors resulting in the various pharmacological effects described in the following chapter.

Among the chemical constituents, those possessing similar molecular skeletons competitively affect the same reaction sites. As a result of the sum total of stimulatory and inhibitory reactions by these components, Sho-saiko-to exhibits a multiplicity of pharmacological effects. Even when the molecular skeleton of one component is different from that of another, it can affect the same reaction site as the result of partial similarity in the third-dimensional structure. These components show systemic and/or local actions in the complex living body to affect the maintenance of homoeostasis.

In herbal drugs, especially in multi-component herbal medicines such as Sho-saiko-to, it is necessary to standardize not only the amount of each active ingredient but also the ratios of the components in order that the herbal medicine possesses desired pharmacological actions. Thus, uniformity of manufacture and tight quality control are important aspects of herbal drug production.

In a multi-component herbal medicine, it is known that, in the process of extraction, one constituent can sometimes affect the chemical nature of one or more of the additional constituents resulting in the formation of new compounds (Akahori *et al*., 1975). It is also known

Table 3.1 Constituent crude drugs of Sho-saiko-to

Plant source	*Part of plant*	*Constituent* (g)
Bupleurum falcatum Linné	Root	7.0
Pinellia ternata Breitenbach	Tuber	5.0
Scutellaria baicalensis Georgi	Root	3.0
Panax ginseng C. A. Meyer	Root	3.0
Zizyphus jujuba Miller var. *inermis* Rehder	Fruit	3.0
Glycyrrhiza uralensis Fisher, *Glycyrrhiza glabra* Linné	Root	2.0
Zingiber officinale Roscoe	Rhizome	1.0

that, in the process of extraction (Kano, 1987), a coexisting crude drug can affect the yield of another constituent. Bupleurum root and Ginseng contain high amounts of saponins, which have strong interactions. As a result of the above considerations, it has been found important that the above seven crude drugs be mixed simultaneously in manufacture of the extract of Sho-saiko-to in order to guarantee proven efficacy.

Bupleurum root

Bupleurum root is a major component of Sho-saiko-to. For Sho-saiko-to of Chinese manufacture, the root of *Bupleurum Chinese*, or that of *B. scorzonerifolium*, is said to be commonly employed (*The Chinese Pharmacopoeia, Part 1* (1977)). But there are many cases in which the origin of the Bupleurum root produced in China is uncertain (Namba and Tani, 1970).

Because the origin of *Bupleurum falcatum* Linné is well established, Amagaya and Ogihara have insisted since 1989 that *Bupleurum falcatum* Linné, or its varieties (*Umbelliferae*), must be used in order to manufacture Sho-saiko-to of uniform quality.

Identification

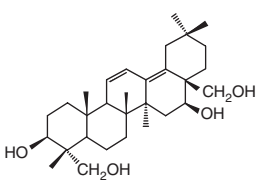
a Shake vigorously 0.5 g of pulverized Bupleurum root with 10 mL of water: a lasting fine foam is produced.

b To 2.0 g of pulverized Bupleurum root add 10 mL of methanol, boil gently under a reflux condenser on a water bath for 15 min, cool, filter and use the filtrate as the sample solution. Dissolve 1 mg of saikosaponin a in 1 mL of methanol and use this solution as the standard one. Perform the test with these solutions. Spot 10 μL each of the sample solution and the standard one on a plate of silica gel for thin-layer chromatography (TLC). Develop the plate with a mixture of chloroform, methanol and water (30 : 10 : 1) to a distance of about 10 cm, air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (1 : 1) on the plate, and warm at 50°C for 5 min. A spot among the several ones from the sample solution and the blue spot from the standard solution show the same Rf value, and the colour tone is blue to purple.

The chemistry of Bupleurum root was reviewed by Takeda (1973) and Akahori (1980).

It has been reported that Bupleurum contains a variety of chemical constituents and that saponins (*c.*3%), sterols (*c.*0.07%), fatty acids, polyhydric alcohols, coumarins, etc. are the major components. Bupleurum, *per se*, is known to have therapeutic actions on liver disease, inflammation and certain tumours, together with effects on the immune system.

Regarding the chemical constituents of Bupleurum root, many studies on saponins and their derivatives have been reported. Thus, seven sapogenins were isolated from the crude drug. They are named saikogenins A (1: mp 287–290°C), B (2: mp 267–269°C), C (3: mp 291–294°C), D (4: mp 256–261°C), E (5: mp 283°C), F (6: mp 265–273°C), and G (7: mp 238–45°C), shown as below.



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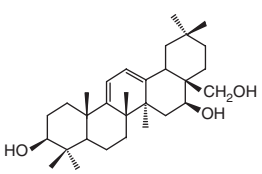

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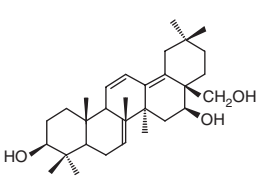

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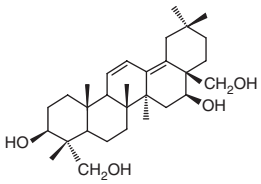

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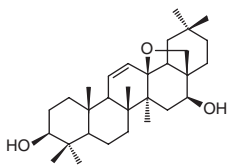

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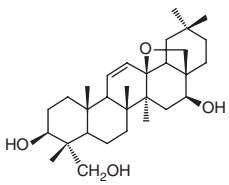

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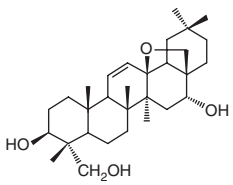

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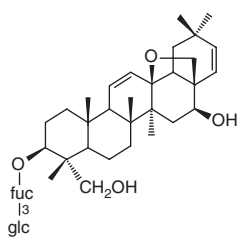
Among these sapogenins, saikogenins A–D are said to be formed secondarily from saikogenins E–G. Studies of saikogenin A and saikogenin E were carried out by Shibata *et al*. (1966) and Aimi *et al*. (1968). Studies of saponins and other sapogenins in Bupleurum root were performed by Kubota and Hinoh (1968), who isolated saikosaponins a (8: mp 225–232°C), b (9), c (10: mp 202–210°C), and d (11: mp 212–218°C) from the extract of Bupleurum root and determined the structures of saikosaponins a, c and d. In addition, saikosaponin e (Ishii *et al*., 1977), saikosaponin f (Tori *et al*., 1976), and partially acetylated saponins are known. All saponins and their aglycones are triterpens having an oleanane skeleton.

Studies of saikosaponins and saikogenins were carried out independently. As a result, the name of a given saikosaponin is not consistent with that of its corresponding saikogenin.



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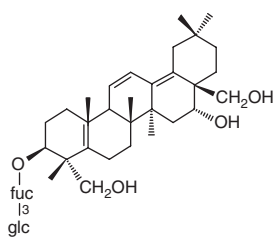
Therapeutic action in liver disease
Anti-inflammatory action
Effects on the immune system



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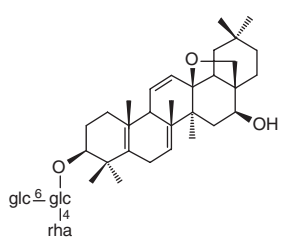
Therapeutic action in liver



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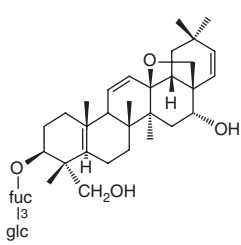
Therapeutic action in liver disease
Effects on the immune system



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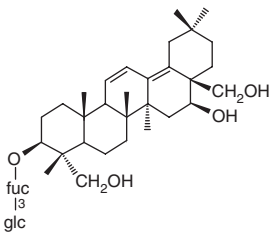
Therapeutic action in liver disease
Anti-inflammatory action

Saikosaponin b was considered a secondary product because its aglycone was saikogenin D derived from saikogenin G. As a result of the study by Shimaoka *et al*. (1975), saikosaponin b was found to be a mixture of four saikosaponins derived from saikosaponin a and saikosaponin d. The two saponins derived from saikosaponin a were named saikosaponin b_1 (12) and saikosaponin b_3 (14), and those from saikosaponin d were named saikosaponin b_2 (13: mp 231–238°C) and saikosaponin b_4 (15: mp 232–236°C).

It can be presumed from the reports of Araki *et al*. and Chi *et al*. that sterols might be contained in Bupleurum root. Takeda *et al*. isolated four sterols from the crude drug in 1953 and in 1958. Among these, the content of α-spinasterol (16: mp 172°C) was 70%, and those of stigmasterol (17: mp 166.5–168°C), Δ^7-stigmastenol (18: mp 145–147°C) and Δ^{22} stigmastenol (19: mp 158–159°C) are about 9–11%, respectively.



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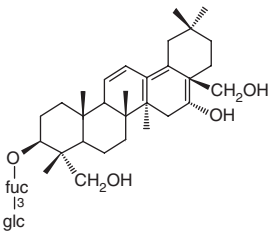

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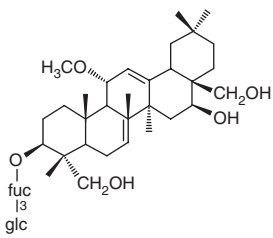

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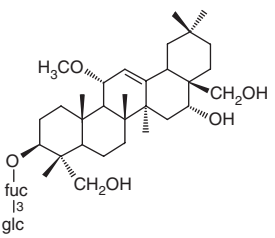

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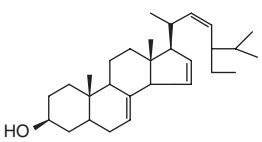

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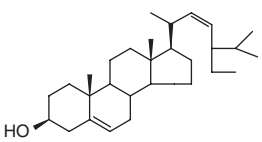

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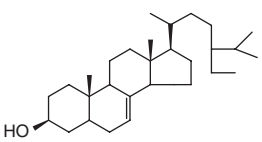

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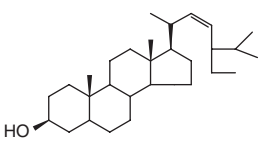
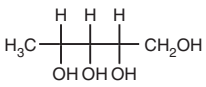


19

Ishii *et al*. (1977) reported that α-spinasterol also exists as its glycoside in Bupleurum root. β-Sitosterol was found in the terrestrial part of the plant by Tomimatsu *et al*. (1972).

Regarding fatty acids, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, etc. were shown to be present.

Polyhydric alcohols were found in Bupleurum root. Nagoshi and Tomimatsu (1969) reported that the content of adonitol (20: mp 102°C), one of the polyhydric alcohols, was high. In addition, coumarine derivatives are known to be contained in the crude drug. Also, angelicin (21: mp 136–137°C) was separated from the crude drug by Nakabayashi *et al*. (1964).



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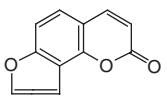


21

Pinellia tuber

Pinellia tuber is the root of *Pinellia ternata* Breitenbach, from which the cork layer has been removed. It was reported that amino acids, choline, fatty acids, β-(β-sitosteryl)-D-glucoside, etc. are found as chemical constituents of this crude drug by Murakami *et al*. (1965), and by Ozeki (1961, 1962). It was determined by Oshio *et al*. (1978) that (−)-ephedrine was also contained in Pinellia tuber. Pinellia tuber has characteristic acrid taste. Chemical constituents of Pinellia tuber were summarized by Shibata and Maki (1988) and later, by Okuyama and Takahashi (1995).

Pinellia tuber is known to have not only an anti-emetic effect but also immunostimulant action and anti-viral effects.

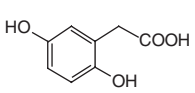
The main pharmacological effect of Pinellia tuber is its anti-emetic action. Many studies have been carried out on constituents, which show such action. As a result, it was reported that both the lipophilic- (Suzuki, 1931) and the water-soluble fractions (Takabe, 1958) have anti-emetic effect. In 1958, Takabe reported that the anti-emetic effect of Pinellia tuber is caused by a water-soluble glucuronic acid derivative. Takabe also reported that proteins and phytosterol are contained in Pinellia tuber. On the other hand, Maki *et al*. (1987) reported that the anti-emetic effect of Pinellia tuber was caused by a water-soluble polysaccharide, which possessed arabinose as a major constituent sugar and also calcium galacturonate.

Nakayama (1924) reported that essential oils, in amounts of 0.003–0.01%, are contained in Pinellia tuber and are said to be the origin of a specific perfume of Pinellia tuber.

Pinellia tuber contains 0.08% of amino acids. Specifically, α-aminobutyric acid, γ-aminobutyric acid, glutamic acid, aspartic acid and arginine have been isolated and identified, as was the presence of ornithine, citrulline, lysine, serine, glycine, alanine, tyrosine, valine and leucine (Ozeki, 1954).

Fatty acids in amounts of 0.85% are contained in this crude drug. Ozeki (1961, 1962) also reported that palmitic acid and stearic acid are contained in Pinellia tuber and, in addition, that there exists oxalic acid, an alkaloid-like substance, β-sitosterol and its glycoside.

In addition, triterpenes, choline, acetylcholine, glucose, rhamnose, glucuronic acid and homogentisic acid (22: mp 152°C) are known to be in Pinellia tuber. Oshio *et al*. (1978) isolated (−)-ephedrine (23: mp 40°C) from a fraction extracted by water/methanol.



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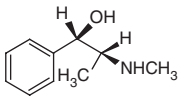
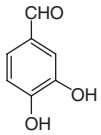


23

As described above, Pinellia tuber has a strong acrid taste. It was found by Suzuki (1969) that 3, 4-diglycosilic benzaldehyde (24: mp 153–154°C) is the origin of the acrid taste.

Recently, Kubo *et al*. reported that C-glycosides of four flavonoids (25)–(28) were found while screening for ACE inhibitors and anti-allergic substances.



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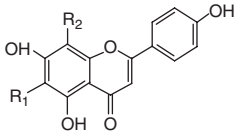
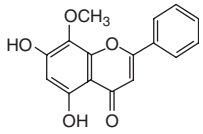


25: R_1 = C-gal, R_2 = C-ara
26: R_1 = C-ara, R_2 = C-gal
27: R_1 = C-gal, R_2 = C-xyl
28: R_1 = C-xyl, R_2 = C-gal

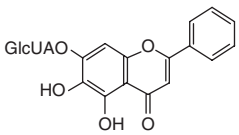
Scutellaria root

Scutellaria root is the root of *Scutellaria baicalensis* Georgi, from which the periderm has been removed. Wogonin (29: mp 203°C) and baicalin (30: mp 230°C(dec)) are known to be the major flavonoids present (0.5% and 4.3%, respectively).



29

Anti-atherosclerotic effect
Anti-tumor effect



30

Protective effect on liver disease
Anti-inflammatory effect
Anti-tumor effect

Identification

a Boil gently 0.5 g of pulverized Scutellaria root with 20 mL of ether under a reflux condenser on a water bath for 5 min, cool and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol, and to 3 mL of the solution add 1–2 drops of dilute ferric chloride TS. A greyish green colour develops, and it changes to purple-brown.

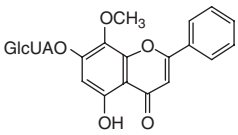
b To 2.0 g of pulverized Scutellaria root add 10 mL of methanol, warm on a water bath for 3 min, cool, filter and use the filtrate as the sample solution. Dissolve 1 mg of baicalin in 1 mL of methanol, and use this solution as the standard one. Perform the test with these solutions. Spot 5 μL each of the sample solution and the standard one on a plate of silica gel for TLC. Develop the plate with a mixture of n-butanol, water and glacial acetic acid (4 : 2 : 1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ferric chloride in methanol (1 in 100) on the plate. A spot among the several ones from the sample solution and a dark green spot from the standard solution show the same colour tone and Rf value.

Many studies on other flavonoids have been carried out (Popova *et al*., 1973; Takido *et al*., 1975, 1979; Horie *et al*., 1979; Takagi *et al*., 1980, 1981; Tomimori *et al*., 1982, 1983, 1984). Chemical constituents in Scutellaria root are reviewed by Takido (1993).

Scutellaria root was found to have an anti-atherosclerotic effect, a protective effect on liver disease, an anti-inflammatory effect, an anti-tumour effect, anti-viral action and other beneficial effects.

Chemical constituents in Scutellaria root have been studied by many investigators over a long period of time, and scutellarin, which is a flavonoid renamed later as wogonin (Shibata *et al*., 1923; Hattori, 1930, 1931; Shibata and Hattori, 1931; Shah *et al*., 1938; Rao and Seshadri 1947), was found.

Since 1970, studies on flavonoids have been carried out by many investigators and the structures of 38 flavonoids have been determined. There are 30 flavones including wogonoside (31: mp 270°C(dec)), baicalein (32: mp 264–265°C(dec)), oroxylin-A (33: mp 201–202°C), oroxylin-A glucuronide, chrysin (34: mp 290–292°C), skullcapflavone II (35: mp 180–181°C) and baicalein-7-*O*-β-glucoside (36: mp 208°C), 5 flavanes including 5,7,2′,6′-tetrahydroxyflavanone (37: mp 211°C(dec)) and dihydrooroxylin-A (38: mp 165–167°C), 1 flavonol, 1 flavanol and 1 chalcone.



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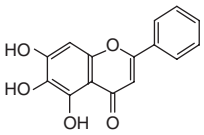

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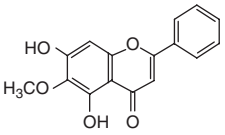
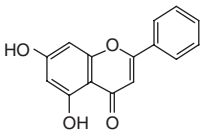


33

Anti-atherosclerotic effect
Anti-tumor effect
Anti-inflammatory effect



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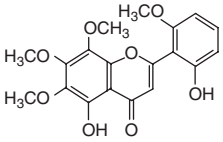

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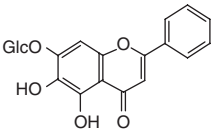
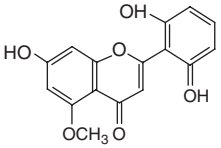


36

Anti-atherosclerotic effect



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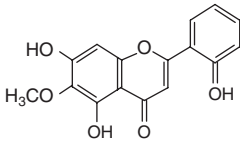
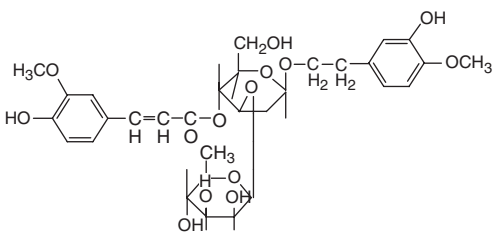


38

Tomimori *et al*. carried out chromatographic studies on 11 major flavonoids contained in 19 kinds of Scutellaria root including marketed products in 1985. The content of these 11 flavonoids varied widely from preparation to preparation. It was found that the crude drug contains a large amount of baicalin, that glycosides are contained more than their aglycone in general, and that there was no relationship between marketed crude preparations and flavonoid content.

Takagi *et al*. found 2-(3-hydroxy-4-methoxyphenyl)-ethyl-1-*O*-α-L-rhamnosyl-(1→3)-β-D-(4-feruloyl)-glucoside (39: mp 115–118°C) in the n-butanol extract from Scutellaria root in 1980.



39

Amino acids in Scutellaria root were also studied by Takagi *et al*. in 1981 and 14 free amino acids were detected in its warm water extract. In the extract, proline represented 80% of the total amino acid content. In addition, the amounts of arginine, alanine, glutamicacid and asparaticacid were found to be high.

In 1987, Fukuhara *et al*. performed GC analysis of the ether-extracted fraction of Scutellaria root to detect 81 essential oils. Among these, the amounts of acetophenone, (E)-4-phenyl-3-butene-2-one, 1-phenyl-1,3-butadione, palmitic acid and oleic acid were larger than those of others.

Furthermore, glucose and sucrose (Takido, 1973), and sitosterol, contaminated by small amounts of camphesterol and stigumasterol, were detected in this herb.

Ginseng

Ginseng is the root of *Panax ginseng* C. A. Meyer, from which either the rootlets have been removed or the root has been quickly passed through hot water.

Identification

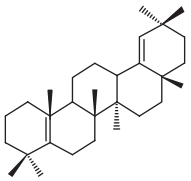
a On a section of Ginseng add dilute iodine TS dropwise; a dark blue colour is produced on the surface.

b To 2.0 g of pulverized Ginseng add 20 mL of methanol, boil gently under a reflux condenser on a water bath for 15 min, cool, filter and use the filtrate as the sample solution. Dissolve 1 mg of ginsenoside Rg_1 for TLC in 1 mL of methanol, and use this solution as the standard one. Perform the test with these solutions. Spot 10 μL each of the sample solution and the standard one on a plate of silica gel for TLC. Develop the plate with the lower layer of a mixture of chloroform, methanol and water (13 : 7 : 2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 5 min; one of the spots from the sample solution and a red-purple spot from the standard solution show the same colour tone and the same Rf value.

The saponins, ginsenoside R0, ginsenosides Ra through Rh etc. have been isolated from ginseng (Nagai *et al*., 1971; Tanaka, 1973; Sanada *et al*., 1974; Sanada and Shoji, 1978; Besso *et al*., 1982; Kitagawa *et al*., 1983), and many other studies report saponins in other plants of the same genus. Furthermore, lipophilic constituents such as panaxynol (farcarinol) (Takahashi and Yoshikura, 1964, 1966; Dabrowski *et al*., 1980) or sesquiterpens (Otsuka *et al*., 1981), saccharides, choline (Takatori *et al*., 1963; Iwabuchi *et al*., 1984), nucleosides (Hiyama *et al*., 1978), etc. are also known to be the constituents of ginseng. The chemistry of ginseng was summarized by Tanaka (1973) and Shibata (1982).

It is known that ginseng has an anti-inflammatory effect, an inhibitory effect on liver pathologies, an anti-ageing effect, an ability to stimulate the immune system, an anti-tumour effect, etc.

Ginseng saponins have been studied for a century in Japan and the study by Asahina and Taguchi (1906) has greatly influenced the results of later investigations. Hörhammer *et al*. (1961) hydrolysed a ginseng saponin to isolate oleanolic acid as its sapogenin. This sapogenin is a popular pentacyclic triterpene and has the oleanane skeleton (40). However, most sapogenins obtained by hydrolysis of ginseng saponins were found to be tetracyclic triterpenoids and to have the dammarane skeleton (41) (Fujita *et al*., 1962). Ginseng contains *c*.4% of saponins.



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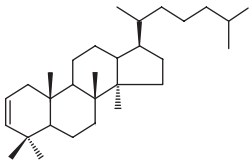
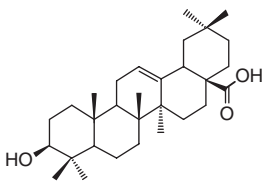


41

Tanaka *et al*. (1973) found various ginseng saponins by TLC analysis. These were named ginsenosides R_0, R_a, R_b, R_c, R_d, R_e, R_f, R_g, and R_h in the order of increasing Rf values. These ginsenosides were then further subdivided. Ginsenoside R_0 (42: mp 239–241°C) and other major saponins (43), (44: mp 197–198°C), (45: mp 200–203°C), (46: mp 199–201°C), (47: mp 206–209°C), (48: mp 201–203°C), (49: mp 197–198°C), (50: mp 194–196.5°C), (51: mp 187–189°C) and (52) are shown (Table 3.2).



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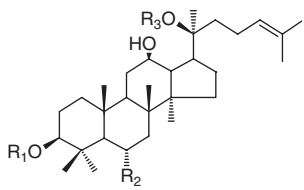


43–52

Anti-inflammatory effect
Inhibitory effect on liver pathologies

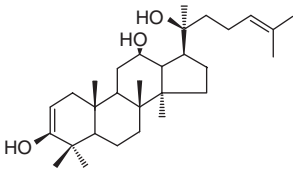
Detailed studies of the structures of saponins were performed by Nagai *et al*. (1971) and by Sanada *et al*. (1974) Subsequently, Yahara *et al*. (1979) conducted an additional studies. As a result, the structures of most ginseng saponins are now established.

Table 3.2 Structures of ginsenosides

Ginsenoside	*R1*	*R2*	*R3*
R_{a1} (43)	Glc^{-2}Glc	H	Xyl^{-4}Ara(p)$^{-6}$Glc
R_{b1} (44)	Glc^{-2}Glc	H	Glc^{-6}Glc
R_{b2} (45)	Glc^{-2}Glc	H	Ara(p)$^{-6}$Glc
R_c (46)	Glc^{-2}Glc	H	Ara(f)$^{-6}$Glc
R_d (47)	Glc^{-2}Glc	H	Xyl^{-6}Glc
R_e (48)	H	Rha^{-2}Glc-O	Glc
R_f (49)	H	Glc^{-2}Glc-O	H
R_{g1} (50)	H	^{2}Glc-O	Glc
R_{g2} (51)	H	Rha^{-2}Glc-O	H
R_{h1} (52)	H	^{2}Glc-O	H

Notes
(44): *Immune stimulation effect*
(45): *Anti-tumor effect*
(46): *Immune stimulation effect, anti-tumor effect*
(47): *Anti-tumor effect*
(49): *Anti-tumor effect*
(50): *Immune stimulation effect*

There are many studies of aglycones of ginseng saponins regarding their reactivities and chemical structures. The sapogenin of ginsenoside R_0 is known to be a true sapogenin (oleanolic acid), while other sapogenins have the dammarane skeleton. An aglycone derived from ginsenosides R_{b1}, R_{b2}, R_c, R_d, etc. is 20(S)-protopanaxadiol (53). On the other hand, that from ginsenosides R_e, R_f, R_{g1}, R_{g2}, etc. is 20(S)-protopanaxatriol (54). The ratio of the total amount of 53 to that of 54, which are derived from ginsenosides, is *c.*3 : 1. Sugars are attached at positions 3, 6 and 20.



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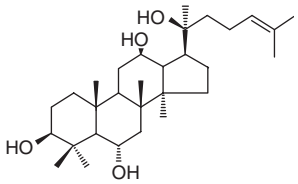
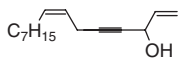


54

As lipophilic constituents of ginseng, stearic acid, palmitic acid and oleic acid were isolated from ginseng root by Kondo and Tanaka in 1915, by Kondo and Yamaguchi (1918) and Kondo and Amano (1920), as well as β-Elemene, a sesquiterpene. Panaxynol (farcarinol) (55: mp 154°C), an essential oil (*c.*0.05%), is also present.



55

β-Sitosterol and daucosterol were isolated from an ether-extracted fraction of ginseng.

Ginseng contains about 5% carbohydrates. Glucose and fructose, as monosaccharides, and sucrose and maltose, as disaccharides, were also separated from this crude drug. Takiura and Nakagawa (1963) investigated trisaccharides in ginseng to determine their chemical structures.

In 1971, Miura and Miyazawa reported the relationship between parts of this plant, ginseng, and their content of free amino acids. Amounts of arginine and alanine were found to be the greatest followed by serine and tyrosine. Peptide content was reported by Gstirner and Vogt (1966).

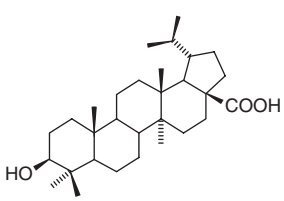
Regarding basic substances, by examining the effect of ginseng extract on blood pressure, it was found that choline (0.1–0.2%) is present in ginseng by Takatori *et al*. (1963).

Additionally, ascorbic acid, vitamin B complex and inorganic ions are detected in this crude drug.

Jujube

Noguchi reviewed in 1983 that Jujube is the fruit of *Zizyphus jujuba* Miller var. *inermis* Rehder or other allied plants and is one of the five crude drugs often used as constituents of Kampo medicines. It was reported by Yagi *et al*. (1978a,b), and by Okamura *et al*. (1981) that Jujube contains pentacyclic triterpenes, triterpene saponins possessing the dammarane skeleton, glycosides of vomifoliol or benzyl alcohol, etc. It also contains large amounts of saccharides, neutral and acidic polysaccharides (Tomoda *et al*., 1969, 1981, 1985; Shimizu and Tomoda, 1983) and a large content of cyclic AMP (Cyong and Hanabusa, 1980; Cyong and Takahashi, 1982b). The chemistry of Jujube was reviewed by Tomoda in 1984.

As pentacyclic triterpenes, betulinic acid (56: mp 293–295°C), aliphitolic acid (57: mp 275–278°C(dec)), 3-*O*-*trans*-p-coumaroyl aliphitolic acid (58: mp 279–282°C), 2-*O*-*trans*-*p*-coumaroyl aliphitolic acid (59: mp 279–280°C), and 3-*O*-*cis*-*p*-coumaroyl aliphitolic acid (60: mp 208–210°C) were isolated from a butanol-soluble fraction of an ethanol extract of Jujube by Yagi *et al*. (1978) and Okamura *et al*. (1981).



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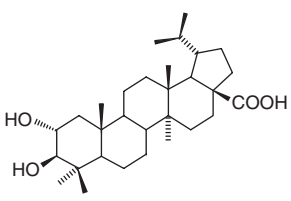

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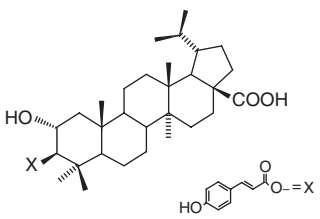

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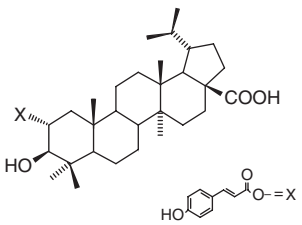

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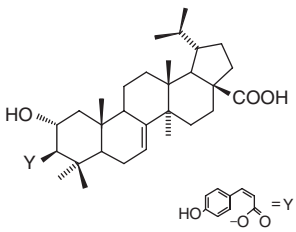
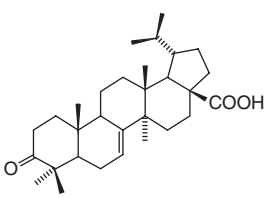


60

Additionally, betulonic acid (61: mp 253°C), oleanonic acid (62: mp 310°C), maslinic acid (63: mp 258–260°C), 3-*O*-*trans*-p-coumaroyl maslinic acid (64: mp 278–282°C), and 3-*O*-*cis*-*p*-coumaroyl maslinic acid (65: mp 190–195°C) were also isolated from Jujube by Yagi *et al*. in 1978.



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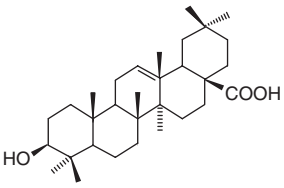

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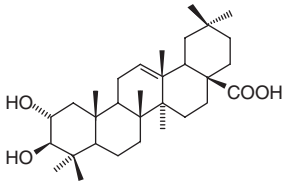

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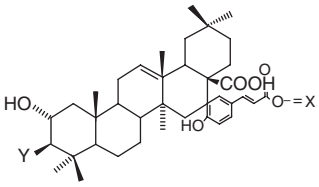

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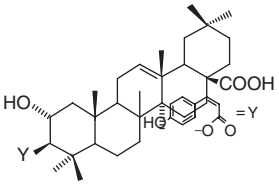
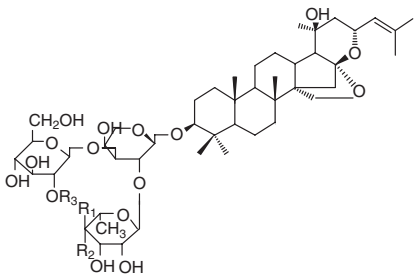


65

Zizyphus saponin I (66: mp 269–272°C), zizyphus saponin II (67: mp 268–269°C), zizyphus saponin III (68: mp 229–233°C) and jujuboside B (69: mp 256–259°C) were obtained from an ethanol extract of this crude drug (by Okamura *et al*., 1981a). These are triterpene saponins possessing the dammarane skeleton. Zizyphus saponins I and III have 6-deoxy-L-talose which is scarce in nature as a sugar.

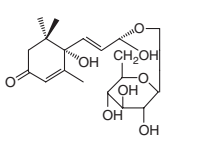


66: R_1=H, R_2=OH, R_3=H
67: R_1=OH, R_2=R_3=H
68: R_1=H, R_2=OH, R_3=X
69: R_1=OH, R_2=H, R_3=X

Furthermore, roseoside (70: mp 153°C), an *O*-glycoside of vomifoliol, zizyboside I (71: mp 192–193°C), zizyboside II (72: mp 237–238°C), and 6,8-di-C-glucosyl-2(S&R)-naringenin (73), were separated from the above extract. In addition, zizybeoside I (74: mp 192–193°C) and zizybeoside II (75: mp 237–238°C) were isolated. Noguchi reported in 1983 that these compounds were *O*-glycosides of benzyl alcohol.

In Jujube, *c.*90% of the content of hot water extracts consists of sugars; three-fourths of which are D-glucose and D-fructose. There exist sucrose and oligosaccharides which contain D-glucose and/or D-fructose (Tomoda *et al*., 1969, 1981 and 1985; Shimizu and Tomoda, 1983). A neutral polysaccharide, zizyphus-arabinan, and two acidic polysaccharides were also isolated.

A hot water extract of Jujube also yielded high concentrations of cyclic AMP and cyclic GMP (Hanabusa *et al*., 1981; Cyong and Takahashi, 1982).



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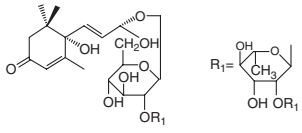

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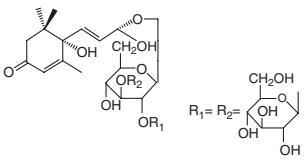

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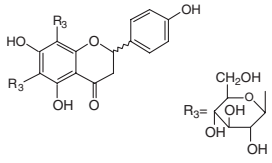

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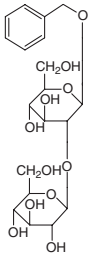

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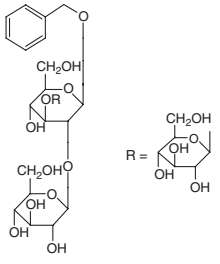


75

Glycyrrhiza

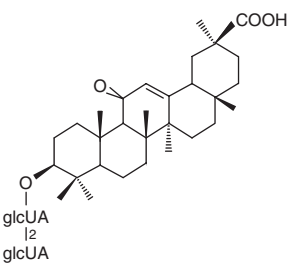
The Japanese Pharmacopoeia, Thirteenth Edition (1996) shows that Glycyrrhiza is the root and stolon, with or without the periderm, of *Glycyrrhiza uralensis* Fisher or *Glycyrrhiza glabra* Linné. Both contain 3–7% glycyrrhizin (glycyrrhizic acid) (76: mp 212–214°C). Glycyrrhizin has 150 times the sweetness of sucrose and forms glycyrrhetic acid upon hydrolysis. Glycyrrhiza contains glycosides of triterpenes, flavanones and chalcone, as well as isoflavanes, coumestan derivatives, isoflavones, polyamines, etc.

Identification

To 2.0 g of powdered Glycyrrhiza add 10 mL of a mixture of ethanol and water (7 : 3), heat by shaking on a water bath for 5 min, cool, filter and use the filtrate as the sample solution. Dissolve 5 mg of glycyrrhizin in 1 mL of a mixture of ethanol and water (7 : 3), and use this solution as the standard one. Perform the test with these solutions. Spot 2 μL each of the sample solution and the standard one on a plate of silica gel with a fluorescent indicator for TLC. Develop the plate with a mixture of n-butanol, water and glacial acetic acid (7 : 2 : 1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (254 nm); one spot among the spots from the sample solution and a dark-purple spot from the standard solution show the same Rf value, and colour tone.

The chemistry of Glycyrrhiza was reviewed by Shibata and Saitoh (1973), and Shibata (1981). In 1993, Kitagawa and Hori summarized those studies of chemical constituents of Glycyrrhiza done subsequent to the publication of the Shibata's review.

Glycyrrhiza contains saponins at 6–14%. Glycyrrhizin is a saponin possessing the triterpenoid skeleton, as determined by Robiquet in 1909. In the crude drug, glycyrrhizin exists as the potassium or calcium salt, and yields, upon hydrolysis, glycyrrhetic acid (77: mp 300°C) and two molecules of glucuronic acid. The structures of glycyrrhizin and glycyrrhetic acid were studied by Ruzicka and Cohen (1937), Ruzicka *et al*. (1937, 1943), by Ruzicka and Jeger (1943) and by Beaton and Spring (1955).



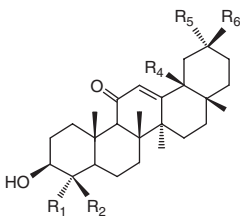
76

Effect on chronic hepatitis
Anti-inflammatory effect
Effect on the immune system
Anti-atherosclerotic effect
Anti-tumour effect
Anti-viral effect

Glycyrrhizin is the major saponin contained in Glycyrrhiza and it shows sweetness, a variety of pharmacological effects and *in vitro* bioactivities, as characteristic of the crude drug. It has been known that Glycyrrhiza has an anti-inflammatory effect, an anti-ulcer effect, an anti-allergic effect, an effect on chronic hepatitis, an effect on the immune system, an anti-atherosclerotic

effect, an anti-tumour effect, an anti-viral effect, etc. On the other hand, glycyrrhizin is known to have an aldosterone-like action. This side-effect prevents administration of large amounts of glycyrrhizin as well as its long-term administration. The aldosterone-like effect is considered present because the structure of the 11-keto-$\Delta^{12(13)}$ C-ring in glycyrrhetic acid is similar to that of the 3-keto-$\Delta^{4(5)}$ A-ring in steroidal hormones, resulting in competition for active sites of the reductase.

More recently, many saponins have been isolated from Glycyrrhiza, however, all of the sapogenins except uralenic acid have an 18 β-H oleanane skeleton. The structures (Table 3.3) of some are shown below ((77)–(90)). Kitagawa *et al*. isolated 13 new saponins and determined their structures.



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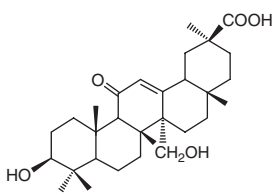

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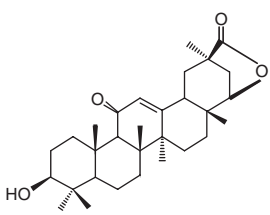

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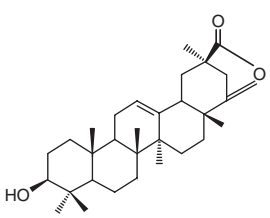

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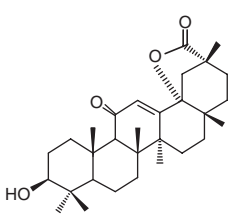

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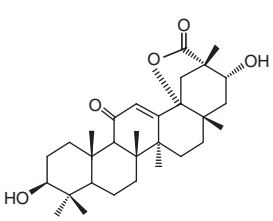

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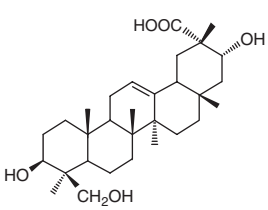


90

Table 3.3 Structures of sapogenins contained in glycyrrhiza

Sapogenin	R^1	R^2	R^3	R^4	R^5	R^6
Glycyrrhetic acid (GA : 77)	CH_3	CH_3	O	β-H	CH_3	COOH
23-Hydroxy-GA (78)	CH_2OH	CH_3	O	β-H	CH_3	COOH
24- Hydroxy-GA (79)	CH_3	CH_2OH	O	β-H	CH_3	COOH
11-Deoxo-GA (80)	CH_3	CH_3	H_2	β-H	CH_3	COOH
24-Hydroxy-11-deoxo-GA (81)	CH_3	CH_2OH	H_2	β-H	CH_3	COOH
Glycyrrhetol (82)	CH_3	CH_3	O	β-H	CH_3	CH_2OH
Uralenic acid (83)	CH_3	CH_3	O	α-H	CH_3	COOH
Liquiritic acid (84)	CH_3	CH_3	O	β-H	COOH	CH_3

Note
(77) Anti-inflammatory effect, anti-atherosclerotic effect.

Table 3.4 Structures of saponins and a sapogenin found in glycyrrhiza

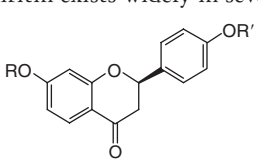
Saponin/Sapogenin	*R*	*R′*
Liquiritin (91)	H	Glucosyl
Liquiritigenin (93)	H	H
Neoliquiritin (95)	Glucosyl	H
Rhamnoriquiritin (97)	H	Glucorhamnosyl

Notes
(91) Anti-inflammatory effect.

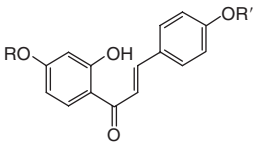
Table 3.5 Additional structures of saponins and sapogenins found in glycyrrhiza

Saponin/Sapogenin	*R*	*R′*
Isoliquiritin (92)	H	Glucosyl
Isoliquiritigenin (94)	H	H
Neoisoliquiritin (96)	Glucosyl	H
Rhamnoisoliquiritin (98)	H	Glucorhamnosyl
Licuraside (99)	H	Glucosyl

The pharmacological effects of Glycyrrhiza or its extract are not explainable simply on the basis of the actions of its saponins, such as glycyrrhizin, or the actions of their sapogenins (Table 3.4). During a study of the effects of Glycyrrhiza on spasm of muscle, liquiritin (91: mp 212°C) (a glycoside of flavanone) (Shinoda and Ueda, 1934), isoliquiritin (92: mp 185–186°C(dec)) (a glycoside of chalcone) (Litvinenko and Obolentseva, 1964; Van Hulle *et al*., 1971) and their respective aglycones, liquiritigenin (93: mp 194–197°C) and isoliquiritigenin (94: mp 191–194°C), were isolated from the crude drug. Also, glycosides consisting of these same aglycones together with another saccharide (90)–(99) were obtained by Litvinenko *et al*. (1964) Liquiritin exists widely in several kinds of Glycyrrhiza (Table 3.5).

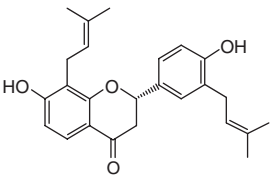


91, 93, 95, 97



92, 94, 96, 98, 99

In addition, flavanones such as glabrol (100: mp 90°C) (Saitoh *et al*., 1976) and shinflavanone were isolated from *G. glabra*, while isobavachin (101: mp 200–206°C) was separated from the root of pallidiflora.



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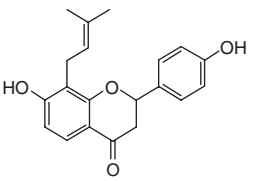
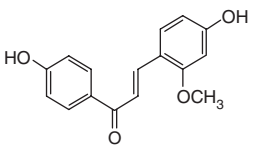


101

Regarding chalcones, Furuya *et al*. (1971) obtained echinatin (102: mp 214–217°C) from *G. echinata* in 1971. Saitoh and Shibata (1975) obtained licochalcone A (103: mp 100°C) and licochalcone B (104: mp 197°C) from a Glycyrrhiza called Sinkiang. Subsequently, licochalcone C and licochalcone D were found in *G. inflata* by Kajiyama *et al*. (1992) and gancaonin in *G. pallidiflora* by Fukai *et al*. (1990).



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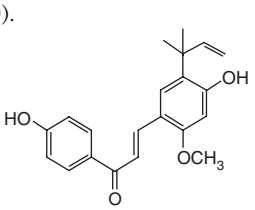

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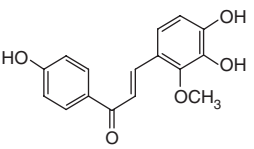
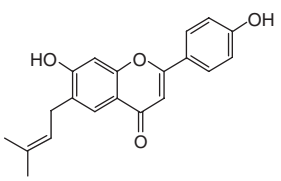


104

Flavones such as licoflavone A (105: mp 246.5°C), 7,4′-dihydroxy-flavone (106: mp 300°C), kumatakenin (107: mp 252–254°C) (Saitoh *et al*., 1976) and prenyllicoflavone, as well as flavonols such as licoflavonol (108: mp 185–187°C(dec)) (Saitoh *et al*., 1976), were obtained from several kinds of Glycyrrhiza.



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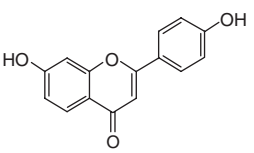

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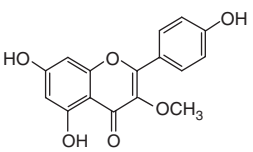

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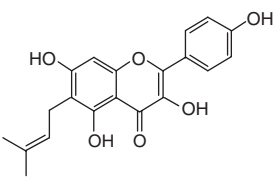
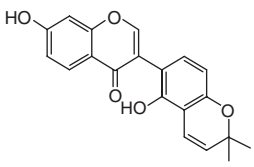


108

Isoflavonoids are widely distributed in plants of *Leguminosae*. Specifically, formononetin is frequently found in most types of Glycyrrhiza. Also found in several kinds of Glycyrrhiza are: glabrone (109: mp 224–226°C) (Kinoshita *et al*., 1976), licoisoflavone A (110: mp 111–113°C) (Kinoshita *et al*., 1978), licoisoflavone B (111) (Saitoh *et al*., 1978), licoisoflavanone (Saitoh *et al*., 1978), licoricone (112: mp 250–251°C) (Kaneda *et al*., 1973; Kinoshita *et al*., 1976), afromosin (113: mp 231–233°C), glyasperin derivative, glicoricone and licofuranone.



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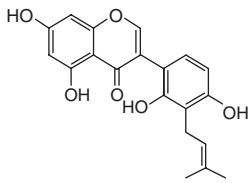

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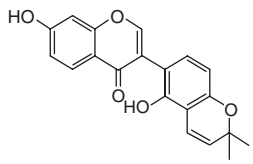

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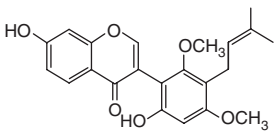

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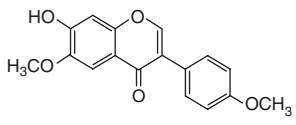
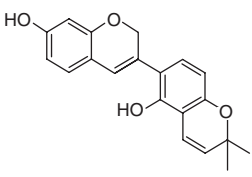


113

Also found were isoflavenes such as glabrene (114: mp 198–202°C(dec)) (Kinoshita *et al*., 1976), and pallidiflorene (115) (Saitoh *et al*. (unpublished)), and isoflavanes such as licoricidin (116: mp 154–156°C), glabridin(117: mp 154–155°C) and vestitol (118).



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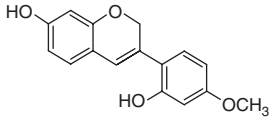

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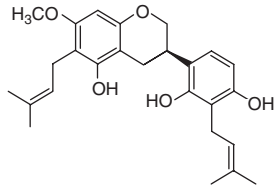

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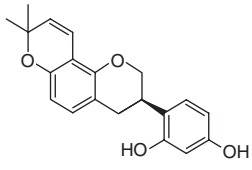

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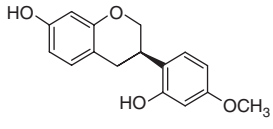
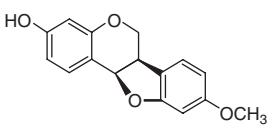


118

In addition, there are known to exist pterocarpans such as medicarpin (119: mp 134–137°C), shinpterocarpin, and methoxyphaseollin, coumestan derivatives such as glycyrol (120: mp 243.5–245°C), 5-*O*-methylglycyrol (121: mp 259–260.5°C), isoglycyrol (122: mp

298–300°C(dec)), glycyrin (123: mp 209–211°C), licopyranocoumarin, and glycycoumarin, as well as several kinds of stilbenes, phenanthrenes, dibenzoylmethanes and polyamines.



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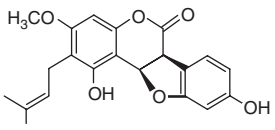

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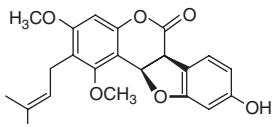

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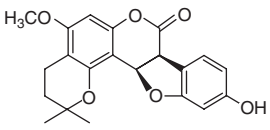

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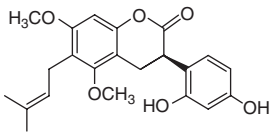


123

Ginger

The Japanese Pharmacopoeia, Thirteenth Edition (1996), shows that Ginger is the rhizome of *Zingiber officinale* Roscoe; it contains 0.25–3% of essential oils and 0.6–1.0% of pungent constituents, as well as other chemicals.

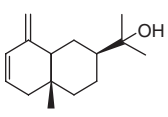
Identification

To 2.0 g of pulverized Ginger add 5 mL of acetone, shake for 3 min, filter and use the filtrate as the sample solution. Dissolve 1 mg of 6-gingerol in 1 mL of acetone, and use this solution as the standard one. Perform the test with these solutions. Spot 10 μL each of the sample solution and the standard one on a plate of silica gel for TLC. Develop the plate with a mixture of hexane, acetone and glacial acetic acid (10 : 7 : 1) to a distance of about 10 cm, and air-dry the plate. Spray the plate evenly with 2,4-dinitrophenyl-hydrazine TS, and heat at 105°C for 10 min; one of the spots from the sample solution and a brown spot from the standard solution show the same Rf value and colour tone.

The chemistry of ginger was studied and a review was reported by Kano (1987). Itokawa (1993) also summarized the chemistry of ginger including related crude drugs.

Ginger is known to have both protective and therapeutic effects in diseases of the liver.

Essential oils contained in ginger have been the subject of study over many years, as reported by Varma *et al*. (1962) and by Kami *et al*. (1972). As essential oils in ginger, a higher content of sesquiterpenes are found compared to other constituents such as zingiberol (124), zingiberene (125: bp 128–129°C/9 mm), α-bisabolene (126), β-bisabolene (127), γ-bisabolene (128: bp 261–262°C/751 mm), α-curcumene (129: bp 130°C/13 mm), β-curcumene (130: bp 142°C/19 mm), farnesol (131: bp 160°C/10 mm), etc. It is also known that monoterpenes such as citral (132: bp 118°C/20 mm), linalool (133: bp 198–199°C/760 mm), camphene (134: mp 51–52°C) and α-phellandrene (135: bp 58–59°C/10 mm) are contained in the crude drug. Nonylaldehyde has also been reported. In 1990, Kano *et al*. found the presence of two diterpenes: galanolactone (136: mp 90–92°C) and (E)-8 β, 17-epoxylabd-12-ene-15, 16-dial (137: mp 135–137°C) in ginger.



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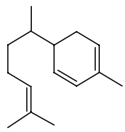

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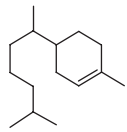

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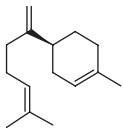

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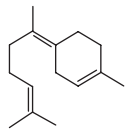

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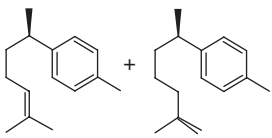

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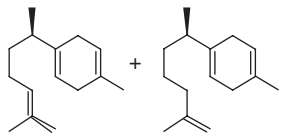

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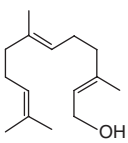

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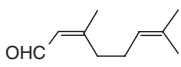

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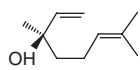

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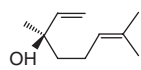

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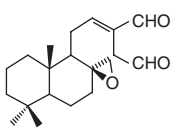

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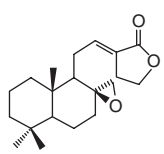
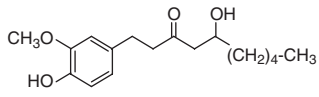


137

A major constituent of pungent substances in ginger is 6-gingerol (138), present at a level of *c.*0.1–0.3%. 6-Shogaol (139: bp 227–229°C/7.5 mm), a dehydrate of 6-gingerol, is present in less than one-tenth that amount. Other components found in ginger are: 6-gingerdion (140), 10-gingerdion (141), 6-dehydrogingerdion (142: mp 83.5–84.5°C), 10-dehydrogingerdion (143: mp 69–69.5°C), 6-gingediol (144), 8-gingediol (145), 10-gingediol (146), 6-methylgingediol (147), 8-gingerol (148), 10-gingerol (149), zingerone (150: mp 40–41°C), 1-(4-hydroxy-3-methoxyphenyl)-3,5-diacethoxyoctane (151), 1-(4-hydroxy-3-methoxyphenyl)-3,5-octanediol (152), 6-gingediacetate (153) and 6-methylgingediacetate (154).



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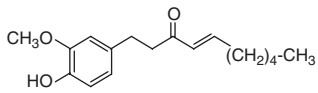

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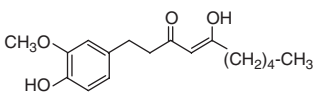

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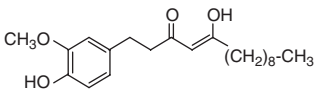

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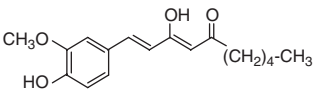

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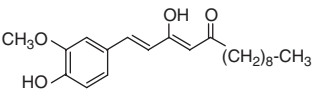

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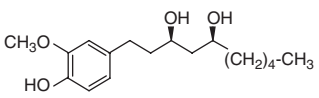

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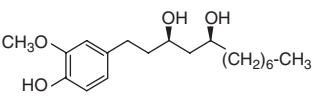

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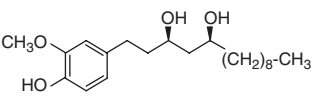

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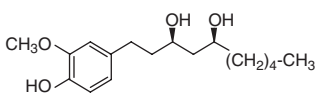

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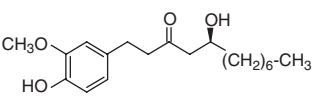

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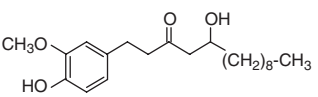

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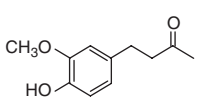

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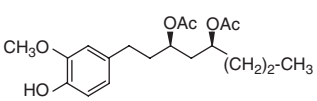

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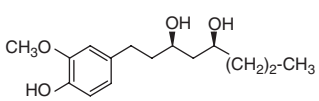

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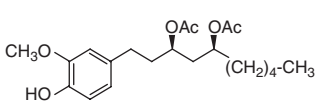

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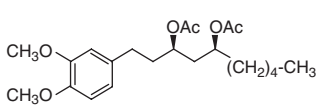
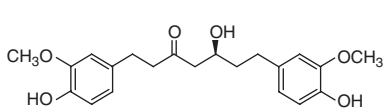


154

The amino acids, γ-aminobutyric acid (155: dec 192–194°C) and pipecolic acid (156: dec 265°C), were also detected.



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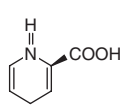

156

Starch, usually found in plants, together with protein, inorganic substances and saccharides are all contained in ginger.

In 1992, Tanabe *et al*., using HPLC and GC/MS, investigated seasonal variations in the content of three pungent constituents, 9 monoterpenes, 2 diterpenes and 4 sesquiterpenes in *Zingiber officinale* var. *rubens* Makino. The amounts of pungent substances, monoterpenes, diterpenes and

sesquiterpenes in the unripe child-rhizoma harvested in June and August were small. In contrast, their maximas were reached in the October–December period. In the mother-rhizoma, however, neither the content of pungent substances nor the amount of the flavour component varied much throughout the year.

Acknowledgement

The author is sincerely grateful to Dr James W. Bastian (North Dakota, USA) for reading the manuscript and his comments on this report.

Crude drugs III

Structural transformation of ingredients

Mitsuhiko Nose

Introduction

Since kampo-hozais (traditional Chinese medicines) are composed of several crude drugs, for example, there are seven medicinal herbs such as *Bupleuri Radix*, *Pinelliae Tuber*, *Zingiberis Rhizoma*, *Scutellariae Radix*, *Zizyphi Fructus*, *Ginseng Radix* and *Glycyrrhizae Radix* in *Sho-saiko-to*, there are numerous ingredients in the decoction. In order to clarify the mechanism of their diverse pharmacological actions on the molecular basis, we first need to know the real active principles in our body. Most kampo-hozais such as Sho-saiko-to are administered orally as a decoction. As a result of oral administration, components, which are involved in kampo-hozais are exposed to gastric juice, other digestive enzymes and intestinal microflora in the gut. Some of their chemical structures should be transformed before the absorption into the blood. The metabolites are absorbed from the alimentary tract *via* the portal vein and then metabolized or detoxicated in the liver. Some of these are excreted into intestine *via* the bile duct, passed into enterohepatic circulation and again meet intestinal bacteria.

Although major ingredients of crude drugs, such as saikosaponins in *Bupleuri Radix*, glycyrrhizin (GL) in *Glycyrrhizae Radix* and baicalin in *Scutellariase Radix*, might be the active principles in Sho-saiko-to, we cannot determine the 'real' active substances without knowing their metabolism. Therefore, studies on the metabolism of ingredients that are involved in kampo-hozais with gastric juice and intestinal flora are important for the evaluation of kampo-hozais. Intestinal microflora are a large environmental factor in the body and almost equal to the liver in weight and function, though their activities are still mostly unclear. Recently, isolation, cultivation and identification techniques of intestinal bacteria in humans and experimental animals have been established. Accordingly, the metabolisms of the major ingredients of crude drugs with intestinal flora can be undertaken, based on chemical, biochemical and molecular biological backgrounds. Among the ingredients of crude drugs, most glycosides are resistant to gastric juice and the other digestive enzymes and pass through the upper intestinal tract without absorption to the lower tract. Glycosides are retained in the tract and mostly hydrolysed by intestinal bacterial glycosidases to the corresponding aglycones and other metabolites.

In this chapter, we will focus on describing metabolisms of major ingredients, which are constituted of Sho-saiko-to, and we will discuss the relationship between the metabolite and their pharmacological actions.

Metabolisms of major ingredients of crude drugs in the alimentary tract

Metabolism of saikosaponins

Saikosaponin a, c and d, constituents of *Bupleuri Radix* (*Bupleurum falcatum* L.), shows various pharmacological effects including anti-inflammatory, anti-hyperchlesteremic and hepatoprotective

actions. The structures of saikosaponin a, c and d are well known to be unstable under the acidic condition because of the presence of 13β-28 epoxy ring moiety in the aglycone. Furthermore, the possibility of the hydrolysis of glycosides with intestinal microflora in the lower alimentary tract is expected. Hence, we supposed that their chemical structures should be transformed to various kinds of metabolites in the gut (Figures 3.15, 3.16 and 3.17).

The first report concerning the structural transformation of saikosaponins by gastric juice and intestinal microflora was conducted in our laboratory (Shimizu *et al*., 1985a,b). Quantitative analysis of saikosaponins and their metabolites was carried out by high-performance liquid chromatography (HPLC) (Shimizu *et al*., 1984).

By the incubation of saikosaponins in rat gastric juice (pH 1.5), saikosaponin a decreased with time. After 3 h, saikosaponin a disappeared completely and saikosaponin b_1, which possessed heteroannular diene moiety at C-11, 13(18) and saikosaponin g which possessed homoannular diene moiety at C-9(11), 12 were detected in the proportion of 3 : 1. On the other hand, saikosaponin d rapidly changed to only saikosaponin b_2, which possessed heteroannular diene moiety, within 30 min after the incubation. Next, by the anaerobic incubation of saikosaponin a with murine intestinal flora, the formation of saikogenin F, a genuine aglycone of saikosaponin a, reached a maximum 1 h after the incubation and its yield was 80%. A minor peak of prosaikogenin F, a monofucoside of saikogenin F, was also detected at 15 min. By the same procedures, saikosaponin b_1, g, d and b_2 were also transferred to the corresponding prosaikogenin A, H, G and D, and saikogenin A, H, G and D with a pattern that was similar to that of saikosaponin a. Finally, the contents of nine excreted metabolites from saikosaponin a, 5 and 20 mg/kg, in faeces after its oral administration was investigated using fasted and non-fasted rats. The total recovery of excreted genuine compounds, saikosaponin a, prosaikogenin F and saikogenin F, were more than 50% of the amount of starting saikosaponin a in the period 0–24 h after the administration. However, the total ratio of the excretion of saikosaponin b_1 and g, prosaikogenin A and H and saikogenin A and H were not more than 1% of the starting amount of saikosaponin a, respectively.

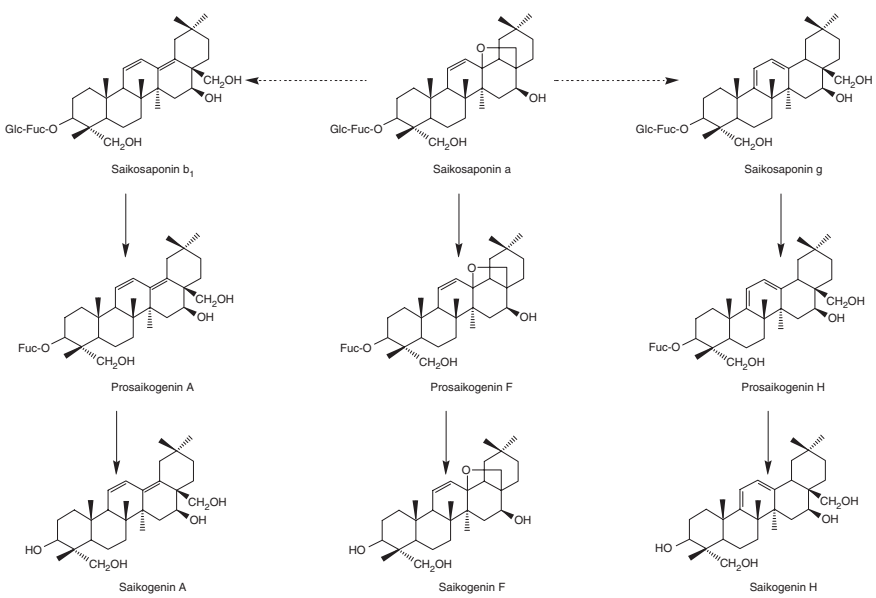

Figure 3.15 Metabolism of saikosaponin a →, gastric juice; →, intestinal flora.

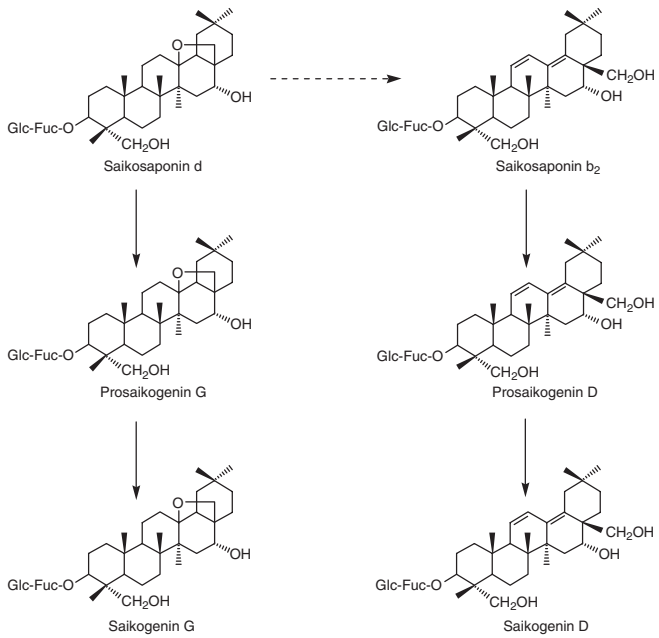

Figure 3.16 Metabolism of saikosaponin d →, gastric juice; →, intestinal flora.

These experiments suggest the possibility that saikosaponin a and d are transformed to at least 13 metabolites in the alimentary tract and absorbed into bloodstream as a mixture. Furthermore, the plasma concentration of saikosaponin a and its metabolites, prosaikogenin F and saikogenin F, after an oral administration of 20 mg/kg of saikosaponin a in rats was determined by HPLC (Fujiwara *et al*., 1986). After the administration, saikosaponin a was detected at 7.4 ± 1.7 and 4.5 ± 2.2 μg/100 ml at 30 min and 1 h, respectively. Prosaikogenin F was detectable between 3 and 12 h, and its peak was at about 8 h with 234 ± 133 ng/100 ml. The concentration of saikogenin F changed in a way similar to those of prosaikogenin F, and its concentration was 167 ± 86 ng/100 ml.

Recently, Kida *et al*. (1997a) studied biotransformation of saikosaponins by human intestinal flora (Figure 3.18). They revealed that saikosaponin a, b_1, b_2 and d were converted to the corresponding prosaikogenins and saikogenins in order. In the case of saikosaponin c, saikogenin E, genuine aglycone of saikosaponin c, was obtained as a sole product. Of 31 defined human intestinal bacterial strains, only *Eubacterium* sp. A-44 could metabolize saikosaponin a and b_1 to the corresponding prosaikogenins and saikogenins. Saikosaponin d and b_2 were hydrolysed to the respective prosaikogenins, but no saikosaponin c by this strain.

After screening bacterial colonies from fresh human faeces for metabolizing activity of saikosaponins, 2 of 60 isolates showed appreciable ability of hydrolysing saikosaponins a, b_1, b_2 and d, except for c, to the corresponding prosaikogenins. However, both strains did not further hydrolyse the prosaikogenins to saikogenins. They were identified as *Bifidobacterium* sp., named *Bifidobacterium* sp. Saiko-1 and Saiko-2, close species to *Bifidobacterium breve* ss *breve* and *B. adolescentis*, respectively. Furthermore, they isolated two glycosidases responsible for the metabolism of saikosaponins from *Eubacterium* sp. A-44 and characterized these enzymes

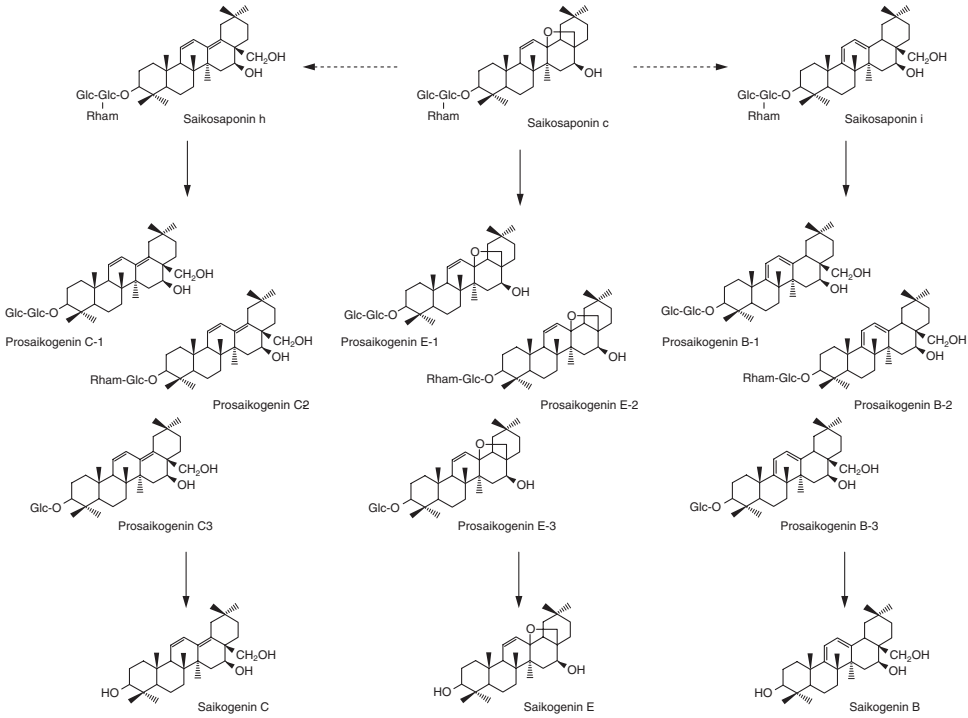

Figure 3.17 Metabolism of saikosaponin c →, gastric juice; →, intestinal flora.

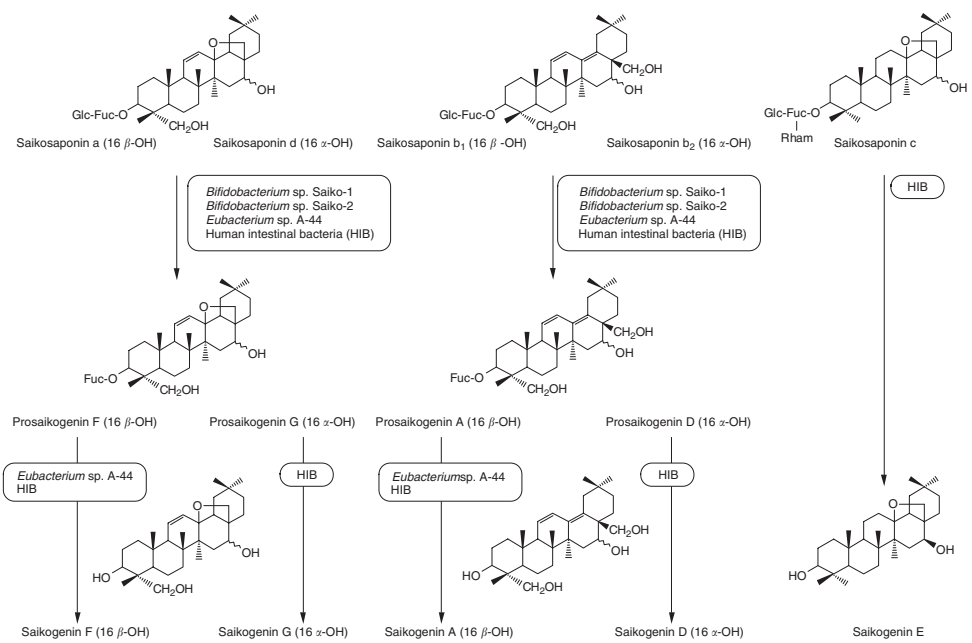

Figure 3.18 Metabolic pathway of saikosaponins by human intestinal bacteria.

(Kida *et al*., 1997b). Relative to the hydrolysing activities with respect to saikosaponin a, b_1 and b_2, the β-D-glucosidase showed lower ability to hydrolyse saikosaponin d, but no ability to hydrolyse saikosaponin c and prosaikogenins. By Sephacryl S-300 column chromatography, the molecular weight of prosaikogenin-hydrolysing β-D-fucosidase was estimated to be about 130 kDa. The β-D-fucosidase could hydrolyse prosaikogenin A and F, but not prosaikogenin D and G or saikosaponins. Relative to *p*-nitrophenyl β-D-fucoside-hydrolysing activity, this enzyme had 32.0% and 22.2% of its hydrolysing ability with respect to *p*-nitrophenyl β-D-glucoside and *p*-nitrophenyl β-D-galactoside, respectively. *p*-Nitrophenyl β-D-fucoside-hydrolysing activity was inhibited by D-fucose, and was weakly inhibited by D-glucose, D-glucono δ-lactone, D-galactose and D-galactono δ-lactone. By combining these two glycosidases, saikosaponins a and b_1 were converted to their saikogenins *via* the corresponding prosaikogenins. More recently, they investigated the metabolic fate of saikosaponin b_1 using conventional, germ-free and *Eubacterium* sp. A-44-infected gnotobiote rats (Kida *et al*., 1998). After the administration of saikosaponin b_1 to germ-free rats at a dose of 50 mg/kg, no metabolite was detected in the plasma, the caecal contents or the cumulative faeces through the experiment. On the other hand, when saikosaponin b_1 was orally given to the *Eubacterium* sp. A-44-infected gnotobiote rats, a considerable amount of its metabolites, prosaikogenin A and saikogenin A, were detected in the rat plasma with the respective $AUC_{0-10\,h}$ values of 17 424 and 22 260 pmol·min/ml, similar to the case of its oral administration to conventional rats ($AUC_{0-10\,h}$ values of 9936 and 12 414 pmol·min/ml, respectively). Furthermore, significant amounts of both metabolites were detected in the caecal contents and the cumulative faeces of the gnotobiote and conventional rats, but not in those of the germ-free rats, within 10 h after the administration. Faecal and caecal activities of hydrolysing saikosaponin b_1 and prosaikogenin A were found in the gnotobiote and conventional rats, though there were no detectable activities in the germ-free rats. Accordingly, both hydrolysing activities in the intestinal bacteria, such as *Eubacterium* sp. A-44, are essential for the appearance of prosaikogenin A and saikogenin A in the rat plasma and cumulative faeces, since orally administered saikosaponin b_1 was poorly absorbed from the gastrointestinal tract. In addition, they suggested that higher concentrations of metabolites could appear in human plasma than in rat plasma since the transformation of saikosaponins by a mixture of bacteria from human faeces was much faster than by bacteria of rat faeces when the saponins were incubated *in vitro* under the same conditions (Kida *et al*., 1997b).

With respect to the pharmacological and biological activities of these metabolites, Fujiwara *et al*. (1986) studied the effects of saikosaponin a and its nine metabolites on protein synthesis by rat hepatocytes *in vitro*. Although saikosaponin a showed no effect on the synthesis of secreted and total protein, its metabolites formed in gastric juice, saikosaponin b_1 and g, increased the synthesis of secreted protein as well as total protein. Their monoglycosides, prosaikogenin F, A and H, also increased the synthesis of secreted and total protein. In contrast, saikogenin F slightly stimulated and saikogenin A and H had no effect on the synthesis of secreted protein, but increased that of total protein. These results suggested that metabolites formed in the alimentary tract were active principles in the protein synthesis in liver, but not saikosaponin a. Furthermore, the corticosterone-inducing activities of saikosaponins and their metabolites were investigated *in vivo*, to identify the active forms of oral saikosaponins (Nose *et al*., 1989). In order to obtain 27 metabolites of saikosaponin a, c and d formed in the alimentary tract, we developed the simple chemical method: an alcoholic alkali metal degradation to cleave the glycosidic bond (Ogihara and Nose, 1986). This new reaction is effective in obtaining partial hydrolysis products of glycosides, especially which is labile in an acidic condition (Chen *et al*., 1987; Ogihara *et al*., 1987). By this method, we have obtained the prosaikogenins from saikosaponins a, c and d in satisfactory yields. Intraperitoneal administration of saikosaponin a and d, and prosaikogenin F and G

showed significant corticosterone secretion-inducing activities, whereas saikogenin F and G, and the metabolites in which an ether ring of aglycones was cleaved, such as saikosaponin b_1, g and b_2, prosaikogenin A, H and D, and saikogenin A, H and D, were inactive. On the other hand, saikosaponin c was inactive but its intestinal metabolites, especially prosaikogenin E-2, showed an activity almost equal to that of saikosaponin a, h and i, gastric metabolites of saikosaponin c, were also inactive, but their prosaikogenins showed slight activities. When these compounds were orally administered, their corticosterone secretion-inducing activities were similar to those observed in the intraperitoneal experiments. Judging from both data, the results obtained in the intraperitoneal treatments with saikosaponin metabolites should accurately reflect the *in vivo* actions of saikosaponin a, c and d. These results indicated that saikosaponin a and d were the active principles in corticosterone secreting-induced activities in the cases of saikosaponin a and d but the activities were weakened through the metabolism of them in the gut, and that the intestinal metabolites such as prosaikogenins were the active principles in the case of saikosaponin c.

Metabolism of glycyrrhizin

Glycyrrhizin, a constituent of *Glycyrrhizae radix* (*Glycyrrhiza glabra* L. and *G. uralensis*, licorice), shows various pharmacological effects including a steroid-like, anti-inflammatory, anti-allergic, anti-cholesteremic and anti-viral action. GL is stable in gastric juice and passes through the upper intestinal tract to the lower tract, where more than hundreds of species and hundred billions of anaerobic bacteria live.

Glycyrrhizin was hydrolysed to an aglycone, glycyrrhetinic acid (GA), which was then transformed to a new compound, 3-epi-18β-GA, *via* a metabolic intermediate, 3-dehydro-18β-GA by incubation with a bacterial mixture from human faeces (Hattori *et al*., 1983) (Figure 3.19). In a survey of human intestinal bacteria capable of metabolizing GL, *Eubacterium* sp., *Ruminococcus* sp. and *Clostridium innocuum* were isolated from human faeces (Hattori *et al*., 1985a; Akao *et al*., 1987).

From *Eubacterium* sp. (named *Eubacterium* sp. strain GLH), GLβ-D-glucuronidase was purified and found to hydrolyse specifically GL to GA. The molecular weight of this novel type β-D-glucuronidase is 65 kDa, the pH optimum and PI values are 5.6 and 4.1, respectively and the Km value for GL is 0.11 mM. This GL-hydrolysing enzyme was separated from the β-D-glucuronidase, which hydrolysed β-D-glucuronides of phenolic compounds by octyl-Sepharose column chromatography.

In addition, GL-β-glucuronidase activity increases during the cultivation of human intestinal flora in the presence of GL (Akao *et al*., 1988a), suggesting that GL-metabolizing activity in intestine is induced by daily oral administration of GL. Recently, Akao *et al*. (1993) showed clearly the significance of this strain responsible for the hydrolysis of GL to GA *in vivo*, using conventional, germ-free and *Eubacterium* sp. strain GLH-infected gnotobiote rats. The faeces and caecal contents of the gnotobiote rats showed GL-hydrolysing activities (31.7 and 31.3 pmol/min/mg protein, respectively) similar to those (81.0 and 39.9 pmol/min/mg protein, respectively) of conventional rats, although there was no detectable activity in germ-free rats. When GL (100 mg/kg) was orally administered to conventional, germ-free and gnotobiote rats, no GL could be detected in plasma 4 or 17 h after the administration, using EIA and HPLC assays. Plasma GA was not detected 4 or 17 h after the administration to germ-free rats nor could GA be detected in caecal contents or in the faeces. However, GA (0.6–2.6 nmol/l) was detected in the plasma of the conventional and the gnotobiote rats 4 and 17 h after the administration, and the caecal contents after 4 h and the cumulative faeces up to 17 h of the conventional and the gnotobiote rats contained considerable amounts of GA. These findings confirm that orally

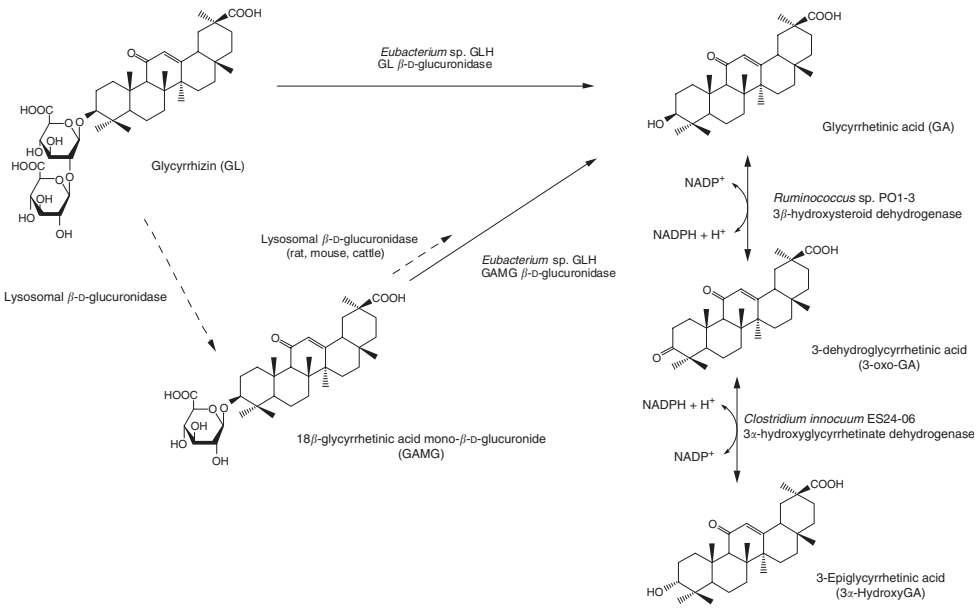

Figure 3.19 Metabolic pathway of GL in the alimentary tract.

administered GL is poorly absorbed from the gut, but is hydrolysed to GA by intestinal flora such as *Eubacterium* sp. strain GLH and that the resulting GA is absorbed.

Ruminococcus sp. capable of oxidizing GA to a 3-dehydro derivative produces a new type of 3β-hydroxysteroid dehydrogenase, which catalyses reversible conversion between 3β-hydroxy and 3-oxo-groups of GAs and has absolute specificity for the β-configuration of a hydroxy group at the 3-position of bile acids and steroids having no double bond in the A/B ring (Akao *et al*., 1986). *Clostridium innocuum* converts 3-dehydroglycyrrhetinic acid to 3-epiglycyrrhetinic acid. The bacterium produces 3α-hydroxyglycyrrhetinate dehydrogenase, which shows absolute specificity for the 3α-hydroxy and 3-oxo-groups of GAs and requires $NADP^+$ and NADPH as cosubstrate (Akao *et al*., 1988b).

It has been believed for a long time that GL is hydrolysed to GA in the liver. Actually, it was reported that GL was hydrolysed to 18β-glycyrrhetinic acid mono-β-D-glucuronide (glycyrrhetyl monoglucuronide (GAMG)) by rat liver homogenate and that the hydrolytic activity was localized in the lysosomes (Akao *et al*., 1991a). Although rat liver lysosomes could also hydrolyse GAMG to GA, the hydrolytic rate was much lower than that of hydrolysis from GL to GAMG. Time-course experiments revealed that GL seemed to be first hydrolysed to GAMG and then GAMG was successively hydrolysed slowly to GA. However, the hydrolysis of GAMG to GA was not detected in lysosomes of human and porcine livers, although hepatic lysosomes from mouse and cattle hydrolysed GAMG to GA in a similar manner to those from rat. Accordingly, lysosomal β-D-glucuronidases from human and porcine livers converted GL to GAMG only.

When GL was injected intravenously to human subjects, GA was observed in their sera (Nakano *et al*., 1980). However, serum concentrations of GA were lower and its appearance time was later after the intravenous injection of GL than those of GA after oral administration of GL.

Moreover, GL in serum seems to be excreted rapidly into the intestinal tract through bile, suggested by the fast disappearance of GL from sera and bile excretion of GL in rat (Ichikawa *et al*., 1984, 1986a). Since β-D-glucuronidase of human liver does not convert GL to GA, GA in serum after the intravenous injection of GL may be derived from GA produced by the bacterial hydrolysis of GL in intestine, in a similar way to GA in serum after oral administration of GL. On the other hand, GAMG was found in the serum of a patient with pseudo-aldosteronism after intravenous administration of large doses of GL (Kanaoka *et al*., 1986). A large amount of GAMG, compared with GA, was present in the serum and little GAMG was produced from GL by intestinal flora. In this case, GAMG seems to be produced from GL by lysosomal β-D-glucuronidase in the liver. GAMG was also hydrolysed to GA by GL β-D-glucuronidase of *Eubacterium* sp. strain GLH (Akao *et al*., 1987). Recently, an enzyme demonstrated to be responsible for the hydrolysis of GAMG to GA was isolated from *Eubacterium* sp. strain GLH and characterized as a GAMG β-D-glucuronidase (Akao, 1997). This enzyme was separated from GL β-D-glucuronidase in *Eubacterium* sp. GLH by Butyl-Toyopearl 650S and Toyopearl HW 55-S column chromatographies, and the molecular weight of GAMG β-D-glucuronidase was 49.5 kDa. These enzymes showed differences in the purification ratio and substrate specificity. GAMG excreted in the bile was then rapidly converted to GA by intestinal flora. These results indicate that intestinal bacteria, such as *Eubacterium* sp. strain GLH, are essential for the metabolism of GL.

Akao *et al*. (1990a) further investigated the metabolic fate of absorbed GA in liver. They revealed that absorbed GA was oxidized to 3-keto GA in rat liver microsomes. They found a second enzyme showing GA-oxidizing activity in addition to 3β-hydroxysteroid dehydrogenase of *Ruminococcus* sp. Interestingly, this glycyrrhetinate dehydrogenase activity was detected in microsomes of adult male rats, but not in those of adult females (Akao *et al*., 1991b). It was not observed in infant males but appeared 6 weeks after birth, after which it increased gradually and reached a maximum level at 12 weeks after birth. These results indicate that GA dehydrogenase in rat liver is male-specific and regulated by sex-hormones through the pituitary. However, since the reductive activity of 3-keto GA was higher than the oxidative activity of GA, a trace amount of 3-keto GA has not been detected in human sera. Although GA is circulated enterohepatically by the conjugation of the 3β-hydroxyl group with glucuronate or sulphate and the 30-carboxyl group with glucuronate followed by biliary excretion (Parke *et al*., 1963; Iveson *et al*., 1971), it is not known whether 3-keto GA is actually circulated, suggesting that its enterohepatic circulation is very low owing to lack of the 3β-hydroxyl group. Furthermore, they also reported that 3-keto GA and 3-epi GA are hydroxylated by rat liver microsomes to form 22α- and 24-hydroxyl derivatives (Akao *et al*., 1990b).

Evidence indicating that GA is the active principle in the body has been accumulated with regard to the pharmacological and biological activities of these metabolites. A comparison of anti-hepatotoxic activities between GL and GA was carried out using *in vivo* and *in vitro* assay methods (Nose *et al*., 1994). The oral administration of GA at 1, 24 and 48 h before D-galactosamine (GalN) treatment significantly reduced the increase of serum transaminase activities 24 h after GalN treatment, whereas GL did not inhibit the increases of those activities. The intraperitoneal administration of GA 1 h before GalN treatment restored the increase of serum transaminase activities with lower doses than GL. Moreover, GA significantly protected the carbon chloride (CCl_4)-induced leakage of transaminase at doses of 5–50 μg/ml, whereas GL inhibited slightly the leakage at a dose of 1000 μg/ml. These results suggest intensively the possibility that GA acts as an active principle in anti-hepatotoxic activity of GL. Recently, Akao *et al*. demonstrated clearly that the hepatoprotective activity of GL was due to GA formed by intestinal flora in the gut, using conventional, germ-free and *Eubacterium* sp. strain GLH-infected gnotobiote rats. The oral administration of GL (100 mg/kg) at 1, 24 and 48 h before CCl_4 treatment significantly

reduced the increase of serum transanimase activities 24 h after CCl_4 treatment in conventional and *Eubacterium* sp. strain GLH-infected gnotobiote rats, whereas GL was not effective in germ-free rats. The degree of hepatoprotective activities was parallel to the plasma concentration of GA in three kinds of rats. Thus, GL orally administered is poorly absorbed from the gut but is hydrolysed to GA by intestinal bacteria and the resulting GA is absorbed and shows the activity.

Metabolism of ginsenosides

Ginseng, the root of *Panax ginseng* C. A. MEYER is an important drug used in kampo-hozai. Its main constituents are glycosides of dammaraene-type triterpenes, protopanaxadiol and protopanaxatriol, and it has been reported that ginsenosides have diverse biochemical and pharmacological action (Figure 3.20). Many investigators have been interested in the metabolism of ginsenosides and a lot of evidence concerning absorption, distribution, excretion and metabolism of ginsenosides has been accumulated.

In 1982, the first report concerning tissue distribution of ginsenoside Rg_1, one of the main protopanaxatriol group saponins in Ginseng, after orally administered to rats, has been published (Takino *et al*., 1982). They developed a quantitative analytical method using a thin-layer chromatography (TLC) combined with a Servachrom XAD-2 resin column, and determined the concentration of Rg_1 in various tissues in rats that had been administered Rg_1 (100 mg/kg) orally. The concentration of Rg_1 in rat tissue and serum was below 10 μg/g or ml. It was found that 77.3 ± 3.9% of the dose remained in the digestive tract such as in the stomach, small and large intestines, at 150 min after the administration. They also measured the cumulative excretion into bile, urine and faeces until 24 h after oral administration of Rg_1 (100 mg/kg) and found that 1.1 ± 0.1%, 0.4 ± 0.04% and 41.2 ± 2.6% of the dose were excreted within 24 h, respectively (Odani *et al*., 1983a). The urinary and biliary excretion of Rg_1 occurred in a 2:5 ratio in both oral and intravenous administration of Rg_1 to rats. As about 80% of the dose of Rg_1 was excreted into urine and bile after intravenous injection to rats, they suggested that Rg_1 was barely metabolized in rat liver. Moreover, they investigated the metabolism of ginsenoside Rb_1, another dammaraene-type

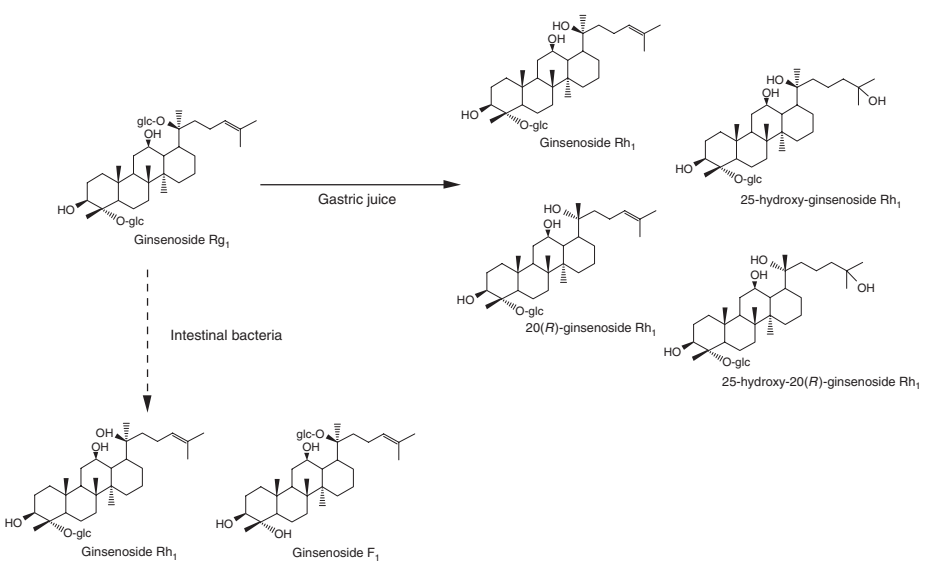

Figure 3.20 Metabolism of ginsenoside Rg_1.

saponin which possesses protopanaxadiol as an aglycone, in rats (Odani *et al*., 1983b). Little Rb_1 was absorbed from the digestive tract after oral administration (100 mg/kg) to rats. The serum level of Rb_1 in rats after intravenous injection (5 mg/kg) declined biexpotentially, and the half-life of the β-phase was 14.5 h. The long persistence of Rb_1 in serum and tissue in rats after intravenous administration was assumed to correlate with its high affinity to plasma proteins. Rb_1 was gradually excreted into urine, but not into bile. Unabsorbed Rb_1 in the digestive tract was rapidly decomposed and/or metabolized mainly in the large intestine. These results are quite different from those of Rg_1 in rats.

Since ginsenosides are poorly absorbed from the gut, many investigators are interested in the metabolic fate of ginsenosides in gastric juice and/or with intestinal microflora (Figure 3.21).

With respect to the metabolites of these ginsenosides in the alimentary tract, Odani *et al*. (1983c) investigated the decomposition of Rg_1 and Rb_1 in the rat stomach and large intestine after oral administration. In the stomach, a part of Rg_1 was decomposed and six decomposition products were observed on a reversed phase thin-layer chromatogram. These six compounds were identical to those which were obtained on hydrolysis of Rg_1 under mild acidic conditions (with 0.1 N HCl, at 37 °C), and four of them were isolated and identified as 20(*R*,*S*)-ginsenoside Rh_1 and 25-hydroxy-20(*R*,*S*)-ginsenoside Rh_1. On the other hand, an unidentified decomposition product of Rb_1 was observed on the TLC of the stomach sample after the oral administration of Rb_1 to rats. The product was different from the decomposition product formed by the hydrolysis of Rb_1 under mild acidic conditions. Later, the major metabolite of Rb_1 in rat gastric juice was identified as 25-hydroperoxy-23-ene derivative of Rb_1, whereas Rb_1 was hydrolysed to 20(*R*,*S*)-ginsenoside Rg_3 in 0.1 N HCl (Karikura *et al*., 1991a). In the large intestine, Rg_1 was decomposed to ginsenoside Rh_1 and ginsenoside F_1 by tetracycline-susceptible bacteria and

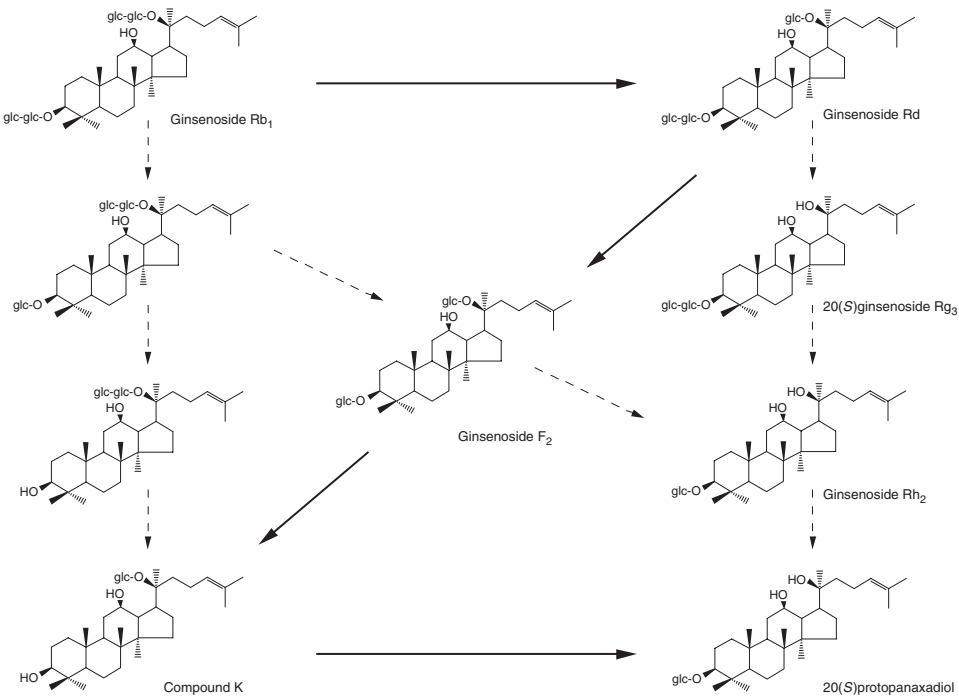

Figure 3.21 Metabolic pathway of ginsenoside Rb_1 by intestinal bacteria.

tetracycline-resistant bacteria, respectively. Rb_1 was decomposed to ginsenoside Rd and two unidentified products by enteric enzyme and tetracycline-resistant bacteria, respectively. Recent study revealed that Rb_1 was metabolized to five compounds, which were identified as gypenoside XVII, ginsenoside Rd, ginsenoside F_2, compound K and 25-hydroperoxy-23-ene derivative of Rb_1, in rat large intestine (Karikura *et al*., 1991b). In the same way, the decomposition of ginsenoside Rb_2 in the rat large intestine after oral administration was investigated (Karikura *et al*., 1990). A part of Rb_2 was decomposed to six products and five products were isolated and identified on the basis of ^{13}C-NMR analysis, as ginsenoside Rd, 3-*O*-β-D-glucopyranosyl-20-*O*-[α-L-arabinopyranosyl(1 $\rightarrow$ 6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol, ginsenoside F_{22}, 20-*O*-[α-L-arabinopyranosyl(1 $\rightarrow$ 6)-β-D-glucopyranosyl]-20(*S*)-protopanaxadiol and compound K, respectively. Moreover, the decomposition of Rb_2 in rat stomach after oral administration and in 0.1 N HCl solution was also investigated (Karikura *et al*., 1991a). By treating with 0.1 N HCl, the acidity of which is similar to that of gastric juice, a part of Rb_2 was hydrolysed to 20(*R*,*S*)-ginsenoside Rg_3. On the other hand, Rb_2 was little decomposed in rat stomach and a small quantity of an unidentified metabolite, which was different from the hydrolysed products in 0.1 N HCl, was detected. The metabolite was separated into four compounds, which were identified on the basis of spectroscopic analysis, as 25-hydroxy-23-ene, 24-hydroxy-25-ene, 25-hydroperoxy-23-ene and 24-hydroperoxy-25-ene derivatives of Rb_2, respectively. In this study, it is suggested that 20(*S*)-protopanaxatriol saponins undergo hydrolysis of the C-20 glycosyl moiety and hydration of the side chain. On the other hand, 20(*S*)-protopanaxadiol saponins undergo oxygenation of the side chain.

Kanaoka *et al*. (1994) reported the metabolism of ginsenosides by human intestinal flora. Ginsenoside Rb_1 and Rg_1 were cultured with fresh human faeces under anaerobic conditions, and Rb_1 was metabolized rapidly within 8 h, whereas Rg_1 was metabolized slowly within 2 days by the successive hydrolysis. They proposed the metabolic pathway by human intestinal flora as follows; ginsenoside $Rb_1 \rightarrow Rd \rightarrow F_2 \rightarrow$ compound K$\rightarrow$20(*S*)-protopanaxadiol, and $Rg_1 \rightarrow Rh_1 \rightarrow$ 20(*S*)-protopanaxatriol, respectively. Hasegawa *et al*. (1996) also demonstrated the metabolisms of ginsenosides by human intestinal bacteria and determined the urinary and blood concentration of their metabolites after oral administration of Ginseng extract and its saponins in humans and rats. They found compound K in urine when Ginseng extract (150 mg/kg) to humans and also compound K mainly in serum and in urine when Ginseng total saponin (1 g/kg) to rats. Akao *et al*. (1998a) also detected compound K in rat plasma after oral administration of Rb_1 using an enzyme immunoassay. Moreover, they revealed that the appearance of compound K in rat plasma was some time later, whereas rapid absorption occurred after oral administration of compound K itself. These results suggest that unabsorbed Rb_1 is transformed into compound K by intestinal bacteria in the lower part of the rat intestine and that compound K is then absorbed. Furthermore, Akao *et al*. (1998b) screened 31 defined strains of human intestinal bacteria for Rb_1-metabolizing activity and found that only strain, *Eubacterium* sp. A-44, was capable of transforming Rb_1 into compound K *via* ginsenoside Rd. They confirmed the role of this strain for the hydrolysis of Rb_1 to compound K, using germ-free and gnotobiote rats mono-associated with *Eubacterium* sp. A-44.

Recently, with respect to the pharmacological and biological actions of metabolites, some remarkable evidence has been reported (Hasegawa and Uchiyama, 1998; Lee *et al*., 1998; Wakabayashi *et al*., 1997). Lee *et al*. reported the anti-genotoxic activities of metabolites formed by intestinal bacteria from ginsenoside Rb_1, Rb_2 and Rc by testing their effects on benzo[*a*]pyrene (B[*a*]P)-induced mutagenicity and clastogenicity *in vitro*. Compound K inhibited the mutagenicity in a dose-dependent manner, and it also reduced the frequency of chromosome aberration by B[*a*]P. Hasegawa *et al*. (1996) and Wakabayashi *et al*. (1997) demonstrated that anti-metastatic action of ginsenoside Rb_1, Rb_2 and Rc were dependent on intestinal-bacterial hydrolysing potential and that compound K was the active principle after oral administration.

Metabolism of baicalin

Baicalin is one of the major flavonoids present in *Scutellariae Radix* and has been shown to possess anti-inflammatory and anti-allergic activity and to have effects on hyperlipidaemia and lipolysis *in vivo* and *in vitro*. However, little information is available on the absorption, metabolism and excretion of baicalin, although considerable information has been accumulated regarding the metabolism of flavonoids in animals and to a very limited extent in humans (Hackett, 1986; Scheline, 1991) (Figure 3.22). Ring fission occurs under the influence of intestinal microflora, which also account for the subsequent demethylation and dehydrogenation of the resulting phenolic acids. Intestinal bacteria also possess glycosidases capable of cleaving sugar residues from flavonoid glycosides. Such glycosidases do not appear to exist in mammalian tissues. Flavonoids can undergo oxidation and reduction reactions as well as methylation, glucuronidation and sulfation in animal species. Biliary excretion and enterohepatic circulation have been well described, but little is known about the absorption, distribution, metabolism and excretion of flavonoids in humans (Hackett, 1986).

With respect to metabolism of baicalin, Abe *et al*. (1990) reported the biliary excretion of its metabolites in rats. They found five major metabolites, which were identified as baicalein 6-*O*-β-glucopyranuroside (M1), 6-*O*-methyl-baicalein 7-*O*-β- glucopyranuro-side (oroxylin A 7-*O*-β-glucopyranuroside (M2)), baicalein 7-*O*-β-glucopyranuroside (M3), 6-*O*-β-glucopyranurosyl-baicalein 7-*O*-sulfate (M4), and baicalein 6,7-di-*O*-β-glucopyranuroside (M5) in the bile of rats administered baicalin orally. The bile of rats treated with baicalein, an aglycone of baicalin, also contained the above metabolites. In addition, the biliary metabolites of both compounds were shown to be mainly composed of M5 and M4, which have high polarities and large molecular weights. Slower biliary excretion of the metabolites after baicalin administration suggested that it was absorbed as baicalein after hydrolysis in the gastrointestinal tract.

Since baicalin is a flavone glucuronide, it has been expected to be hydrolysed by intestinal flora to baicalein as well as in the case of GL. However, HPLC analysis with electrochemical detection of the plasma of rats that had been administered orally revealed that only baicalin was found in the plasma (Wakui *et al*., 1992). Interestingly, almost the same pattern of plasma concentration of baicalin was observed when baicalein was administered orally in rats. These results suggested that baicalin was first hydrolysed by intestinal flora, absorbed as

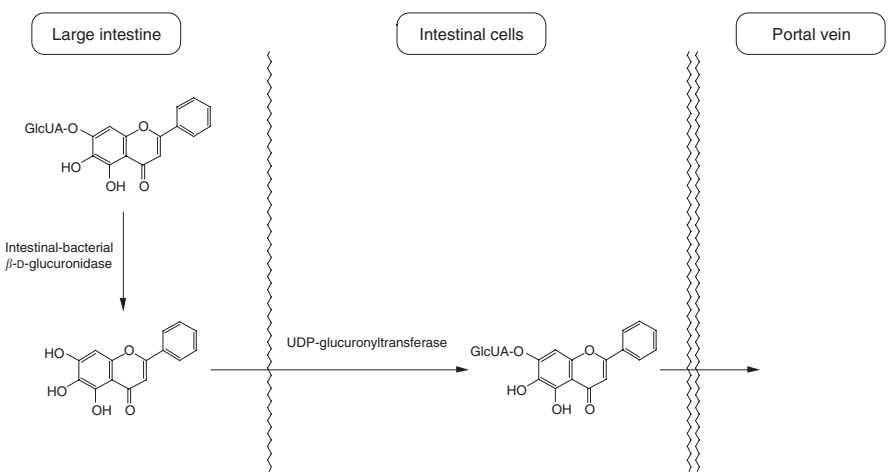

Figure 3.22 Metabolism of baicalin.

baicalein and then conjugated with glucuronic acid to form baicalin. In order to confirm this hypothesis, the same experiment was carried out using germ-free rats (Kobashi, 1998). When baicalin was administered orally to germ-free rats, baicalin was not detected in the plasma. These results indicate that baicalin is poorly absorbed from the gut, but is hydrolysed to baicalein by intestinal flora, and the resulting baicalein is absorbed. Baicalein is then conjugated with glucuronic acid to return baicalin. Since baicalin was found in the portal vein, it was supposed that baicalein was conjugated with glucuronic acid by UDP-glucuronyltransferase in the mucosa of the small intestine.

Metabolism of other ingredients which are involved in kampo-hozais

Kobashi *et al*. (1992) have done much work in this area and demonstrated the importance of intestinal flora in the metabolism of plant constituents.

Sennosides, the main ingredients of *Sennae Folium* (*Cassia angustifolia* Vahl and *C. acutifolia* Delile) and *Rhei Rhizoma* (*Rheum palmatum* L.), are considered to be inactive as laxative themselves but metabolized to active principles by intestinal flora (Ippen *et al*., 1936; Fairbairn *et al*., 1965; Sasaki *et al*., 1979; Dressen *et al*., 1981) (Figure 3.23). Kobashi *et al*. revealed the metabolic pathway of sennosides by intestinal bacteria and determined the active laxative metabolites (Kobashi *et al*., 1980; Hattori *et al*., 1982). They proposed two different pathways for the metabolism of sennosides; one is that sennosides are hydrolysed in a stepwise fashion to sennidins *via* sennidin-8-monoglucosides by bacterial β-glucosidase. The resulting sennidins are reduced to rhein anthrone, an active laxative principle (pathway I), and other is that sennosides are at first reductively cleaved to 8-glucosyl-rhein anthrone, which is subsequently hydrolysed to rhein anthrone (pathway II). They isolated a *Bifidobacterium* sp. (named *Bifidobacterium* sp. SEN) capable of hydrolysing sennosides to sennidin and *Peptostreptococcus intermedius*, which has the activity to reduce sennidins and sennosides from human faeces. Enzymatic analysis revealed that hydrolysis of sennosides to the aglycone (pathway I) may be more predominant in gut than reductive cleavage of sennosides to 8-glucosyl-rhein anthrone (pathway II) (Hattori *et al*., 1988a).

Paeoniae Radix (*Paeonia lactiflora* Pall.) has been used for treatment of abdominal pain and syndromes such as stiffness of abdominal muscles in traditional Chinese medicine. The monoterpene glucosides such as paeoniflorin, oxypaeoniflorin and benzylpaeoniflorin are isolated as

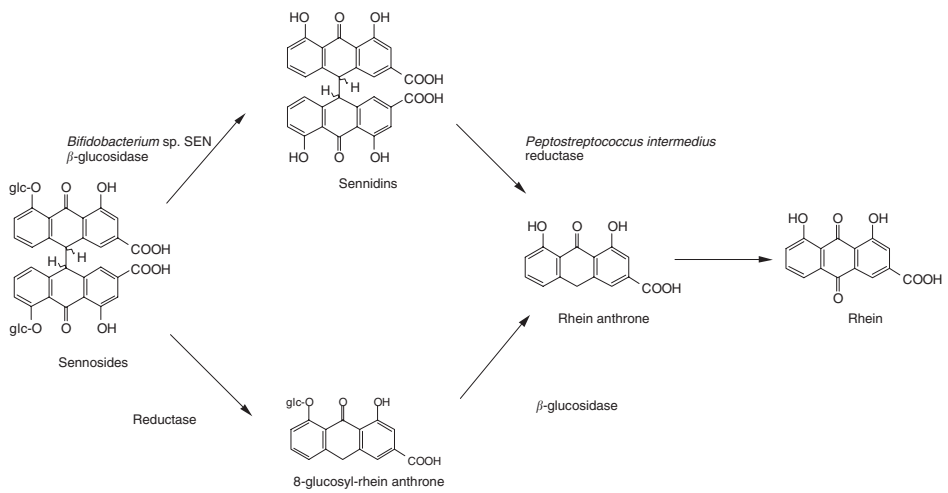

Figure 3.23 Metabolic pathway of sennosides by human intestinal bacteria.

physiologically active ingredients. By anaerobic incubation with human intestinal bacteria, paeoniflorin is transformed to at least two metabolites named paeonimetabolins I and II (Hattori *et al*., 1985b) (Figure 3.24). Paeoniflorin is first hydrolysed to glucose, benzoic acid and aglycone by membrane-bound β-glucosidase and esterase. Following ring cleavage and stereochemical new ring formation, a double bond is reduced to give an epimeric mixture of paeonimetabolins by bacterial reductase. Paeonimetabolins I and II are new compounds having two epimeric 7*R*- and 7*S*-structures. 7*S*-Paeonimetabolin I shows anti-convulsive effect that is more active than paeoniflorin on EI mice, and β-glucosidase of animal liver origins does not hydrolyse paeoniflorin, suggesting the significance of intestinal bacterial role in the metabolism.

Furthermore, they also clarified the metabolic fate of geniposide, a major iridoid glucoside in *Gardeniae Fructus* (*Gardenia jasminoides* Ellis) (Kawata *et al*., 1991) and barbaloin, a popular laxative compound from *Aloe* (*Aloe ferox* Mill.) (Hattori *et al*., 1988b; Che *et al*., 1991a,b) (Figures 3.25 and 3.26).

Lignans are one of the most important ingredients in crude drugs and their metabolism has been of interest to researchers for a long time because of the formation of mammalian lignans. Mammalian lignans such as enterolactone and enterodiol differ from the plant lignans in having phenolic hydroxy functions only in the *meta*-position of the aromatic rings and it has been proposed that they should be derived from dietary precursors, such as secoisolariciresinol and matairesinol (Setchell *et al*., 1980; 1981a,b; Borriello *et al*., 1985) (Figure 3.27). However, there is little information concerning the metabolism of the plant lignans. Arctiin and tracheloside are major ingredients of seeds of *Arctium lappa* and *Carthamus tinctorius*, which are used as a herbal medicine in Japan and China. Their structural transformation in the alimentary tract was investigated using rat gastric juice and intestinal flora (Nose *et al*., 1992). By the incubation of arctiin and tracheloside with rat gastric juice, both lignans were not converted into any metabolites. These results show that both lignans pass through stomach without structural transformation. By the incubation of arctiin and tracheloside with rat intestinal flora, the corresponding aglycones, arctigenin (arctiin metabolite 1, AM1) and trachelogenin (tracheloside metabolite 1, TM1), were obtained first. Then, the major metabolites, arctiin metabolite 2 (AM2) and tracheloside metabolite 2 (TM2), which underwent demethylation at C-3″ position of their aglycones by subsequent metabolism,

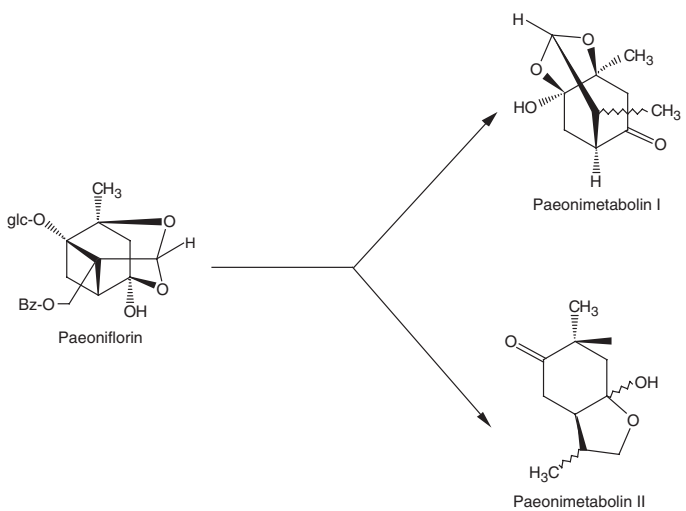

Figure 3.24 Metabolism of paeoniflorin by intestinal bacteria.

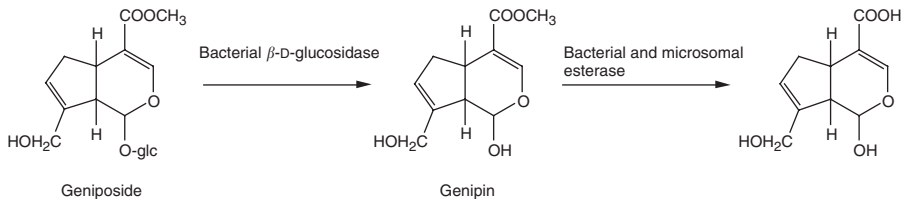

Figure 3.25 Metabolism of geniposide.

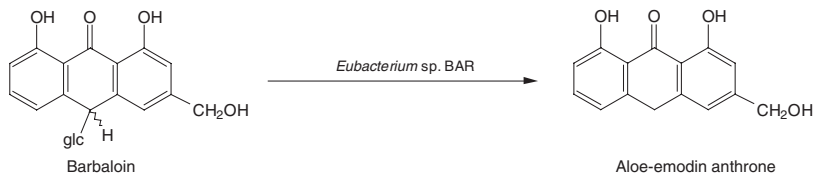

Figure 3.26 Metabolism of barbaloin.

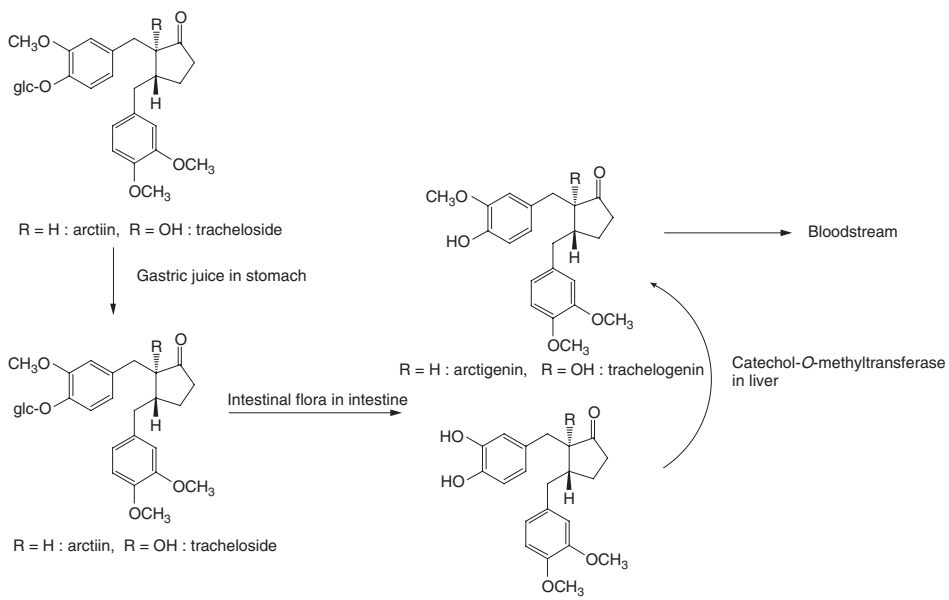

Figure 3.27 Metabolism of lignans in the alimentary tract.

increased gradually. The pattern of the formation of these metabolites between arctiin and tracheloside was almost the same, whereas TM2 was more stable than AM2 in the bacterial mixture. These results suggested that the initiation of the metabolism of arctiin and tracheloside in the gut was cleavage of the glycosidic bond and that further demethylation at C-3″ position of their aglycone occurred. Furthermore, the serum concentrations of these lignans and their metabolites was measured in rats (Nose *et al*., 1993). Arctiin or tracheloside was not detected in the serum after oral

administration (200 mg/kg), but each metabolite 1, their genuine aglycones, appeared, and they seemed to exist as conjugated forms in the serum. The serum concentration of AM1 reached its peak at 4 h and that of TM1 reached its peak at 8 h after administration. On the other hand, both metabolites 2, which possess a catechol moiety, were not detected in the serum, whereas they found in the large intestinal contents after oral administration. Since it was presumed that the metabolites 2 might have been converted into metabolites 1 through C-3″ methylation by catechol-*O*-methyltransferase (COMT) in rat liver, each metabolite 2 was incubated with rat liver cytosol in the presence of *S*-adenosyl-L-methionine. It was proved that metabolite 2 was rapidly converted into metabolite 1. Thus, it has been suggested that arctiin or tracheloside was transformed to at least two metabolites in the gastrointestinal tract, and after absorption from the gut, metabolite 2 was converted into metabolite 1 through methylation by COMT in the liver, and arctiin and tracheloside existed as metabolites 1, the genuine genin, in the blood stream. The pharmacological and biochemical activities of these lignans have been reported by many investigators. They revealed that their genuine genins, arctigenin and trachelogenin, had inhibitory effects on cyclic-AMP phosphodiesterase (Nikaido *et al*., 1981), Ca^{2+} antagonist activities (Ichikawa *et al*., 1986b), platelet activating- factor (PAF) antagonist activities (Iwakami *et al*., 1990), and inhibitory effects on the histamine release in rat mast cells (Tsuruga *et al*., 1991). Furthermore, they reported that arctiin and tracheloside did not have those activities or had only slight activities. Thus, we think that arctigenin and trachelogenin may be the active forms when arctiin or tracheloside are administered orally.

Concluding remarks

As mentioned above, most research on the structural transformation and metabolism of plant ingredients in the alimentary tract has been done with plant glycosides. Most ingested glycosides, except saikosaponins and ginsenosides, are resistant to gastric juice and other digestive enzymes and pass through the upper intestinal tract without absorption to the lower tract. Glycosides are retained in the tract and mostly hydrolysed by intestinal bacterial glycosidases to the corresponding aglycones, and some aglycones are sequentially reduced by oxidoreductases of the intestinal bacteria. Finally, metabolites are absorbed slowly and continuously to exhibit pharmacological and biological actions. Most glycosides are activated through the structural transformation in the gut, whereas some of their pharmacological actions are weakened. Thus, plant glycosides are sometimes called the natural prodrugs.

Some investigators have examined the role and/or mechanism of major ingredients in pharmacological actions of kampo-hozais using an *in vitro* assay system. In this kind of *in vitro* assay system, compounds are usually added directly to the media of cells or enzymes. Because of their complex chemical nature and metabolism, the clinically useful effects of kampo-hozais may be lost in an *in vitro* assay. Since *in vivo* experiments limit the study of the mechanism of pharmacological actions, investigations must inevitably require some *in vitro* works. Considering the metabolism of plant glycosides, the metabolites, especially corresponding aglycones, should be used in *in vitro* assay system but not glycosides themselves, in order to clarify the mechanism of their pharmacological and biological activities on the molecular basis. Otherwise, the serum that is collected from the animals administered kampo-hozai orally should be subject to an *in vitro* assay system as previously proposed (Iwama *et al*., 1987; Umeda *et al*., 1988; Amagaya *et al*., 1989).

Crude drugs IV

Pharmacological and biochemical studies of medicinal plants of Sho-saiko-to

Makoto Inoue

Introduction

Sho-saiko-to, a Kampo prescription, which is widely used for treatment of chronic hepatitis, cirrhosis and acute inflammation, consists of seven medicinal crude drugs, Bupleuri radix, Pinelliae tuber, Scutellariae radix, Ginseng radix, Zingiberis rhizoma, Zizyphi fructus and Glycyrrhizae radix. In general, the study of crude drugs of Kampo prescriptions has been performed extensively, as compared with Kampo prescriptions. That is why the necessity of the study of Kampo prescriptions as a combined medicine has not been widely noticed. Pharmacological activities of Sho-saiko-to as a Kampo prescription have been investigated as noted in another chapter. In order to better understand Kampo prescriptions, although the pharmacological and biological activities of Kampo prescriptions are not necessarily explained by those of each crude drug, it is necessary for us to learn the biological activities of each crude drug. In this chapter, important activities so far reported on each crude drug are mentioned.

Bupleurum root

Bupleurum radix, which is prepared from the roots of *Bupleurum falcatum* L. has been widely used in oriental medicines as a major component in many prescriptions for the treatment of hepatitis and renal disease for more than 1000 years (Table 3.6).

Immunomodulating activity

Saikosaponins are the main components in the root of *Bupleurum falcatum* L. and saikosaponins a, b1, b2, c and d have so far been obtained as the biologically active ingredients. *In vitro* study demonstrated that saikosaponin d (SSd) increased phagocytic activities of murine peritoneal macrophages such as spreading activity, phagocytosis, lysosomal enzyme activity and intracellular killing activity of living yeast (Ushio and Abe, 1991a). On the other hand, intramuscular injection

Table 3.6 Pharmacological and biological activities of Bupleurum root

Immunomodulating activity
Anti-inflammatory activity
Anti-nephritic activity
Hepatoprotective effect
Anti-ulcer activity
Anti-hypercholesterolemic action
Anti-platelet activity

of SSd also increased the antibody response in plaque-forming cell numbers after *in vivo* immunization with sheep red blood cells (SRBC) and an augmentation of spleen cell proliferation responses to stimulation with T- or B-cell mitogens both before and after immunization. Furthermore, after SRBC immunization, the macrophages from mice treated intramuscularly with SSd revealed significant increases in spreading activity and lysosomal enzyme activity. Intramuscular treatment of mice induced the Fc receptor (FcR) expression on macrophages and interleukin-1 (IL-1) production (Ushio and Abe, 1991b). These results demonstrate that SSd may stimulate *in vivo* immunological lymphocyte functions, partly by activating some macrophage functions (Ushio *et al*., 1991).

The immunoregulatory action of SSd was examined on splenic T lymphocytes of C57BL/6 mice (Kato *et al*., 1994). SSd displayed a definite action *in vitro* to bidirectionally control the growth response of T lymphocytes stimulated by concanavalin A, anti-CD3 monoclonal antibody (mAb), and calcium ionophore A23187 plus phorbol 12-myristate 13-acetate. Low concentrations (1–3 μg/ml) of SSd up-regulated the responses to suboptimum stimuli of agonists, particularly during the relatively late stage of the responses, whereas it down-regulated the responses to supraoptimal stimuli. Under appropriate experimental conditions, SSd promoted interleukin-2 (IL-2) production and IL-2 receptor expression. It also accelerated *c-fos* gene transcription, but it did not modulate the level of tyrosine phosphorylation of cellular proteins. These results indicate that SSd uniquely modulates the T lymphocyte function and that at least one target of the action of SSd is located at or before the step of *c-fos* gene transcription and after T-cell receptor/CD3-mediated protein tyrosine kinase activation. In addition, the immunoregulatory action of SSd was examined on murine thymocytes, compared with that on spleen cells (Kato *et al*., 1995). Constitutive DNA synthesis or the growth response stimulated with anti-CD3mAb of thymocytes were down-regulated by 3 μg/ml SSd, whereas with spleen cells these were up-regulated by the same concentration of SSd. On the other hand, 3 μg/ml of SSd greatly up-regulated the growth response and IL-2/interleukin 4 (IL-4) production, which were induced through a receptor-independent pathway by calcium ionophore A23187 plus phorbol 12-myristate 13-acetate (PMA) in thymocytes, whereas it only slightly up-regulated them in spleen cells. Moreover, the same concentration of SSd inhibited DNA fragmentation in thymocytes induced by A23187 or PMA. These results suggest a unique cell type-dependent immuno-modulatory action of SSd.

The FcR on macrophages plays an important role in the host defence system; clearance of immune complexes, antibody-dependent cell-mediated cytotoxicity, tumouricidal action and prostaglandin release. An acidic pectic polysaccharide, bupleuran 2IIb, has a molecular weight of 23 000 and consists of a highly methyl esterified and a less esterified galacturonan region and a rhamnogalacturonan core, which is substituted by neutral carbohydrate side chains such as galactan, arabinogalactan or arabinopyranan. It increased glucose oxidase-anti-glucose oxidase complexes (GAG) binding to macrophages in a dose-dependent manner (Matusmoto *et al*., 1993). Scatchard analysis indicated enhanced expression of the FcR on the surface and bupleuran-2IIb stimulated the expression of both FcRI and FcRII mRNAs. These results suggested that the endo-polygalacturonase-resistant carbohydrate portion of bupleuran 2IIb is important for the expression of the activity, and that the activity of bupleuran 2IIb on GAG binding was mediated by receptors for polysaccharide on the cells. The up-regulation of the FcR by bupleuran 2IIb was also suggested to mediate by *de novo* synthesis of the receptor protein. In addition, the study on the mechanism by which bupleuram 2IIb induced FcR revealed that bupleuram 2IIb enhanced FcR expression on macrophages, depending on an increase in intracellular Ca^{2+}, followed by activation of the calmodulin (Matsumoto and Yamada, 1995). These results suggest that the acidic pectic polysaccharides from *Bupleurum falcatum* L. may be a part of the active principles of Kampo prescriptions for immune-complexes-mediated autoimmune diseases.

Anti-inflammatory activity

Anti-inflammatory action of saikosaponins was examined by using female albino rats (Yamamoto *et al*., 1975a). The anti-exudative action shown by granuloma pouch method and the anti-granulomatous action shown by cotton pellet method were demonstrated with intramuscular and oral administrations of saikosaponins. The oral administration of saikosaponins in ten times the dosage of intramuscular injection showed almost the same effect. Among saikosaponins isolated from *Bupleurum falcatum* L., saikosaponins a and d, not c, were demonstrated to have anti-inflammatory action. Saikosaponin a, d and their metabolites were reported to increase the plasma adrenocorticotropin and corticosterone levels when given intraperitoneally (i.p.) (Hiai *et al*., 1981; Nose *et al*., 1989) and to show anti-inflammatory effect.

Anti-nephritic activity

Administration of the amino nucleoside of puromycin to rats results in the symptoms of proteinuria, hypoproteinaemia and hypercholesterolaemia resembling the changes observed in the nephrotic syndrome that occurs spontaneously in humans (Frenk *et al*., 1955). When the effects of SSd on aminonucleoside nephrosis were studied in rats, intramuscular administration of SSd was found to prevent the development of proteinuria induced by aminonucleoside in the rat (Abe *et al*., 1986). Urine protein excretion in rats receiving aminonucleoside alone was significantly elevated on the 2nd day after the last injection of aminonucleoside and reached a peak on the 11th day. Urinary protein on the 11th day was reduced by 48% and 46%, respectively, in animals treated with SSd from the 2nd and 8th day after the last injection of aminonucleoside. Electron microscopically, the degree of abnormality, for example, loss or fusion of foot processes, in the glomerular epithelial cells was significantly lower in the rats treated with SSd after aminonucleoside injection than in the rats treated with aminonucleoside alone. In this model, since glucocorticoids have been reported to be ineffective, SSd was considered to prevent proteinuria in the nephrotic syndrome by a different mechanism from that of glucocorticoids. Hattori *et al*. also reported that i.p. administration of crude saikosaponin at 1.0 mg and 5.0 mg/kg prevented urinary protein excretion and elevation of serum cholesterol content on the 10th day after the injection of anti-GBM serum and that saikosaponin a (5.0 mg/kg, i.p.) and d (1.0 mg and 5.0 mg/kg, i.p.) also prevented urinary protein excretion, elevation of serum cholesterol content, and histopathological changes (Hattori *et al*., 1991). A detailed study of the mechanism revealed that crude saikosaponin and SSd significantly inhibited the increase in platelet aggregation, and SSd enhanced the serum and intra-adrenal corticosterone levels. In addition, crude saikosaponin and saikosaponin a inhibited the decrease in activity of scavengers (superoxide dismutase (SOD), catalase, glutathione peroxidase). These results indicated that saikosaponins showed an anti-nephritic effect, which was partly due to anti-platelet, corticosterone releasing and enhancing action on the activity of reactive-oxygen-species scavengers. Accordingly, saikosaponins, especially saikosaponin d and a, are expected to prevent proteinuria in the nephrotic syndrome.

Hepatoprotective effect

Saikosaponin d protected against experimental hepatitis induced by D-galactosamine or carbon tetrachloride (CCl_4). Pre-treatment with SSd greatly decreased the sensitivity to the hepatotoxicity of D-galactosamine (Abe *et al*., 1980). In addition, pre-treatment with SSd produced a remarkable inhibitory action on acute hepatic injury by CCl_4. A significant inhibition of lipid peroxidation induced by an acute dose of CCl_4 in the liver of rats pre-treated with SSd was also noted. Continuous injection of CCl_4 caused liver cirrhosis in rats but the severity of cirrhosis was reduced

in rats treated simultaneously with CCl_4 and SSd (Abe *et al*., 1982). Protective effects are observed in two types of intoxications which have different induction mechanisms of liver injury.

Anti-ulcer activity

Sun *et al*. studied effects of an acidic polysaccharide fraction, BR-2, from the roots of *Bupleurum falcatum* L., on HCl-ethanol, ethanol and water immersion stress-induced gastric lesions in mice and pylorus-ligated ulcers in rats (Sun *et al*., 1991). Oral administration of BR-2 at doses of 50–200 mg/kg inhibited the formation of the gastric lesions induced by necrotizing agents such as HCl-ethanol and ethanol in a dose-dependent manner. This protective effect was observed after oral, i.p. and subcutaneous administration of BR-2 (25–100 mg/kg). BR-2 also inhibited the formation of gastric ulcers which were induced by water immersion stress or pylorus-ligation. BR-2 is consistently active in various experimental ulcer animal models and may be useful to treat peptic ulcer in man. Oxygen radicals derived from neutrophils play an important role in the pathogenesis of gastric mucosal lesions induced by HCl/ethanol. Anti-ulcer pectic polysaccharide, bupleuran 2IIc, which was recently isolated from the roots of *Bupleurum falcatum* L., showed potent inhibition of HCl/ethanol-induced gastric lesions in mice (Matsumoto *et al*., 1993). Bupleuran 2IIc is an effective scavenger of hydroxyl radical generated by Fenton reaction, as certain carbohydrates and related compounds are known to scavenge hydroxyl radicals. Taken together, it is suggested that the oxygen radical-scavenging activity seems to be the most likely mode of action of the anti-ulcer polysaccharides, in addition to the protective coating effect.

Anti-hypercholesterolemic action

Elevation of plasma levels of cholesterol, triglycerides and phospholipids by cholesterol feeding was reduced by saikosaponins. Although hepatic lipogenesis and cholesterogenesis from acetate-1-^{14}C of glucose-^{14}C(U) were stimulated, the elimination of i.p. injected cholesterol-4-^{14}C from plasma was accelerated by saikosaponins treatment. Faecal excretion of i.p. injected cholesterol-4-^{14}C, expressed as total-^{14}C including bile acids-^{14}C and neutral sterols-^{14}C, was increased. These results suggested that the anti-hypercholesterolemic effect shown by saikosaponins was in part due to the increase of excretion of cholesterol as bile acids (Yamamoto *et al*., 1975b).

Anti-platelet activity

Chang and Hsu studied the effects of saikosaponins on platelet aggregation, platelet thromboxane biosynthesis and H_2O_2-induced endothelial cell injury (Chang and Hsu, 1991). Among three saikosaponins, saikosaponin a, c and d, saikosaponin a significantly inhibited human platelet aggregation elicited by adenosine diphosphate (ADP), and the potency of inhibition was comparable with aspirin. Saikosaponin a dose-dependently inhibited the platelet thromboxane formation from exogenous and endogenous arachidonic acid. The inhibitory effect of saikosaponin a on platelet activation could explain the possible role of saikosaponins in maintaining vascular homoeostasis.

Ginger

Zingiberis Rhizoma (ginger), which is prepared from the rhizome of *Zingiber officinale* ROSCOE, is one of the best known crude drugs and it is prescribed in Chinese and Japanese traditional medicines used for treatment of headache, nausea, stomach-ache and colds. In addition, ginger is consumed world-wide as a spice and a flavouring agent. In this chapter, we discuss its pharmacological and biological activities (described in Table 3.7).

Table 3.7 Pharmacological and biological activities of ginger

Anti-emetic activity
Anti-inflammatory activity
Anti-thrombotic activity
Anti-hypercholesterolemic action
Anti-tumour activity
Anti-ulcer activity
Anti-oxidative activity
Cardiotonic activity
Analgesic effect
Stimulatory effect on digestion
Cytocidal effect on Anisakis
Suppression of headache

Anti-emetic activity

Nausea and vomiting are a common and sometimes severe complication of anaesthesia and surgery, in addition to a common complaint of early pregnancy. Most of the currently available anti-emetics act as central dopaminergic receptors and may produce extrapyramidal side effects. Any sedative effects are particularly undesirable in day-case patients. The powder rhizoma of *Zingiber officinale*, has been a traditional remedy for gastrointestinal complaints. It is generally regarded as an excellent carminative and intestinal spasmolytic. Phillips reported that the prophylactic use of powdered ginger root reduced the incidence of post-operative nausea and vomiting without toxic effects (Phillips *et al*., 1993). The incidence of post-operative nausea or vomiting in the placebo group was 41%, similar to that reported elsewhere. The oral administration of 1 g of *Zingiber officinale* reduced this incidence by 50% and appears to be as effective as metoclopramide 10 mg when given by mouth 1 h before anaesthesia. In addition, the need for post-operative anti-emetics was significantly reduced by the administration of ginger but not metoclopramide. Besides, Bone *et al*. reported that there were statistically, significantly fewer recorded incidences of nausea in 60 women who had major gynaecological surgery and who received ginger root compared with a placebo (Bone *et al*., 1990). Fischer *et al*. found that powdered root of ginger in daily doses of 1 g during 4 days was better than the placebo in dimin-ishing or eliminating the symptoms of hyperremesis gravidarum (Fischer *et al*., 1991). Sharma reported that acetone and 50% ethanolic extract at the doses of 25, 50, 100 and 200 mg/kg p.o. exhibited significant protection against cisplatin emesis, while aqueous extract at these doses was ineffective (Sharma *et al*., 1997). Additionally, Mowrey showed that Zingiber officinale was superior to dimenhydrinate in reducing motion sickness (Mowrey and Clayson, 1982). In contrast, there are several reports that powdered ginseng is not effective for motion sickness (Stewart *et al*., 1991), post-operative nausea and vomiting (Arfeen *et al*., 1995), and post-operative nausea and vomiting after day-case gynaecological laparoscopy (Visalyaputra *et al*., 1998). Consequently, the effect of ginseng on nausea and vomiting induced by various causes is controversial and should be evaluated in more detail.

Anti-inflammatory activity

Mycobacterial adjuvant inflammation of the paw is an accepted and well-established experimental model for prediciting the clinical value of anti-inflammatory and anti-rheumatic drugs. Severe arthritis was induced in the right knee and right paw of male Sprague–Dawley rats by injecting 0.05 ml of a fine suspension of dead Mycobacterium tuberculosis bacilli in liquid paraffin, and the effect of oral administration of ginger oil (33 mg/kg) on this model was studied. Ginger oil,

given orally for 26 days, caused a significant suppression of both paw and joint swelling, suggesting that ginger oil has potent anti-inflammatory and anti-rheumatic activities (Sharma *et al*., 1994). Srivastava reported that powdered ginger was effective for the patient with rheumatoid arthritis, osteoarthritis and muscular discomfort (Srivastava *et al*., 1992). In all 56 patients (28 with rheumatoid arthritis, 18 with osteoarthritis and 10 with muscular discomfort) used powdered ginger against their afflictions. Amongst the arthritis patients more than three-quarters experienced, to varying degrees, relief in pain and swelling. All the patients with muscular discomfort experienced relief in pain. None of the patients reported adverse effects during the period of ginger consumption which ranged from 3 months to 2.5 years. It is suggested that at least one of the mechanisms by which ginger shows its ameliorative effects could be related to inhibition of prostaglandin and leukotriene biosynthesis; that is, it works as a dual inhibitor of eicosanoid biosynthesis. In fact, a hot aqueous extract of ginger was highly inhibitory against prostaglandin synthetase and gingerols and diarylhepatanoids were identified as active compounds in *in vitro* assay (Kiuchi *et al*., 1982, 1992). IC_{50}s of [6]-gingerol against prostaglandin synthetase and 5-lipoxygenase were 4.6 and 3.0 μM, respectively. IC_{50} of [6]-Shogaol against prostaglandin synthetase was 1.6 μM. In addition, [6]-Shogaol inhibited carrageenin-induced swelling of the hind paw in rats and arachidonic acid-induced platelet aggregation in rabbits (Suekawa *et al*., 1986).

Anti-thrombotic activity

Ginger extract inhibited platelet aggregation induced by ADP, collagen, epinephrine and arachidonate; it did not inhibit Ca^{2+} ionophore A23187-induced aggregation. It reduced the formation of thromboxane and prostaglandin in platelets (Srivastava, 1984). A lot of evidence based on *in vitro* study has been accumulated to date. However, ginger has been claimed to exert an anti-thrombotic effect in humans as ginger extracts inhibit cyclo-oxygenase activity of platelets *in vitro*. Verma reported that, in volunteers supplemented with 100 g of butter for 7 days, platelet aggregation was found to enhance to a significant extent ($P < 0.001$), and that addition of 5 g of dry ginger in two divided doses with a fatty meal (in 10 individuals) significantly ($P < 0.001$) inhibited the platelet aggregation induced by ADP and epinephrine (Verma *et al*., 1993). Bordia *et al*. represented that in patients with coronary artery disease (CAD) administration of powdered ginger at a dose of 4 g daily for 3 months did not affect ADP- and epinephrine-induced platelet aggregation and also the fibrinolytic activity and fibrinogen level. However, a single administration of 10 g of powdered ginger to CAD patients produced a significant reduction in platelet aggregation induced by ADP and epinephrine (Bordia *et al*., 1996). In contrast, Janssen concluded that the putative anti-thrombotic activity of ginger in humans cannot be confirmed (Janssen *et al*., 1996). Lumb found that in a randomized double-blind study on eight healthy male volunteers, the intake of 2 g of dried ginger did not affect bleeding time, platelet count, thromboelastography and whole blood platelet aggregometry, although larger (clinically impractical) doses may do so (Lumb, 1994). In these studies, the doses used in the studies and the duration of administration were different and it remains to evaluate the potential of an anti-thrombotic agent.

Anti-hypercholesterolemic action

The effect of ginger on serum lipids has not been reported sufficiently. Bhandari studied the effects of ethanolic extract of ginger (200 mg/kg, p.o.) in cholesterol-fed rabbits (Bhandari *et al*., 1998). The marked rise in serum and tissue cholesterol, serum triglycerides, serum lipoproteins and phospholipids that followed 10 weeks of cholesterol feeding was significantly reduced by the ethanolic ginger extract and results were compared with gemfibrozil, a standard orally effective

hypolipidemic drug. The severity of aortic atherosclerosis as judged by gross grading was more marked in pathogenic (i.e. the hypercholesterolmic group), while animals receiving ginger extract along with cholesterol showed a lower degree of atherosclerosis. The results indicate that ginger is definitely an anti-hyperlipidemic agent. When the effect of ginger on normal rats was examined, a significant decrease in blood glucose, serum total cholesterol was found. In addition, HDL-cholesterol, VLDL-cholesterol and atherogenic index were significantly decreased (Ahmed and Sharma, 1997). The acetone extracts of ginger, which contain essential oils and pungent principles, caused an increase in bile secretion (Yamahara *et al*., 1985). [6]-Gingerol and [10]-gingerol were mainly responsible for the cholagogic effect of ginger. The mechanism by which ginger shows anti-hypercholesterolemic action was fully understood; however, the activity of hepatic cholesterol 7a-hydroxylase, the rate-limiting enzyme of bile acid synthesis, was elevated in ginger-treated rats, suggesting this is a reason why ginger enhanced the excretion of cholesterol as bile acid from liver to result in the decrease of serum cholesterol (Srinivasan and Sambaiah, 1991).

Anti-tumour activity

Detection and evaluation of plant components as chemical mutagens have been actively conducted and a number of the components have been shown to be potently mutagenic and some of them to be carcinogenic to higher animals. Nakamura and Yamamoto found that rhizome juice of ginger, *Zingiber officinale*, contained both accelerative and suppressive factor(s) to mutagenesis in bacteria (Nakamura and Yamamoto, 1982). In addition, when ginger extract and its constituents (gingerol, shogaol and zingerone) were tested in *Salmonella typhimurium* strains TA 100, TA 98, TA 1535 and TA 1538 in the presence and in absence of S9 mix (Nagabhushan *et al*., 1987), it was observed that ginger extract, gingerol and shogaol were mutagenic in metabolic activation in strains TA 100 and TA 1535, but zingerone was non-mutagenic in all the four strains with or without S9 mix. When mutagenicity of gingerol and shogaol was tested in presence of different concentrations of zingerone, it was observed that zingerone suppressed mutagenic activity in both the compounds in a dose-dependent manner.

There is considerable emphasis on identifying potential chemopreventive agents present in plants. The effect of ethanol extract of ginger on tumour promotion was therefore studied in a mouse skin tumourigenesis model (Katiyar *et al*., 1996). Skin tumour promoters are known to induce epidermal ornithine decarboxylase (ODC), cyclo-oxygenase, and lipoxygenase activities and oedema and hyperplasia are conventionally used markers of skin tumour promotion. When the effect of ethanol extract of ginger on these parameters was assessed, pre-application of ethanol extract of ginger onto the skin of SENCAR mice resulted in significant inhibition of 12-0-tetradecanoylphorbol-13-acetate (TPA)-caused induction of epidermal ODC, cyclo-oxygenase, and lipoxygenase activities and ODC mRNA expression in a does-dependent manner. Pre-application of ethanol extract of ginger to mouse skin also afforded significant inhibition of TPA-caused epidermal oedema (56%) and hyperplasia (44%). In long-term tumour studies, topical application of ethanol extract of ginger 30 min prior to that of each TPA application to 7,12-dimethylbenz(a)anthracene-initiated SENCAR mice resulted in highly significant protection against skin tumour incidence and its subsequent multiplicity. The animals pre-treated with ethanol extract of ginger showed substantially lower tumour body burdens compared with controls. A two-stage mouse skin carcinogenesis model was used to determine the anti-tumour promotional activity of [6]-gingerol, a major pungent principle of ginger (Park *et al*., 1998). Topical application of [6]-gingerol onto shaven backs of female ICR mice prior to each topical dose of TPA significantly inhibited 7,12-dimethylbenz[a]anthracene-induced skin papillomagenesis. The compound also suppressed TPA-induced epidermal ODC activity and inflammation.

These studies provide useful basic understanding of the compounds present in ginger useful for chemoprevention in addition to promoting the study on chemopreventive agents.

Anti-ulcer activity

Yamahara *et al*. examined the effects of ginger on HCl/ethanol-induced gastric lesions in rats (Yamahara *et al*., 1988). The orally administered acetone extract at a dose of 1000 mg/kg and zingiberene, the main terpenoid from acetone extract, at a dose of 100 mg/kg significantly inhibited gastric lesions by 97.5% and 53.6%, respectively. 6-Gingerol, the pungent principle, at 100 mg/kg significantly inhibited gastric lesions by 54.5%. These results suggest that zingiberene, the terpenoid and 6-gingerol are important constituents in stomachic medications containing ginger. In addition, they further isolated anti-ulcer constituents such as 6-gingesulfonic acid, gingerglycolipids A, B, C, β-sesquiphellandrene, β-bisabolene, ar-curcumene and 6-shogaol (Yamahara *et al.*, 1992; Yoshikawa *et al*., 1994). Among them, 6-gingesulfonic acid exhibited weaker pungency and more potent anti-ulcer activity than any other constituents.

Anti-oxidative activity

Zingerone, an ingredient of ginger, inhibited ascorbate/Fe^{2+}-induced lipid peroxidation in rat liver microsome at high concentration ($>$150 mM) and nitrobluetetrazolium reduction in xanthine–xanthine oxidase to a maximum of 23% (Reddy and Lokesh, 1992; Krishnakantha and Lokesh, 1993).

Cardiotonic activity

Over the recent years there have been numerous efforts to find and develop novel cardiotonic agents that are more effective and more selective than cardiac glycosides or catecholamines. In the course of search for agents, Kobayashi *et al*. found that gingerol activates the Ca^{2+}-ATPase activity of skeletal muscle sarcoplasmic reticulum (SR) (Kobayashi *et al*., 1987). Gingerol stimulated the Ca^{2+}-pumping activity of fragmented SR prepared from rabbit skeletal and dog cardiac muscles. The extravesicular Ca^{2+} concentrations of the heavy fraction of the fragmented SR (HSR) were measured directly with a Ca^{2+} electrode to examine the effect of gingerol on the SR. Gingerol (3–30 μM) accelerated the Ca^{2+}-pumping rate of skeletal and cardiac SR in a concentration-dependent manner. The rate of $^{45}Ca^{2+}$ uptake of HSR was also increased markedly by 30 μM gingerol without affecting the $^{45}Ca^{2+}$ efflux from HSR. Furthermore, gingerol activated Ca^{2+}-ATPase activities of skeletal and cardiac SR (EC50, 4 μM). The activation of SR Ca^{2+}-ATPase activity by gingerol (30 μM) was completely reversed by 100-fold dilution with the fresh saline solution. Kinetic analysis of activating effects of gingerol suggests that the activation of SR Ca^{2+}-ATPase is uncompetitive and competitive with respect to Mg^{2+}, ATP at concentrations of 0.2–0.5 mM, and above 1 mM, respectively. Kinetic analysis also suggests that the activation by gingerol is mixed-type with respect to free Ca^{2+} and this enzyme is activated probably due to the acceleration of enzyme–substrate complex breakdown. Gingerol had no significant effect on sarcolemmal Ca^{2+}-ATPase, myosin Ca^{2+}-ATPase, actin-activated myosin ATPase, and cAMP-phosphodiesterase activities, indicating that the effect of gingerol is rather specific to SR Ca^{2+}-ATPase activity. In addition, [8]-gingerol was found to produce a concentration-dependent positive inotropic effect on the guinea-pig isolated left atria at concentrations of 1×10^{-6} to 3×10^{-5} M (Kobayashi *et al*., 1988). Gingerol also exhibited positive inotropic and chronotropic effects on the guinea-pig right atria. The gingerol-induced inotropic effect was abolished by ryanodine, but was little affected by propranolol, chlorpheniramine, cimetidine, tetrodotoxin, diltiazem or reserpine. The time to peak tension and relaxation time within

a single contraction were shortened by gingerol (1×10^{-5} M) as well as isoproterenol, whereas they were prolonged by BAY K 8644. In guinea-pig isolated atrial cells, gingerol (3×10^{-6} M) caused an increase in the degree and the rate of longitudinal contractions. In guinea-pig left atria, gingerol (1×10^{-6} to 3×10^{-5} M) had little influence on the action potential, although it increased the contractile force of the atria. Whole-cell patch-clamp experiments showed that the slow inward current was little affected by gingerol (1×10^{-6} to 3×10^{-5} M) in voltage-clamped guinea-pig cardiac myocytes. The measurement of extravesicular Ca^{2+} concentration using a Ca^{2+} electrode indicated that gingerol (3×10^{-6} to 3×10^{-5} M) accelerated the Ca^{2+} uptake of fragmented SR prepared from canine cardiac muscle in a concentration-dependent manner. Gingerol may provide a valuable chemical tool for studies aimed at clarifying the regulatory mechanisms of SR Ca^{2+}-pumping systems and the causal relationship between the Ca^{2+}-pumping activity of SR and muscle contractility. These results show that gingerol is a novel leading compound for potent cardiotonic drug.

Analgestic effect

As other characteristic activities, ginger shows analgestic action, stimulation of digestion, killing activity of Anisakis and suppression of headache.

In the study of analgestic action, [6]-shogaol was found to share the sites of action with capsaicin on the terminals of substance-P-containing primary afferents and inhibit the release of substance-P, by subsequent stimulation of the primary afferents. The latter action of [6]-shogaol might be relevant to its analgestic effect.

Stimulatory effect on digestion

Dietary ginger prominantly enhanced intestinal lipase activity and also the disaccharidases sucrase and maltase. The positive influences of a good number of spices on these terminal enzymes of digestive process could be an additional feature of species that are generally well recognized to stimulate digestion (Platel and Srinivasan, 1996).

Cytocidal effect on Anisakis

An extract of *Zingiber officinale* is traditionally eaten with raw fish in Japan. The wisdom of ancient people, that ginger and raw fish are eaten together, is proven to be effective for avoiding intake of parasites. [6]-Shogaol and [6]-gingerol were found to be the most prevalent components to kill Anisakis larvae at a minimal effective dose of 62.5 and 250 μg/ml, respectively (Goto *et al*., 1990).

Suppression of headache

Migraines are considered a neurological disorder with little convincing evidence of the involvement of some vascular phenomenon. Recent understanding of the mechanisms behind migraine pain generation and perception have considerably helped the development of modern migraine drugs. Most migraine drugs in use (i.e. ergotamine and dihydroergotamine, iprazochrome, pizotifen and diazepam) and non-steroidal anti-inflammatory drugs (i.e. aspirin, paracetamol, persantin, etc.) have side-effects and are prescribed with caution for a limited duration. Ginger is reported in the Ayurvedic and Tibb systems of medicine to be useful in neurological disorders. It is proposed that administration of ginger may exert abortive and prophylactic effects in migraine headache without any side-effects (Mustafa and Srivastava, 1990).

Ginseng

Panax ginseng C. A. Meyer has long been used as a tonic in traditional Chinese medicine. At present, its medicinal utilization has been adequately explored and extended not only to China and Korea but also Japan, European countries, and the United States. The studies of ginseng in botany, phytochemistry, pharmacology and clinical application are advanced most among crude drugs. Some review papers summarize the achievements of ginseng research, and Liu summarized some recent achievements from some of the published papers in the studies on ginseng research from 1979 to 1990 in China (Liu *et al*., 1992). So this chapter focuses on the research from 1991 to date and summarizes them according to the order of activities listed in Table 3.6.

Immunomodulating activity

Ginseng administration to mice induces increases in various parameters of the immune functions, such as number of plaque-forming cells to specific antigens, specific antibody to antigen, number of antigen-reactive T cells, natural killer activity and phagocytes (Singh *et al*., 1984; Park *et al*., 1988; Kim *et al*., 1990). In addition, oral administration of hot-water extract from wild *Panax ginseng* to mice increased the percentages of Thy 1.2-, L3T4- and Lyt2-positive cell population as compared to control by 31.2%, 17.9% and 30.1%, respectively (Mizuno *et al*., 1994). Cyclophosphamide (CY) treatment markedly decreased the number of peritoneal macrophages and their chemotactic activity. An extract of ginseng radix showed protective effects on the number and functions of macrophages by treatment with CY but did not show any effects on the lymphocytic blastogenesis (Jin *et al*., 1994). Thus, the beneficial effects of ginseng seem to have an influence on the systems involved in the homoeostasis of the organisms, such as the immune system (Table 3.8).

Then, when ginseng extract was applied orally to young healthy men, there were no significant changes in peripheral blood leucocyte counts and lymphocyte subsets (Srisurapanon *et al*., 1997), whereas chemotaxis and phagocytosis indices of polymorphonuclear leucocytes were enhanced significantly (Scaglione *et al*., 1990). These results may suggest a possibility that pharmacological activities are different among animal species used in these experiments and more extensive studies are required. The activities of a number of components of *Panax ginseng* have also been reported. According to those reports, ginsenoside Rg1 and Rb1 are the most pharmacologically active saponins even though they are found only in trace amounts. Ginsenoside Rg1 increased the number of T-helper cells with respect to the whole T-cell number and the splenocyte NK activity induced an augmentation of the production of IL-1 by macrophages, and

Table 3.8 Pharmacological and biological activities of ginseng

Immunomodulating activity
Improvement of learning and memory
Anti-ageing effect
Anti-diabetic activity
Anti-atherosclerotic activity
Anti-cancer activity
Inhibitiory activity of ion channel
A functional ligand of glucocorticoid receptor
Anti-platelet activity
PAF antagonist
Hepatoprotective effect
Anti-ulcer activity

exerted a direct mitogenic effect on microcultured thymus cells, and partly restored the impaired immune reactivity by cyclophosphamide treatment (Kenarova *et al*., 1990). In addition, ginsenoside Rg1 was found to enhance the expression of IL-2 receptor α chain and inhibit the release of soluble IL-2 receptor. In *in vitro* experiment, Rg1 showed no influence on Con A-induced increase of cytoplasmic-free calcium concentration, but significantly increased the levels of intracellular cAMP and cGMP in aged animals. In view of the important role of cAMP and cGMP as second messengers in the regulation of immune system, the results of the present studies suggest that one of the mechanisms by which Rg1 enhances immune function in old rats might be mediated by increase of cAMP and cGMP contents, resulting in IL-2 gene expression and splenocyte proliferation (Liu and Zhang, 1996). The proliferation of splenic lymphocytes, the humoral immune response to SRBCs, and the phagocytotic function of i.p. macrophages were all suppressed by cold water (4°C) swim stress (CWSS) for 5 min in rats and for 3 min in mice. Meanwhile, the levels of serum corticosterone increased. Ginsenoside Rb1 10 mg/kg i.p. or i.g. completely antagonized the immunosuppression induced by CWSS, and inhibited the increase of serum corticosterone in CWSS rats, but increased the level of serum corticosterone further in CWSS mice (Luo *et al*., 1993). Several acidic polysaccharides, which were isolated from the root of *Panax ginseng*, were found to have immunological activities. Ginsenan PA and PB showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test, pronounced anti-complementary activity and alkaline phosphatase-inducing activity (Tomoda *et al*., 1993). Ginsenan S-IIA was found to be a potent inducer of IL-8 production by human monocytes and THP-1 cells, and this induction is accompanied by increased IL-8 mRNA expression (Sonoda *et al*., 1998). In addition, ginsan induces the expression of mRNA for IL-2, interferon-g, IL-1a, and GM-CSF, resulting in LAK cell induction to lead to its effectiveness in the immunoprevention and immunotherapy of cancer (Kim *et al*., 1998).

Improvement of learning and memory

One-week administration of ginseng at 200 mg/kg/day before the occlusion produced an improvement of local cerebral glucose utilization, as compared with the untreated group. This result indicates that ginseng has some protective effects against brain damage in transient global cerebral ischaemia (Choi *et al*., 1996). In addition, oral administration of red ginseng powder significantly prevented the ischaemia-induced decrease in response latency, as determined by the passive avoidance test, and rescued a significant number of ischaemic hippocampal CA1 pyramidal neurones in a dose-dependent manner. In the same experiment, ginsenoside Rb1 significantly prolonged the response latency of ischaemia gerbils and rescued a significant number of ischaemic CA1 pyramidal neurones, whereas ginsenoside Rg1 and Ro were ineffective. These findings suggest that red ginseng powder is effective in the prevention of delayed neuronal death, and that ginsenoside Rb1 is one of the neuroprotective molecules within ginseng root (Wen *et al*., 1996). The central infusion of ginsenoside Rb1 after forebrain ischaemia protects hippocampal CA1 neurone against lethal ischaemic damage possibly by scavenging free radicals which are overproduced *in situ* after brain ischaemia and reperfusion (Lim *et al*., 1997). In addition, Rb1 and Rb3 were found to significantly attenuate glutamate-induced neurotoxicity and their effects were partly due to the protection of neurone from oxidative damage which is produced by exposure to excess glutamate. The protective effect might be derived from the inhibition of the overproduction of nitric oxide, the maintenance of superoxide dismutase levels, and the inhibition of lipid peroxidation (Kim *et al*., 1998).

Ginseng ethanol extract and saponins possess a beneficial effect regarding spatial cognitive impairment, evidenced by the results in radial maze performance and one-way avoidance in rats

(Ma *et al*., 1991; Nitta *et al*., 1995). Ginsenoside Rb1 is able to prevent the memory deficits induced by a cholinergic agent (scoplamine) in rats. When its mechanism was studied, ginsenoside Rb1 facilitated the release of acetylcholine from hippocampal slices, which is associated with an increased uptake of choline into nerve endings; however, calcium influx is unaltered (Benishin *et al*., 1991). Analysis of choline uptake kinetics indicated that Rb1 increases the maximum velocity of choline uptake, while the affinity of the choline uptake carriere for choline (Km) was not significantly altered. Three-day administration of Rb1 also increases the number of choline uptake sites in the hippocampus and to a lesser extent in the cortex (Benishin, 1992). *In situ* hybridization studies show that Rb1 increases the expression of choline acetyltransferase and trkA mRNAs in the basal forebrain and nerve growth factor mRNA in the hippocampus (Salim *et al*., 1997).

Ginseng saponins are known to have various pharmacological actions on the central nervous system. When the effects of ginsenoside Rb1 and malonylginsenoside Rb1 were studied on the induction of long-term potentiation (LTP) in the dentate gyrus using anaesthetized rats, injection (i.c.v.) of ginsenoside Rb1 did not affect the basal synaptic responses evoked by low-frequency test stimulation, but significantly attenuated the magnitude of LTP induced by strong tetanus (100 pulses at 100 Hz). The inhibitory effect of ginsenoside Rb1 depended on its doses (0.5–50 nmol, i.c.v.). On the other hand, malonylginsenoside Rb1 did not affect the LTP induced by the strong tetanus, but facilitated the generation of LTP by the weak tetanus (20 pulses at 60 Hz) that produced only short-lasting potentiation in normal conditions. The LTP-facilitating effect of ginsenoside malonylginsenoside Rb1 was seen maximally at a dose of 5 nmol (i.c.v.) and diminished at a higher dose (50 nmol, i.c.v.). Since another ginseng saponin ginsenoside Rg1 did not affect the induction of LTP at all, the inhibition and facilitation of LTP induction are probably specific actions of ginsenoside Rb1 and malonylginsenoside Rb1, respectively. These results indicate that ginseng saponins affect the activity-dependent synaptic plasticity in the brain (Abe *et al*., 1994).

Anti-ageing effect

Ageing is known to be a declining process associated with dysfunction of neuro-endocrino-immuno-system network. Functional changes of the central-nervous-system neurotransmitter systems by ageing bring about memory loss. In animal models, aged rats showed significantly impaired learning performance in the radial maze and operant discrimination tasks. Daily administration of ginseng extract (8 g/kg/day, p.o. for 12–33 days) ameliorated the impairment of learning performance in the radial maze task but not in the operant discrimination task, thus suggesting that subchronic treatment with ginseng extract improves spatial cognitive impairment in aged rats (Nitta *et al*., 1995). In another study, oral intake of a 1.8% water extract of *Panax ginseng* for 4 weeks produced an increase in spontaneous motor activity during the dark period in old rats, while it caused a decrease in the activity in young rats (Watanabe *et al*., 1991). These results suggest that subchronic intake of ginseng extract inhibits the activity of nigrostriatal dopamine neurones in the daytime and activates spontaneous motor activity during the dark period in old rats, while it produces opposite effects in young rats. Hong found atrial natriuretic peptide (ANP) gene expression declined during ontogenic ageing development and i.p. injection with ginseng extract reversed the reduction, suggesting that ginsenosides possesses anti-ageing effects in the heart endocrineous function aspect (Hong *et al*., 1992). It has been well documented that the immune function declines with age in the human and animals. The possible causes for the decline are the inability of lymphocytes to proliferate in response to mitogenic stimulation and the decrease of IL-2 production. Ginsenoside Rg1 was shown to selectively enhance the proliferation of lymphocytes and the production of IL-2 in aged rats.

Using Northern blot and Western blot analyses, Rg1 was found to promote IL-2 gene expression which showed increase of IL-2 mRNA and IL-2 protein contents. On the other hand, under the same conditions, ginsenoside Rg1 showed no effect on the immune function of young adult rats, thus indicating that ginsenoside Rg1 is regarded as an immunoregulator rather than an immuno-potentiating agent (Liu and Zhang, 1995).

Anti-diabetic activity

The water extract of *Panax ginseng* exhibits a significant hypoglycaemic effect after i.p. administration to normal and diabetic mice (Konno *et al*., 1985), and it has an effect on the liver glucose metabolism (Yokozawa *et al*., 1985). The study on ginseng saponins revealed that among ginsenosides, only ginseng Rb2 can normalize such dysbolism to some extent in streptozotocin-induced diabetic rats. In addition, ginseng glycans designated panaxans have been demonstrated to elicit hypoglycaemia in both normal and diabetic mice (Konno *et al*., 1985; Ng and Yeung, 1985; Oshima *et al*., 1985). In the study to investigate the effect of ginseng on non-insulin-dependent diabetes mellitus (NIDDM) patients, ginseng therapy (100 or 200 mg for 8 weeks) improved psychophysical performance and reduced fasting blood glucose and body weight, indicating that ginsenoside may be a useful therapeutic adjunct in the management of NIDDM (Sotaniemi *et al*., 1995). In addition, the oral administration of the water extract of ginseng to normal and epinephrine-induced hyperglycaemic mice caused a significant decrease in the blood glucose level 4 h after its administration. The hepatic content of glucose transporter isoform 2 (GLUT2) from mouse liver significantly increased in the orally ginseng extract-treated normal and epinephrine-induced hyperglycaemic mice compared to that in the control. These results suggest that the hypoglycaemic activity of ginseng extract is presumably due, at least in part, to the increment of GLUT2 protein content (Ohnishi *et al*., 1996). Furthermore, Takaku *et al*. reported the existance of insulin-like substances in Korean red ginseng (Takaku *et al*., 1990) and Kimura *et al*. showed that some ginseng fractions stimulated insulin release, especially glucose-induced insulin release from pancreatic islets and thereby lowered the blood glucose level (Kimura *et al*., 1981). These findings suggest that hypoglycaemic action shown by ginseng partially depend on the increase in insulin level.

Anti-atherosclerotic activity

There are some reports concerning the effects of ginseng or ginseng saponins on cholesterol metabolism. Administration of ginseng powder or ginseng saponins lowered serum cholesterol, especially LDL cholesterol, and increased HDL-cholesterol in humans, chickens or rats (Qureshi *et al*., 1983; Yamamoto *et al*., 1983). In addition, it reduced β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase and cholesterol-7α-hydroxylase activities. On the other hand, ginseng saponins injected i.p. into rats or treated liver slices in *in vitro* culture increased cholesterol synthesis and enhanced the HMG-CoA reductase activity, showing that the effect of ginseng and ginseng saponins on the enzymes related to cholesterol metabolism was still not definite (Sakakibara *et al*., 1975; Gommori *et al*., 1976; Ikehara *et al*., 1978). Recent studies reported that the polyacetylene compounds such as panaxydol, panaxydiol and panaxytriol, which were isolated from Panax ginseng roots, inhibit cholesteryl ester transfer protein and acyl-CoA: cholesterol acyltransferase (ACAT) *in vitro* (Kwon *et al*., 1996, 1997) thus suggesting that compounds other than saponins also contribute to anti-hyperlipidemic action exhibited by ginseng.

Nitric oxide (NO), described as an endothelium-derived relaxant, has been demonstrated to play a key role in the control vascular tone and peripheral blood flow (Palmer *et al*., 1987). Recent

accumulating evidence indicates that NO is an anti-atherogenic molecule. A spontaneous NO donor or an enhancer of serum NO would be a good candidate for agents to treat hypertension and atherosclerosis. Kim *et al*. reported that ginsenosides caused vasodilation and stimulated NO production in bovine aortic endothelial cells (Kim *et al*., 1992). Subsequently, some studies reported that ginsenosides elicit NO production to cause physiological responses. For example, ginsenosides can produce an inhibitory effect on the vasodilator response prejunctionally in the rat perfused mesentery by NO released from non-adrenergic, non-cholinergic nerves (Peng *et al*., 1994). Ginsenosides stimulate endogenous production of NO in rat kidney (Han and Kim, 1996), aorta (Kang *et al*., 1995) and in rabbit corpus cavernosum (Chen and Lee, 1995). Among ginsenosides, ginsenoside Rg1, but not Rb1, enhanced the release of NO from endothelial cells and may contribute to the beneficial effect of ginseng on the cardiovascular system.

Recent progress in the study on atherogenesis found that oxidized LDL plays critical roles in the development of atherosclerosis. Anti-oxidant as probucol and vitamin E can protect LDL from oxidation, resulting in the prevention of atherosclerosis. In fact, ginsenoside Rb1 and Rg1 showed anti-oxidative effect on vitamin C-NADPH and Fe^{2+}-cysteine-induced lipid peroxidation and liver microsome-NADPH-gossypol-induced superoxide generation (Deng and Zhang, 1991). This effect was observed in *in vivo* experiment after i.p. injection of ginsenoside Rb1. Besides their anti-oxidative effect ginsenoside Rb2 was reported to activate Cu, Zn-superoxide dismutase gene (SOD1) transcription through a transcription factor AP2 binding site, although total saponins from *Panax ginseng* did not show such an activity. This result suggests the existence of antagonist in ginseng saponin components or complex mechanism underlying the activation of gene transcription (Kim *et al*., 1996). These observations strongly suggest that these activities contribute to the anti-atherogenic and anti-hypertensive effect shown by ginseng or ginseng saponins.

Anti-cancer activity

Chemoprevention is still the most prevailing measure to treat cancer, regardless of its serious side-effects. New cancer preventive agents are expected to be developed. To date, anti-tumour effects of ginseng have been supported by animal studies. Those studies revealed that ginseng showed anti-tumour activities in slow growing tumours but not in rapidly growing tumours (Lee and Huemer, 1971). And red ginseng extract could significantly decrease the incidence of papilloma, prolong the latent period of tumour occurrence and reduce tumour number per mouse in a two-stage model by using DMBA/Croton oil (Xiaoguang *et al*., 1998). In addition, there are some reports concerning the substances in ginseng with anti-tumour activity. Polyacetylene compounds from red sinseng, panaxynol, panaxydol and panaxytriol inhibited the cell growth of several tumour cells (Matsunaga *et al*., 1990). The ethanol-insoluble fraction of ginseng showed anti-tumour effects as an immunomodulator (Yun *et al*., 1993). Ginsenoside Rh2 causes the differentiation of F9 teratocarcinoma stem cells, HL-60 and B16 cells (Lee *et al*., 1996; Kim *et al*., 1998d). In an experimental lung metastasis model using B16-BL6 melanoma, multiple administrations of ginsenosie Rh2 after the intravenous inoculation of B16-BL6 melanoma cells resulted in a significant inhibition of lung metastasis and this inhibition may be partly due to the inhibition of tumour-associated angiogenesis (Sato *et al*., 1994). A major intestinal bacterial metabolite of ginsenoside, M1, is considered to be a causal substance to inhibit lung metastasis, because M1 can inhibit lung metastasis of melanoma cells and *in vitro* tumour cell invasion and migration, although ginsenoside Rb1, Rb2 and Rc hardly inhibited the invasion and migration *in vitro* (Wakabayashi *et al*., 1997). In addition, M1 induced apoptosis in B16-BL6 cells at 40 μM and the regulation of apoptosis-related proteins by M1 is responsible for the induction (Wakabayashi *et al*., 1998). It was also reported that ginsenoside Rh2 also

induced apoptosis in human hepatoma SK-HEP-1 cells. The mechanism by which ginsenoside Rh2 induces apoptosis is to stimulate the proteolytic cleavage of cyclin kinase inhibitor, $p21^{WAFa/CIP1}$, by caspase-3 during the early stage of apoptosis, resulting in the elevated levels of cyclin/Cdk kinase leading to apoptosis (Park *et al*., 1998a). On the other hand, i.p. or oral administration of ginseng extract suppressed apoptotic cell death of hair follicle induced by irradiation (Kim *et al*., 1998a). Besides these studies on animal models and *in vitro* experiment, a case-control study has shown a significant reduction in the risk of cancer development among those who regularly consumed ginseng, and a cohort study strongly suggested that *Panax ginseng* has a non-organ specific preventive effect against cancer (Yun and Choi, 1990, 1998). These epidemiological results may promote basic study on the relationship between ginsenosides and cancer prevention.

Inhibitory activity of ion channel

Ca^{2+} is an important regulator for many neuronal functions, including exocytosis and excitability. Voltage-gated Ca^{2+} channels play a key role in control of free cytosolic Ca^{2+} opioids, GABA, and norepinephrine all inhibit Ca^{2+} channels in sensory neurones through pertussis toxin-sensitive GTP-binding protein. Inhibition of voltage-gated Ca^{2+} channels in sensory neurones by opiates and endogenous opioids may contribute to the analgesic action of these compounds. Both ginseng and opioids inhibit the response to several forms of stress. However, the receptor for ginseng root extract is not an α2-adrenergic, GABAB, muscarinic or opioid receptor (Nah and McCleskey, 1994). At saturating concentrations, a saponin within ginseng, Rf, rapidly and reversibly inhibits N-type, and other high thresholds, Ca^{2+} channels in rat sensory neurones to the same degree as a maximal dose of opioids. The effect is dose-dependent (half-maximal inhibition: 40 μM) and is virtually eliminated by pre-treatment of the neurones with pertussis toxin, an inhibitor of G(o) and Gi GTP-binding proteins. Other ginseng saponins – ginsenosides Rb1, Rc, Re and Rg1 – caused relatively little inhibition of Ca^{2+} channels. Antagonists of a variety of neurotransmitter receptors that inhibit Ca^{2+} channels fail to alter the effect of Rf, raising the possibility that Rf acts through another G protein-linked receptor. Rf might be useful in itself or as a template for designing additional modulators of neuronal Ca^{2+} channels (Nah *et al*., 1995).

Rat brain microsomal Na^+, K^+-ATPase activity was inhibited significantly by ginsenoside Rb1 at the IC_{50} of 6.3 μM. However, the inhibitory effect of Rg1 was much weaker than that of Rb1. In addition, ginseng saponin reduced acetylcholine (ACh)-evoked Na^+ influx and catecholamine secretion in bovine adrenal chromaffin cells. The order of inhibitory potency was as follows: Rg2 > Rf > Re > Rh1 > Rh2, Rg1 > Rb1 > Rc > Rb3, Rd, Ro, Rs1. Ginseng saponins, especially ginsenoside Rg2, block the nicotinic ACh receptors or the receptor-operated Na^+ channels, inhibit Na^+ influx through the channels and, consequently, reduce both Ca^{2+} influx and catecholamine secretion in bovine adrenal chromaffin cells (Tachikawa *et al*., 1995).

Functional ligand of glucocorticoid receptor

Several reports suggested that ginsenoside Rg1 regulated the induction of tyrosine aminotransferase gene transcription through glucocorticoid receptor; however, direct evidence was missing (Kang *et al*., 1994; Kim *et al*., 1994). A recent remarkable finding in ginseng research is that ginsenoside Rg1 acts by binding to the glucocorticoid receptor. This finding was elucidated by the evidence as follows. (1) Ginsenoside Rg1 competed for [^{3}H]dexamethasone binding to glucocorticoid receptor. (2) Glucocorticoid Rg1 activated a glucocorticoid responsive element-containing luciferase reporter gene. (3) The growth of FT02B cells, a rat hepatoma-derived cell line that contains physiological levels of glucocorticoid receptor, was suppressed by ginsenoside

Rg1 as well as by dexamethasone, each of whose effects were abolished by RU486, a specific glucocorticoid receptor antagonist. (4) Ginsenoside Rg1 treatment led to the down-regulation of intracellular glucocorticoid receptor content in FT02B cells, which was similar to the effect of dexamethasone (Lee *et al*., 1997; Chung *et al*., 1998).

Anti-platelet activity

Teng *et al*. reported anti-platelet actions of panaxynol and ginsenosides isolated from ginseng (Teng *et al*., 1989). Panaxynol (0.1 mg/ml) markedly inhibited the aggregation of washed platelets induced by collagen, arachidonic acid, ADP, ionophore A23187, PAF and thrombin, while ginsenosides had no significant effect on the aggregation. In addition, thromboxane B_2 formation of platelets was inhibited by panaxynol but not by ginsenosides. These results concluded that panaxynol is the most potent anti-platelet agent in ginseng and its mechanism of action is chiefly due to the inhibition of thromboxane formation. On the other hand, non-saponin fraction from ginseng also showed anti-platelet action by increasing cGMP concentration in human platelets, inhibition of Ca^{2+} influx into platelets and potent inhibition of thromboxane A_2 production (Park *et al*., 1995).

PAF antagonist

Platelet activating factor (PAF) is a biologically active phospholipid that plays important physiological and pathological roles in a hypotensive, increasing vascular permeability, acute inflammation, asthma, cardiac anaphylaxis, thrombosis, gastrointestinal ulceration, endotoxin shock, allergic skin disease and transplanted organ rejection. PAF receptor antagonist is thought to be a potent anti-allergy agent. Ginkgolide B is isolated from Ginkg biloba leaves and a well known potent PAF antagonist with an IC_{50} value of 1.9×10^{-7} M. On the other hand, 20(S)-ginsenoside Rg3 and Δ20-ginsenoside Rg3 also showed inhibitory activity of PAF binding to platelets with IC_{50}s of 4.9×10^{-5} and 9.2×10^{-5} M, respectively, although it is less potent than ginkgolide B (Jung *et al*., 1998).

Hepatoprotective effect

There are some reports concerning anti-hepatotoxic activity of ginseng saponins and ginsenoside R_0. Ginsenoside R_0 inhibited the increase of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels in D-galactosamine- and carbon tetrachloride-induced acute hepatitic rats. This activity was stronger than those of the aglycone of ginsenoside R_0, oleanolic acid, or glycyrrhizic acid and its aglycone, glycyrrhetinic acid (Matsuda *et al*., 1991; Jeong *et al*., 1997).

Anti-ulcer activity

Using the fact that *Panax ginseng* has been used for the treatment of gastroenteric diseases, Sun *et al*. purified some acidic polysaccharides with anti-ulcer activity (Sun *et al*., 1992). Oral administration of such acidic polysaccharides, especially, GL-4, markedly prevented the formation of acute gastric ulcers induced by HCl/ethanol, absolute ethanol, restraint water immersion stress, indomethacin and pylorus-ligated rats. Although the precise mechanism has not yet been determined, prostaglandin did not seem likely to mediate the cytoprotective action of GL-4.

A number of pharmacological and biological activities other than those described here were also reported to date.

Glycyrrhiza

Liquorice root (*Glycyrrhiza uralensis* FISCHER; *Glycyrrhiza glabra* L.) has been used as medicine and a sweetening agent in food products. In Japan and the United States, it is currently used as a sweetening and flavouring agent in candy, chewing gums, chocolate, cigarettes, smoking mixtures, liquors and beer. The main water soluble constituent of liquorice is glycyrrhizin, a pentacyclic triterpene derivative of 8-amyrin type (oleanane). Glycyrrhizin has been shown to possess several beneficial pharmacological effects including anti-inflammatory, anti-ulcerous, anti-viral, anti-allergic and anti-cholesterolemic activities as described in Table 3.9.

Liquorice has been used medicinally for at least 5000 years but scientific interest was aroused in 1946 in the small Dutch village of Heerenven. It was here that Reevers studied patients suffering from peptic ulcers who improved after taking a proprietary liquorice preparation from the local chemist. Subsequently, Reevers reported that one in five of these patients developed oedema, shortness of breath and hypertension (Reevers, 1948). Since his observation, there have been numerous world-wide reports of liquorice-induced hypertension with hypokalaemia complicated in some cases by myopathy and cardiac arrythmias (Molhuysen *et al*., 1950; Koster and David, 1968). Patients exhibit a mineralocorticoid excess state with sodium retention, suppression of the renin–aldosterone system and hypokalaemia. The condition appears to be reversible, because patients recover by stopping liquorice intake or by administering aldosterone antagonist and spironolactone administration. A recent study indicated that excessive ingestion of liquorice induces a syndrome of hypokalaemia and hypertension that reflects increased activation of renal mineralocorticoid receptors by cortisol (Walker and Edwards, 1994). A similar syndrome of cortisol-dependent mineralocorticoid excess occurs in congenital deficiency of the enzyme 11 β-hydroxysteroid dehydrogenase (11β-HSD), which normally inactivates cortisol to cortisone. It has been shown that liquorice inhibits 11β-HSD, preventing local inactivation of cortisol and allowing cortisol inappropriate access to intrinsically non-specific renal mineralocorticoid receptors. Cortisol binds with the same affinity as aldosterone to the mineralocorticoid receptor, resulting in a hypermineralocorticoid condition. Ingestion of liquorice may therefore result in retention of sodium and water, hypertension, hypokalaemia, alkalosis and suppression of the renin–aldosterone system (Heikens *et al*., 1995). In addition, when the effect of regular moderate liquorice consumption (50 and 100 g daily) was examined on blood pressure (BP) in a normotensive population, ingestion of 100 g of liquorice daily ($n = 30$) caused a significant rise in systolic blood pressure (SBP) by a mean of 6.5 mm Hg ($P < 0.001$) and a fall in plasma potassium by 0.24 mmol/l ($P < 0.001$); the highest rise in SBP observed was 19 mm Hg. In a subgroup of 13 women the consumption of 50 g of liquorice daily also caused a significant rise in SBP of 5.6 mm Hg ($P < 0.001$) and diastolic blood pressure (DBP) of 3.4 mm Hg ($P = 0.002$). A significant change in the cortisol/cortisone ratio in urine was observed during 100 g liquorice consumption indicating inhibition of 11β-HSD in kidneys. The results indicate that liquorice-induced

Table 3.9 Pharmacological and biological activities of glycyrrhiza

Hypotensive activity
Immunomodulatory activity
Anti-oxidative activity
Anti-inflammatory activity
Anti-tumourigenic and anti-mutagenic activity
Drug metabolism
Miscellaneous activities

hypertension might be more common than has been appreciated and it is important for medical doctors to be on the alert for this effect in both the prevention and treatment of hypertension (Sigurjonsdottir *et al*., 1995).

Enzyme 11 β-hydroxysteroid dehydrogenase is a microsomal membrane-bound enzyme and is responsible for the conversion of the physiologically active C-11 hydroxylated glucocorticoids, cortisol and corticosterone to inactive metabolites, cortisone and 11-dehydrocorticosterone, respectively. Congenital deficiency of 11β-HSD exhibits a rare, often fatal, cause of mineralocorticoid hypertension, that is, severe hypertension with suppression of the renin–angiotensin–aldosterone axis and hypokalaemia. The active apparent mineralocorticoid in liquorice is the aglycone of glycyrrhizic acid, and glycyrrhetinic acid. Inhibition of 11β-HSD by glycyrrhetinic acid results in elevation of cortisol acting as a potent mineralocorticoid. 11β-HSD is ubiquitously expressed and, by converting active glucocorticoid to inactive metabolites, may be an important prereceptor regulator of ligand access to the glucocorticoid receptor. Glycyrrhizin administration to rats *in vivo* (75 mg/kg day for 5 days) resulted in not only inhibition of 11β-HSD activity, but also a significant reduction in steady-state 11β-HSD mRNA levels in both predominantly mineralocorticoid (kidney and distal colon) and glucocorticoid (liver and pituitary) target tissues. *In vitro*, 11β-HSD mRNA and activity of 11β-HSD were detected in rat pituitary GH3 cells and inhibited by glycyrrhetinic acid in a dose-dependent fashion. While corticosterone or glycyrrhetinic acid alone (10^{-8}–10^{-5} M) had little or no effect on PRL mRNA (a known glucocorticoiod target gene) levels or immunoassayable PRL, combinations of glycyrrhetinic acid plus corticosterone resulted in marked inhibition of PRL mRNA levels and secretion, to such an extent that a concentration of 10^{-6} M corticosterone with 10^{-6} M glycyrrhetinic acid was more potent than equimolar concentration of the synthetic glucocorticoid receptor agonist RU 28362. This inhibitory effect on PRL mRNA levels was blocked by a 10-fold excess of the glucocorticoid receptor antagonist RU 38486, but not by a 10-fold excess of the mineralocorticoid receptor antagonist RU 26752, confirming that this potentiation of glucocorticoid hormone action was operating through the glucocorticoid receptor and not the mineralocorticoid receptor. In addition to its established role as a competitive inhibitor of 11β-HSD, liquorice results in pre-translational inhibition of 11β-HSD both *in vitro* and *in vivo*. 11β-HSD is clearly an important mechanism in regulating tissue levels of active glucocorticoid and, hence, ligand supply to the glucocorticoid receptor (Whorwood *et al*., 1993). A complementary DNA of 11β-HSD was cloned from a rat liver cDNA library and its sequence indicated that 11β-HSD is a single protein catalysing both dehydrogenation and reduction (Tannin *et al*., 1991). However, several lines of evidence suggest the existence of several species of 11β-HSD (Seckl, 1993).

Enzyme 18β-Glycyrrhetinic acid has been thought to be one of the major metabolites that causes liquorice-induced pseudoaldosteronism. However, the difference of the blood level of 18β-glycyrrhetinic acid between patients with and without pseudoaldosteronism was not found. When the blood concentration of 3β-D-(monoglucuronyl)18β-glycyrrhetinic acid (3MGA), another metabolite of 3β-D-diglucuronyl-18 β-glycyrrhetinic acid (glycyrrhizin) was measured by high performance liquid chromatography, 3MGA concentration was found to be high in 10 patients with liquorice-induced pseudoaldosteronism, but not in 11 patients without pseudoaldosteronism. In addition, 3MGA was found to be a potent inhibitor of 11β-HSD, allowing cortisol to exert its full mineralocorticoid effects. These results suggest that liquorice-induced pseudoaldosteronism is due to an increased concentration of 3MGA, but not 18β-glycyrrhetinic acid, in the circulating blood of these patients (Kato *et al*., 1995).

Carbenoxolone, which is synthesized from glycyrrhetinic acid, is the succinic acid ester of 18β-glycyrrhetinic acid and is used for gastric ulcers. Its effectiveness appears to be related to its ability to enhance mucus secretion, increase the life span of gastric epithelial cells, inhibit

back-diffusion of hydrogen ions induced by bile, and possibly inhibit peptic activity. On the basis of the results of controlled clinical trials, it can be concluded that carbenoxolone is effective in accelerating the healing of gastric ulcers (Lewis, 1974). Although these side-effects of Glycyrrhiza extract and its ingredients are widely known, Kobuke *et al*. has reported that the long-term oral administration of glycyrrhizin as sodium salt in drinking water (up to 0.5%) to mice did not yield any evidence of chronic toxicity, which sugests that the extracts of liquorice and its major constituent glycyrrhizin are devoid of systemic toxicity and, therefore, may be useful as therapeutic agents against several diseases (Kobuke *et al*., 1985).

Immunomodulatory activity

When the effect of glycyrrhizin on the production of interferon-γ in human peripheral lymphocyte-macrophage cultures by concanavalin A (Con A) was examined, interferon-γ production in normal lymphocyte-macrophage cultures treated with 10–100 μg/ml of glycyrrhizin at 37°C for 12 h or longer, followed by the treatment with 10 μg/ml of Con A, was enhanced 4–8 times compared to control cell cultures (Shinada *et al*., 1986). Lymphocyte-macrophage cultures treated with 10–100 μg/ml of glycyrrhizin alone did not produce interferon. No significant difference in the adsorption of [^{3}H]-Con A to glycyrrhizin-treated and control lymphocyte-macrophage cultures was found, but RNA and protein synthesis of the treated lymphocytes was increased compared to control cells; DNA synthesis, however, was reduced. Collaboration between enriched T-lymphocytes and macrophages, both treated with glycyrrhizin, was needed for the enhancement of interferon-γ production. A smaller increase in interferon production was also observed in the glycyrrhizin-treated peripheral lymphocyte-macrophage cultures derived from an asymptomatic carrier of hepatitis B virus, in response to Con A and surface antigen of hepatitis B virus. In addition, Pompei *et al*. reported that glycyrrhetinic acid directly inhibits growth and cytopathology of several unrelated DNA and RNA viruses, while not affecting cell activity and ability to replicate (Pompei *et al*., 1979). In addition, glycyrrhetinic acid inactivates the herpes simplex virus particles irreversibly. These results suggest that glycyrrhetinic acid shows anti-virus activity through both interferon-γ and direct growth inhibitory activity against viruses.

Glycyrrhiza polysaccharide was reported to increase the phagocytosis of macrophages, induce macrophages to secret IL-1, enhance both NK and ADCC activities, behave as a mitogen of B lymphocytes, inhibit the multiplication of several viruses, and induce the release of IFN from spleen cells (Yang and Yu, 1990). An acidic polysaccharide, named glycyrrhizan GA, was isolated from the stolon of Glycyrrhiza glabra and its molecular mass was estimated to be 85 000. Glycyrrhizan GA is composed of L-arabinose : D-galactose : L-rhamnose : D-galacturonic acid : D-glucuronic acid in the molar ratio of 22 : 10 : 1 : 2 : 1, in addition to small amounts of *O*-acetyl groups. Part of the hexuronic acid residues exist as methyl esters. Methylation analysis, carbon-13 nuclear magnetic resonance and periodate oxidation studies indicated that its structural features include mainly α-arabino-β-3,6-galactan-type structural units. Glycyrrhizan GA showed remarkable reticuloendothelial system-potentiating activity in a carbon clearance test (Shimizu *et al*., 1991).

The complement plays an important role in immune reaction. Two pathways exist in the activation pathway of the complement; that is, the classical and alternative pathways. β-Glycyrrhetinic acid was found to be a potent inhibitor of the classical complement pathway (IC_{50}: 35 μM), whereas no inhibitory activity was observed towards the alternative pathway ($IC_{50} > 2.5$ mM). This inhibitory activity was dependent on its conformation, since the a-form was not active. Further, detailed studies revealed that β-glycyrrhetinic acid acts at the level of complement component C2 (Kroes *et al*., 1997).

Anti-oxidative activity

A critical event in the pathogenesis of atherosclerosis is subendothelial accumulation of lipid-laden foam cells, which are primarily derived from monocytes/macrophages by uptake of oxidatively modified LDL (ox-LDL). Ox-LDL not only leads macrophages to foam cells, but also impairs several macrophage functions. Ox-LDL enhances monocyte chemoattractant protein-1 synthesis, IL-8 production, cell adhesion molecules expression such as VCAM-1, ICAM-1, and suppresion of NO production in endothelial cells and macrophages, all of which are considered to stimulate atherosclerosis. In fact, ox-LDL was detected in human and animal atherosclerotic lesions and is therefore considered to be a causal substance to induce atherosclerosis. Consequently, reduction of ox-LDL production should be beneficial to prevention or treatment of atherosclerosis. Glabridin, which is an isoflavan isolated from *Glycyrrhiza glabra*, was found to be potent anti-oxidant, evidenced by the inhibitory activity of cholesteryl linoleate hydroperoxide formation in an 2,2′-azobis(2-amidinopropane)dihydrochloride-induced LDL oxidation (Vaya *et al*., 1997) and the reduction in copper-ion-induced LDL oxidation (Belinky *et al*., 1998). As an *ex vivo* study, dietary supplementation of each apolipoprotein E-deficient mice with liquorice (200 μg/day) or pure glabridin (20 μg/day) for 6 weeks resulted in a substantial reduction in the susceptibility of their LDL to oxidation along with a reduction in the atherosclerotic lesion area (Fuhrman *et al*., 1997). This study also showed that the absorption and binding of glabridin to the LDL particle led to subsequent protection of the LDL from oxidation by multiple modes.

Anti-inflammatory activity

Selectins are a family of adhesion receptors implicated in the initial interaction between leucocytes and vascular endothelium leading to an inflammatory response. At present, three selectins, E-selectin, L-selectin and P-selectin were identified and all these selectins are known to recognize sialyl Lewis X and sialyl Lewis A and related oligosaccharides. Binding of selectins to their carbohydrate ligands appears to be required for neutrophil rolling and extravasation and plays a major role in lymphocyte recirculation and platelet adhesion. The biology of the selectins suggests that compounds that block their function may serve as anti-inflammatory agents. Rao *et al*. reported that glycyrrhizin was identified and found to block selectin binding to sialyl Lewis X in a dose-dependent manner *in vitro*, whereas glycyrrhetinic acid is relatively ineffective for blocking selectin activity (Rao *et al*., 1994). Glycyrrhizin selectively blocked L-selectin and P-selectin binding to sialyl Lewis X in the micromolar range (Foxall *et al*., 1992). In addition, the study of derivatives indicated that the substitution of L-fucose for the glucuronic acid of glycyrrhizin made a most effective derivative *in vitro* and *in vivo*. Fucose glycosidically linked to glycyrrhetinic acid demonstrated an increased ability, relative to glycyrrhizin, to block swelling and neutrophil influx in an arachidonic acid-stimulated inflammation model in mice.

As another interesting activity shown by glycyrrhizin, glycyrrhizin was identified as a new thrombin inhibitor (Mauricio *et al*., 1997). It prolonged plasma recalcification and thrombin and fibrinogen clotting times, and inhibited thrombin-induced, but not collagen-, PAF- or convulxin-induced platelet aggregation. These results strongly suggest that glycyrrhizin is a selective inhibitor of thrombin (the first one isolated from plants) that is able to exert its anti-thrombin action by interacting with the enzyme's anion binding exosite 1. A pharmacophoric search identified glycyrrhizin as a sialyl Lewis X mimetic compound able to inhibit selectin binding to sialyl Lewis X. However, sialyl Lewis X did not affect thrombin clotting activities, which indicates a lack of its interaction with thrombin and distinguishes both molecules. These activities may be partly related to the anti-inflammatory effect of glycyrrhizin.

Anti-tumourigenic and anti-mutagenic activity

Anti-mutagenic activity of *Glycyrrhiza glabra* extract, glycyrrhizin and glycyrrhetinic acid were reported by using a modified method of the Ames' test (Tanaka *et al*., 1987) and the Salmonella/microsome reversion assay (Zani *et al*., 1993).

Liquorice (*Glycyrrhiza glabra*), a Mediterranean plant, has been used as an antidote, demulcent and elixir folk medicine for generations in China. The main water-soluble constituent of liquorice is glycyrrhizin, which has been shown to possess several pharmacological properties. In this study, we show that oral feeding of glycyrrhizin to Sencar mice resulted in substantial protection against skin tumourigenesis caused by 7,12-dimethyl-benz [α]anthracene (DMBA) initiation and TPA promotion. The latent period prior to the onset of tumour development was considerably prolonged in glycyrrhizin-fed animals compared with animals not fed glycyrrhizin and resulted in significant decrease in the number of tumours per mouse, during and at the termination of the experiment. Oral feeding of glycyrrhizin in drinking water also resulted in inhibition in the binding of topically applied [^{3}H]benzo[a]pyrene and [^{3}H]DMBA to epidermal DNA. The possible mechanism(s) of the anti-tumour-initiating activity may be due to the involvement of glycyrrhizin as an inhibitor of the carcinogen metabolism followed by DNA adduct formation. Our results suggest that glycyrrhizin possesses considerable anti-tumourigenic activity and could prove useful in killing some forms of human cancer.

Drug metabolism

As an approach to elucidate the possible *in vivo* interaction of synthetic drugs and herbs which are frequently used in combination in Asia, the effect of *Glycyrrhiza glabra* on the metabolism of acetaminophen (AAP) was examined in male Sprague–Dawley rats (Moon and Kim, 1997). The pre-treatment of the methanol extract of *Glycyrrhiza glabra* roots (1 g/kg, p.o.) for 6 days significantly increased the cumulative biliary (156%) and urinary (132%) excretions of AAP-glucuronide conjugate within 120 min after the administration of AAP (150 mg/kg, i.v.) without affecting thioether and sulfate conjugates. Therefore, the enzymatic activity of *p*-nitrophenol UDP-glucuronosyltransferase (UGT) and intracellular concentrations of hepatic UDP-glucuronic acid were examined after 6-day administration of *Glycyrrhiza glabra* extract (1 g/kg p.o.) or glycyrrhizin (23 mg/kg, p.o.). *Glycyrrhiza glabra* extracts and glycyrrhizin caused increases in specific activities of UGT by 111% and 96%, respectively. In addition, the concentration of UDP-glucuronic acid was increased 257% by *Glycyrrhiza glabra* extract and 484% by glycyrrhizin. These data indicate that *Glycyrrhiza glabra* extracts and glycyrrhizin activated glucuronidation, thus suggesting the possibility that *Glycyrrhiza glabra* may influence detoxification of xenobiotics in rat liver. Paolini *et al*. reported the effect of the prolonged intake of conspicuous amounts of liquorice, or glycyrrhizin on murine liver cytochrome P-450 (CYP)-catalysed drug metabolism (Paolini *et al*., 1998). For this purpose the modulation of the regio- and stereo-selective hydroxylation of testosterone, together with the use of highly specific substrates as probes for different CYP isoforms such as ethoxyresorufin (CYP1A1), methoxyresorufin (1A2), pentoxyresorufin (2B1), *p*-nitrophenol (2E1) and aminopyrine (3A), were investigated. Daily doses of liquorice root extract (3138 or 6276 mg/kg b.w./os) or glycyrrhizin (240 or 480 mg/kg b.w./os) were administered to different groups of Swiss Albino CD1 mice of both sexes for 1, 4 or 10 consecutive days. While a single liquorice root extract or glycyrrhizin dose was unable to affect the multienzymatic CYP-system, long-term treatment with liquorice root extract or glycyrrhizin was able to significantly induce hepatic CYP3A- and, to a lesser extent, 2B1- and 1A2-dependent microsomal mono-oxygenase activities as well as 6β- (mainly associated to CYP3A), 2α-, 6α- (CYP2A1, 2B1), 7α-, 16α- (CYP2B9), and 16β-testosterone hydroxylase (TH) activities

in male and female mice. Data on CYP3A modulation, the major isoform present in human liver, was confirmed by using Western blotting with anti-CYP3A1/2 rabbit polyclonal antibodies raised against purified rat CYP3A. Northern blotting analysis using a CYP3A cDNA biotinylated probe showed that the expression of such an isozyme is regulated at the mRNA level. These results suggest that the induction of cytochrome P450-dependent activities by the prolonged intake of high liquorice root extract or glycyrrhizin doses may result in accelerated metabolism of co-administered drugs with important implications for their disposition. The adverse effects associated with CYP changes, such as toxicity/co-toxicity and co-mutagenicity, may also have clinical consequences.

In addition, when the 12 liquorice constituents were examined for the inhibition of monoamine oxidase (MAO), genistein, licopyranocoumarin, licocoumarone and glycyrrhisoflavone inhibited the enzyme with the IC_{50} values of 6.0×10^{-5} to 1.4×10^{-4} M. Glycyrrhizin also inhibited MAO with the IC_{50} value of 1.6×10^{-4} M, even though the concentration was considerably high (Hatano *et al*., 1991).

Miscellaneous activities

As other activities noted in clinical importance, anti-hypercholesterolemic action of liquorice roots (Yamamoto *et al*., 1970; Sitohy *et al*., 1991) and hepatoprotective acitvity (Wang and Han, 1993) have been reported.

Pinellia tuber

Pinelliae tuber, the tuber of *Pinellia ternata* Breit, is distributed widely in China, Korea and Japan and has been used in traditional Chinese medicine. Pinellia pedatisecta Schott tuber is also found in the drug market. The tuber is globular and ellipsoid in shape, white or grey-white in colour, with no characteristic smell, but an acrid taste. This crude drug contains homogentisic acid and its glucoside, 3,4-dihydroxybenzaldehyde, and its diglucoside, ephedrine, β-sitosterol, a lection and polysaccharide. The prescriptions containing Pinellia tuber show anti-emetic, anti-tussive, sedative, anti-inflammatory and anti-implantation effects. The most remarkable effect of this crude drug has been demonstrated for morning sickness when it was applied to the patients in the form of a prescription containing it as the main component. However, the pharmacological property and active ingredients corresponding its actions have not been fully understood yet.

Maki *et al*. (1987) isolated a polysaccharide fraction from the aquerous extracts of the tuber of *Pinellia ternata* as an anti-emetic principle.They established an assay method for the anti-emetic action using leopard and ranid frogs as the test animals. The test solution was orally administered to the frogs, and then they were allowed to stand for 30 min to stabilize. Apomorphine hydrochloride was given orally to the frogs, and the behaviour was observed for 60 min at intervals of 5 min to see whether the gastric contents were vomited out of their mouths. The result was judged by opening the mouth of each frog 15 min after the administration of the emetic agent. The anti-emetic polysaccharide, named PT-F2-I, separated by means of chromatography over sephacryl S-300 and TSK-G4000SW columns. PT-F2-I showed the highest anti-emetic activity (100%) against 1 mg/frog (p.o.) of apomorphine at a dose of 100 μg. It was represented by an arabinan consisting of a(1→4)-linkages with a (1→2)-side chains as the main part, and oligosaccharide chains as the subunits involving fucose, glucose, galactose, rhamnose and ribose in the ratio of 0.12, 0.1, 0.09, 0.05 and 0.05 provided that arabinose was 1.00.

Tomoda *et al*. (1994) reported that a glucan, called pinellian G, was isolated from the tuber of *Pinellia ternata* Breit. It was homogenous in electrophoresis and gel chromatography, and its

molecular mass was estimated to be 15 000. It is composed solely of D-glucose, in addition to a few O-acetyl groups. Methylation analysis, nuclear magnetic resonance and enzymic degradation studies indicated that it is a branched glucan mainly composed of a-1,4-linked D-glucopyranose residues with partially a-1,3-linked units and 4,6-branching points. The glucan showed significant reticuloendothelial system-potentiating activity in a carbon clearance test by i.p. injection. Furthermore, Gonda *et al*. (1994) isolated an acidic polysaccharide, named pinellian PA, and this polysaccharide also showed significant potentiation of the reticuloendothelial system and potent anti-complemenatry activity. Tachibana and kawanishi (1992) extracted proteins with hot water and saline, and prepared protein fractions by ammonium sulfate precipitation from 18 different crude drugs. They reported the protein fraction of Pinelliae tuber showed potent miogenic activity for both human and/or murine lymphocytes.

Nijima *et al*. (1993) demonstrated the effect of intraduodenal infusion with hot aqueous extract of *Pinellia ternata* tuber on the efferent discharges in the gastric branch of the vagus nerve by using anaesthetized rats. The infusion of the extract in doses of 2–150 mg per animal resulted in a dose-related increase in efferent activity of vagal gastric nerve. The enhancement of the nerve activity following administration of 150 mg of this extract lasted longer than 90 min. It was observed that the suppressive effect on vagal gastric activity due to apomorphine and copper sulfate was antagonized by prior administration of the extracts. They suggested that Pinellia tuber acts as a facilitatory agent on gastric function. Recently, they reported the effect of taste stimulation of Pinelliae tuber, Zingiberis Rhizoma and their mixture on the efferent activity of the gastric branch of the vagus nerve in anaesthetized rats (Niijima *et al*., 1998). Taste stimulation by *Pinellia ternata* (50 mg/ml, 10 min) resulted in a suppression in vagal gastric nerve activity. The mixture of Pinelliae tuber and Zingiberis Rhizoma (5 : 1, 50 mg/ml, 10 min stimulation) demonstrated no suppressive effect on gastric nerve activity. These observations indicate the existence of interaction between Pinelliae tuber and Zingiberis Rhizoma, which are often used in combination in Chinese herbal medicine.

Scutellaria root

The root of *Scutellariae baicalensis* Georgi has been used clinically in China and Japan as a therapeutic drug for treatment of allergic inflammatory diseases such as asthma, atopic dermatitis, hyperlipidaemia and arteriosclerosis. Here we review the various pharmacological and biological activities so far reported.

Anti-viral activity

Influenza A and B viruses express two envelope glycoporteins: hemagglutinin and sialidase. The hemagglutinin is known to mediate the attachment of the virus to the host cells *via* sialic acid residue in glycoconjugate receptors and the subsequent fusion of viral and host cell membranes. While sialidase catalyses cleavage of terminal sialic acid residue of the sialoglycoconjugate receptors, resulting in the release of newly formed viruses from the infected host cells and the destruction of sialic acid containing mucus glycoproteins that can act as receptor analogues located on the host cell surface. Therefore, influenza virus sialidase inhibitors may inhibit the virus infection. Anti-viral activity of several ingredients isolated from *Scutellariae baicalensis* has been reported to date. 5,7,4′-Trihydroxy-8-methoxyflavone showed potent inhibitory activity against the influenza virus sialidase (IC_{50}, 55 μM) and appeared to be a non-competitive inhibitor (Nagai *et al*., 1990, 1995). However, negligible or weak inhibitory activities were observed for mouse liver sialidase, β-galactosidase and α-mannosidase. This flavone inhibited the infection

by influenza virus A/PR/8/34 and B/Ibaraki of Madin–Darby canine kidney cells, and replication of the virus in the allantoic sack of embryonated egg. And 5,7,4′-trihydroxy-8-methoxyflavone is suggested to inhibit the replication of A/Guizhou and B/Ibaraki viruses at least partly by inhibiting the fusion of viral envelopes with the endosome/lysosome membrane, which occurs at the early stage of the virus infection cycle. In addition, Nagai reported that 5,7,8,4′-tetrahydroxyflavone (isoscutellarein) also inhibited (IC_{50}, 20 μM) influenza virus sialidase non-competitively and negligible inhibitory activity was observed for mouse liver sialidase at a concentration of 79 μM (Nagai *et al*., 1992). Isoscutellarein also inhibited the replication of influenza virus A/WSN/33 in Madin–Darby bovine kidney cells with 50% virus inhibitory dose at 16 nmol/well and influenza virus A/PR/8/34 in the allantoic sac of embryonated egg with little toxic effects. The flavone showed significant anti-influenza virus activity *in vitro* similar to 5,7,4′-trihydroxy-8-methoxyflavone and more potent virucidal activity in ovo than 5,7,4′-trihydroxy-8-methoxyflavone. However, 5,7,4′-trihydroxy-8-methoxyflavone completely prevented proliferation of mouse-adapted influenza virus A/PR/8/34 in mouse lung by the intranasal (0.5 mg/kg) and i.p. (4 mg/kg) administrations, and it was more potent than the known anti-influenza virus substance, amantadine. Intranasal administration of 5,7,4′-trihydroxy-8-methoxyflavone (0.5 mg/kg) also protected mice against a lethal influenza virus A/PR/8/34 infection. Isoscutellarein significantly inhibited lung virus proliferation when administered intranasally or orally to mice. And 5,7,4′-trihydroxy-8-methoxyflavone and isoscutellarein showed negligible toxic effect against mice. These results suggested that flavones, which have potent influenza virus sialidase inhibitory activity, have anti-influenza virus activity *in vivo*.

Human T-cell leukaemia virus type I (HTLV-I) initiates the development and progression of adult T-cell leukaemia as well as the neurologic disorder tropic spastic paraparesis. 7-glucuronic acid, 5,6-dihydroxyflavone (baicalin) concentration-dependently inhibits HTLV-I replication in productively infected T and B cells (Baylor *et al*., 1992). Moreover, baicalin treatment selectively reduced the detectable levels of HTLV-I p19 gag protein in infected cells by greater than 70% at concentrations that produced insignificant effects on total cellular protein and DNA synthesis with no loss of cell viability. Resistance to HTLV-I infection and virus-mediated transformation was noted in uninfected peripheral blood lymphocytes pre-treated with baicalin before co-cultivation with lethally irradiated chronically infected cells. Baicalin inhibited reverse transcriptase activity in HTLV-I-infected cells as well as the activity of purified reverse transcriptase from Moloney murine leukaemia virus and Rous-associated virus type 2. These results suggest that baicalin may be a potential therapeutic agent against HTLV-I-associated T-cell diseases. Baicalin also inhibits human immunodeficiency virus (HIV-1), the etiological agent of acquired immunodeficiency syndrome, infection, and replication as measured by (1) a quantitative focal syncytium formation on CEM-ss monolayer cells; and (2) HIV-1 specific core antigen p24 expression and retroviral reverse transcriptase (RT) activity in the HIV-1-infected H9 cells (Li *et al*., 1993). The enzymatic activity of purified recombinant HIV-1/RT was inhibited by baicalin. In addition to lymphoid cell lines, the anti-HIV-1 activity of baicalin was also observed in cultures of primary human peripheral blood mononuclear cells infected with HIV-1 *in vitro*. This data suggests that baicalin may serve as a useful drug for the treatment and prevention of HIV infections.

Anti-inflammatory activity

The eicosanoids, leukotriene C4 (LTC4) and LTB4, which are released as proinflammatory mediators after activation of macrophages and mast cells, have been shown to play important roles in the pathogenesis of inflammatory disorder such as arthritis. Kimura *et al*. reported that baicalein (5,6,7-trioxyflavone-7-*O*-beta-D-glucuronide) isolated from Scutellariae radix inhibited LTB4

and C4 syntheses, de-granulation in human polymorphonuclear leucocytes (Kimura *et al*., 1986). Anti-inflammatory activity of baicalein or baicalin was observed in the rat carrageenan-induced paw oedema and ajuvant-induced arthritis (Kubo *et al*., 1984; Butenko *et al*., 1993). A comparative study of the 5-lipoxygenase (5-LO) inhibitory activity of baicalein, BW 755 C, and hydroxamic acid arachidonate on LTC4 biosynthesis by rat resident peritoneal macrophages stimulated with calcium ionophore (A 23186) showed that these drugs significantly inhibited LTC4 production, IC_{50}: 9.5, 41.8 and 2.8 mM, respectively. This finding suggests that inhibition of the 5-LO pathway of arachidonic acid metabolism may be one of the mechanisms of anti-inflammatory activity shown by baicalein and baicalin. The migration of leucocytes into tissues is the central event in the inflammatory response. Leucocyte emigration is responsible for the host response to tissue injury and infection but is also potentially harmful and contributes to the pathology of many diseases and inflammatory disorders. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and endothelial leucocyte adhesion molecules-1 (ELAM-1) were expressed into inflamed tissue of inflammatory disorders such as asthma, rheumatoid arthritis and atherosclerosis. And it is well known that IL-1b and TNF-α stimulate the ELAM-1 and ICAM-1 expression. Kimura *et al*. reported that baicalein dose-dependently inhibited IL-1β and TNF-α-induced ELAM-1 and ICAM-1 expressions (Kimura *et al*., 1997a). Its IC_{50} for the IL-1β -induced ELAM-1 and ICAM-1 expressions were 2.3×10^{-5} M and 4.0×10^{-5} M, respectively. The IC_{50} for the TNF-α-induced ELAM-1 and ICAM-1 expressions were 1.5×10^{-5} M and 3.1×10^{-5} M, respectively. In addition, in LPS-induced production of IL-1β, three flavonoids of *Scutellariae baicalensis* Georgi, wogonin, baicalein, and baicalin at 1 μg/ml expressed a significant (>50%) inhibitory effect, similar to that of prednisolone (Chung *et al*., 1995). Moreover, the flavonoids inhibited IL-1β -induced synthesis of PGE2 and LTB4 considerably, although the effect of wogonin on LTB4 synthesis was marginal. These findings have potentially important implications for the therapeutic use of *Scutellariae baicalensis* and its flavonoids in the inflammatory diseases.

Anti-atherosclerotic activities

Tissue-type plasminogen activator (t-PA) is a highly specific protease synthesized by vascular endothelial cells and secreted into the bloodstream. The enzyme plays a key role in the fibrinolytic system, the natural counterpart of the blood coagulation system, and is responsible for timely degradation of fibrin structures in blood clots and thrombus. Plasminogen activator inhibitor-1 (PAI-1) is also synthesized by vascular endothelial cells and is secreted into the bloodstream. This protein is the main physiological inhibitor of both t-PA and urokinase-type plasminogen in plasma. Thus t-PA and PAI-1 play an important role in the regulation of the blood coagulation system in the bloodstream. Thrombin is a serine protease, which also plays a central role in blood coagulation, platelet activation and pulmonary vascular injury, and causes production of t-PA and PAI-1 in endothelial cells. Thrombin and thrombin receptor agonist peptide induced production of both t-PA and PAI-1 and the elevation of intracellular free calcium concentration ($[Ca^{2+}]i$). Baicalein dose-dependently inhibited PAI-1 production induced by thrombin and thrombin receptor agonist peptide; inhibitory concentrations for 50% inhibition (IC_{50}) were 6.8 and 3.5 μM, respectively. In addition, baicalein strongly inhibited the reduction of t-PA production and the elevation of PAI-1 production induced by trypsin. IC_{50} for PAI-1 production was 3.7 μM. In addition, wogonin, oroxylin A, skullcapflavone II and 2′,5,5′,7-tetrahydroxy-6′,8-dimethoxyflavone inhibited the elevation of PAI-1 induced by trypsin, though less strongly; their IC_{50} values were 105, 61, 110 and 88 μM, respectively. These findings suggest that baicalein prevents the thrombotic tendency induced by thrombin or

trypsin. Baicalein inhibited the elevation of $[Ca^{2+}]i$ induced by thrombin and thrombin receptor agonist peptide and, at a concentration of 1000 μM, slightly increased t-PA production. These findings suggest that the mechanism by which baicalein inhibits PAI-1 production induced by thrombin and thrombin receptor agonist peptide might be by reduction of $[Ca^{2+}]i$ elevation and that baicalein in Scutellariae Radix might be active as a drug in the treatment of arteriosclerosis and thrombosis (Kimura *et al*., 1997b,c). In the study using C6 rat glioma cells (Kyo *et al*., 1998), baicalin and baicalein caused concentration-dependent inhibition of a histamine-induced increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$). The potency of baicalein was significantly greater than that of baicalin. The noradrenaline- and carbachol-induced increase in $[Ca^{2+}]i$ was also inhibited by baicalein and both drugs inhibited histamine-induced accumulation of total [^{3}H]inositol phosphates, consistent with their inhibition of the increase in $[Ca^{2+}]i$. These results suggest that baicalin and baicalein inhibit $[Ca^{2+}]i$ elevation by reducing phospholipase C activity. The inhibitory effects of baicalin and baicalein on $[Ca^{2+}]i$ elevation might be important in the interpretation of their pharmacological action.

Intimal smooth muscle cell proliferation is closely linked to the development of atherosclerosis. Its suppression is a major target of anti-atherosclerotic agents. When the effects of baicalein, baicalin and wogonin on the proliferative responses of cultured rabbit vascular smooth muscle cells were studied, all three flavonoids dose-dependently inhibited the proliferative response induced by 5% foetal calf serum at the dose range of 10^{-6} to 10^{-4} M (Huang *et al*., 1994). Baicalin and wogonin were less effective than baicalein as inhibitors of the serum-induced smooth muscle cell proliferation, indicating that the three hydroxyl groups on positions 5, 6 and 7 seem to be necessary and sufficient for full inhibitory activity against the proliferative response of smooth muscle cells. Baicalein had a greater inhibitory effect on the proliferative reponse stimulated by platelet-derived growth factor than on serum-stimulated proliferation. In addition, baicalein decreased ACAT activity in a dose-dependent fashion from the level of 10^{-6} M in human hepatoma HepG2 cells (Yotsumoto *et al*., 1997). This result suggests that baicalein may suppress cholesteryl ester production in liver and macrophages, resulting in the reduction of plasma cholesterol levels and the inhibition of foam cell formation, respectively, although ACAT in liver and macrophages is found to be structurally different. Baicalein, a flavonoid with anti-proliferative and lipoxygenase-inhibitory activities may be useful as another template for the development of better drugs to prevent the pathological changes of atherosclerosis and retenosis.

Miscellaneous activities

Other activities reported to date include anti-oxidative activity of baicalin and baicalein, inhibitory activity of glucosidase by baicalein and thermoregulation of *Scutellariae baicalensis* extract.

Jujube

Zizyphi Fructus, the fruits of *Zizyphus jujuba* Miller var. inermis Rehder, is a well-known crude material which has been used for the purpose of analeptic and palliative treatments. It contains mainly carbohydrates (more than 85% in water extract) such as D-glucose, D-fructose, oligosaccharide and polysaccharide. Some triterpenoid acids and dammarane-type saponins were also isolated from ethenol extracts of the dried fruits, however, the pharmacological property has not yet been fully understood yet. Koda *et al*. (1982) demonstrated that the water extract of Zizyphi Fructus showed a suppressive effect on homologous passive cutaneous anaphylaxis by oral administration. In the survey of ingredients which showed anti-allergic activity, Yagi *et al*. (1981) isolated ethyl α-D-fructofuranoside from ethanol extract of Zizyphi Fructus by the

screening for passive cutaneous anaphylaxis and haemagglutination anaphylaxis. Niwa *et al*. (1985) reported that the water extract of Zizyphi Fructus prolonged aPTT and PT *in vitro*. Yamada *et al*. (1985) reported zizyphus-arabinan [(1→5)-linked a-L-arabinofuranosyl main-chain having (1→2)-linked a-arabinofuranosyl side-chains], one of the polysaccharides isolated from Zizyphi Fructus, showed considerable anti-complementary activity. The complement system plays an important role in host defence, inflammation, or allergic reactions and activation occurs *via* both the classical and alternative pathways. The classical pathway is activated by an immune complex containing IgM and IgG antibodies, the acute phase protein, C-reactive protein and RNA tumour virus. The alternative pathway does not require antibodies and is directly activated by polysaccharides, certain immunoglobulins, viruses, fungi, bacteria, certain animal cells and parasites. The activation of complement by Zizyphus-arabinan was both *via* the alternative and classical pathways. Many polysaccharides from medicinal plants, for example, algae and fungi are known to have various biological activities, especially immunopotentiating and immunomodulating activity. It has been recently reported that Sho-saiko-to augmented NK (NK) activity in the peripheral blood (Kaneko *et al*., 1994) and the activation of NK activity is due to an acidic polysaccharide fraction of Sho-saiko-to (Yamaoka *et al*., 1995). When the active components in the crude drugs in Sho-saiko-to were examined, the extracts of Zizyphi Fructus, Zingiberis Rhizoma, Scutellariae Radix, Glycyrrhizae Radix and Pinelliae tuber augmented NK activity by oral administration, and the high molecular weight fraction of Zizyphi Fructus was the most effective in augmenting NK activity. This polysaccharide fraction with a high molecular weight of approximately 43 000 contained 54.7% carbohydrate as glucose, 61.8% uronic acid as galacturonic acid and 20.9% protein as bovine serum albumin. The sugar moiety was composed of rhamnose, arabinose, xylose, fucose, mannose, galactose, glucose and galacturonic acid in molar ratios of 28 : 59 : 11 : 9 : 7 : 32 : 20 : 100 (Yamaoka *et al*., 1996). These results suggest that high molecular weight substances play important roles in the pharmacological action of Zizyphi Fructus in Sho-saiko-to. Furthermore, Tamaoki *et al*. (1996) investigated the effect of Zizyphi Fructus on ciliary motillity and NO generation in canine cultured tracheal epithelium by the microphoto-oscillation method and the specific amperometric method, respectively. They revealed that Zizyphi Fructus enhanced airway ciliary motility and that this effect was exerted through the stimulation of epithelial NO generation. Yoshikawa *et al*. (1997) isolated new dammarane-type triterpene oligoglycosides, jujubosides A1 and C, and acetyljujuboside B1 from the seeds of *Zizyphus jujuba* MILL var. *spinosa* Hu, which is used as sedative or hypnotic as another crude drug clled Zizyphi Spinosi Semen in Japanese Kampo prescription. In addition, these saponins were found to inhibit the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction.

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4 Formulation I

Quality assessment, manufacturing technology and quality control of Sho-saiko-to extract formulation

Takeshi Yamamoto

Introduction

Kampo medicines (oriental medicines), developed as a result of a long history of use, have been used in various forms such as decoction, pills, powder and liquor extract to fit the need. In the recent years, the majority has been used as a decoction (in which the chopped raw herbs are placed in an infusing pot and decocted by boiling to about one-half the volume), with a few others being used as a powder or pill formulation. The selection of raw herbs, method of decoction and method of ingestion, while based on the medical texts, are often left up to the raw herb practitioner, physician, pharmacist or the patient, so that it is often difficult to preserve and maintain the quality of the Kampo decoction for the entire duration of the use by the patient.

In addition, Kosuge pointed out in today's fast-paced society that there were additional problems, such as complexity of the decoction process and long-term storage of the decoction preparation. To correct the shortcomings and inconvenience of the decocting process, manufacturers have prepared Kampo extract formulations as Kampo medicines for medicinal use (ethical use) to meet the needs of today's society.

These Kampo extract formulations for medicinal use (ethical extract products in Kampo medicine formulations) are being used therapeutically along with Western medicines and have clinical applications especially in those diseases that are poorly understood and where Western medicines are often not sufficiently effective, such as chronic illnesses, gynaecological diseases, and immunological and allergic diseases. Along with the increasing numbers of patients with such diseases being treated with Kampo extract formulations, greater emphasis is being placed on maintaining and ensuring quality control, efficacy and safety.

Regulatory notices regarding the quality control of Kampo extract formulations for medicinal use (for ethical use)

In this chapter, we review the studies conducted by a study group of the Ministry of Health and Welfare and discuss an interpretation of the notices as they relate to the Sho-saiko-to extract formulation.

The objective of the study group of the Ministry of Health and Welfare was to ensure that the quality control (assurance) of Kampo extract formulation as a medical product for medicinal use permits the use of these products in medical treatment and other clinical applications. The study was conducted as a scientific study of the Ministry of Health and Welfare to ensure the quality of Kampo extract formulation for medicinal use. The details of this study are now discussed by Nogami *et al*. (1985) and by Miyake (1986).

The notice, 'Requirements for Prescription Extract Products in Kampo Medicine Formulation' [PAB/ERD-2 Notification No. 120 (May 31, 1985)] was published by the Medical

and Safety Bureau of the Ministry of Health and Welfare, and a request was made for submission of data regarding comparative testing of standard decoction preparations.

Thus, the objectives of the 'Assessment of Kampo extract formulation for medicinal use' in the 'Pharmaceutical Affairs Council 2, No. 120' are listed in the section, 'Data regarding comparative testing of standard decoctions' of this notice.

The objectives are summarized as follows:

1 The prescribing methods of the standard decoction formulation based on the classical medical texts.
2 Comparative study of the Kampo extract formulation to ensure quality using a decoction standard formulation for comparison.
3 Conducting a quantitative analysis of the index components of two or more components in one prescription to compare the extract and the final product against the decoction standard.
4 Bring together manufacturing facility and manufacturing conditions that will permit the manufacture of Kampo extract formulations with the quality equivalent to the standard decoction.
5 Performance of the manufacturing control and quality control in the manufacturing process of extract for Kampo medicine formulations.

That is, based on the conditions under which a decoction has been used therapeutically by Kampo physicians and the efficacy is recognized, decoction standards are established, and by conducting a comparative study of the quality against the decoction standard, one can manufacture an extract formulation of a decoction that is of the same quality. Therefore, to achieve this aim, quality evaluation and quality control are conducted, the manufacturing methods and conditions are examined, and the manufacturing facility is assembled in order to manufacture a Kampo extract formulation of desired quality.

Furthermore, in comparison with a standard decoction formulation, those components which are present as two or more components in the active raw herbs in a prescription are designated as 'index components', and these must be quantitated and the equivalence must be ensured.

Upon receiving this notice, the Kampo drug manufacturers conducted studies on standardization of decoctions and studies on manufacturing conditions and quality assessment of Kampo extracts, gathered information on the manufacturing facilities and conditions required for the manufacture of Kampo extract formulations, and organized how to conduct the quality control and test methods. In addition, medical product manufacturing guidelines, 1995 edition satisfaction of GMP standards as indicated in 'Kampo GMP Self regulatory standards' (Pharmaceutical Supervisory Notice no.72) was also planned, so that new Kampo extract formulations for medicinal use became available to the medical field.

In this chapter, the author discusses the data, provided by Nagasawa, M. and the author, on the quality assessment of Kampo extract formulations for medicinal use, manufacturing methods for Kampo extract formulation for medicinal use, and quality control of Kampo extract formulation for medicinal use (Nagasawa and Yamamoto, 1983).

Quality assessment of Kampo extract formulation for medicinal use

Comparative assessment with the standard decoction

Based on the notification (Pharmaceutical Affairs Notice no. 120) of the Ministry of Health and Welfare regarding the assessment of the Kampo extract formulation for medicinal use,

the following items were examined with respect to the manufacturing of Kampo extract formulation (Figure 4.1) for medicinal use to ensure adequacy of the manufacturing methods, manufacturing facilities and quality control assessment:

1 Origin, producer region, chemical compositional studies and marketability of the raw herbs.
2 Studies on the establishment of decoction standard based on the classical medical texts.
3 Studies on the manufacturing techniques and formulation techniques of Kampo extracts.
4 Studies on quality control assurance.
5 Data collection on efficacy and safety.

Understanding of factors affecting quality and manufacturing conditions of Kampo extract formulation

Table 4.1 shows the conditions in the manufacturing steps of the extract powder which affect the extract quality. The major factors affecting the quality of the extract solution during the extraction step are the extractability, ascending heating time, and extraction time, while the important factor in the concentrating step is the concentrating temperature. The drying method and drying time are important in the drying step. By maintaining these conditions constant, the

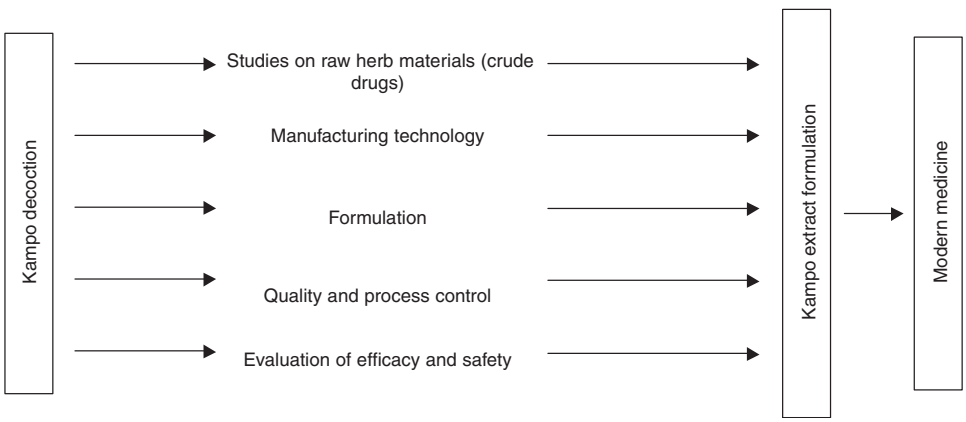

Figure 4.1 Drug design for formulation of Kampo extracts for medical use.

Table 4.1 Manufacturing conditions affecting product quality at each manufacturing step (influential variable factors)

Extraction	*Separation*	*Concentration*	*Drying*
Extraction solvent	Separation method (centrifugal separation)	Concentration method (reduced pressure concentration)	Drying method (spray dry)
Extraction volume	Separation speed	Concentration temperature	Drying temperature
Extraction temperature	Centrifugal speed	Vacuum strength	
Ascending heating time	Feed temperature	Extent of concentration	
Extraction time			
Extraction mix speed			

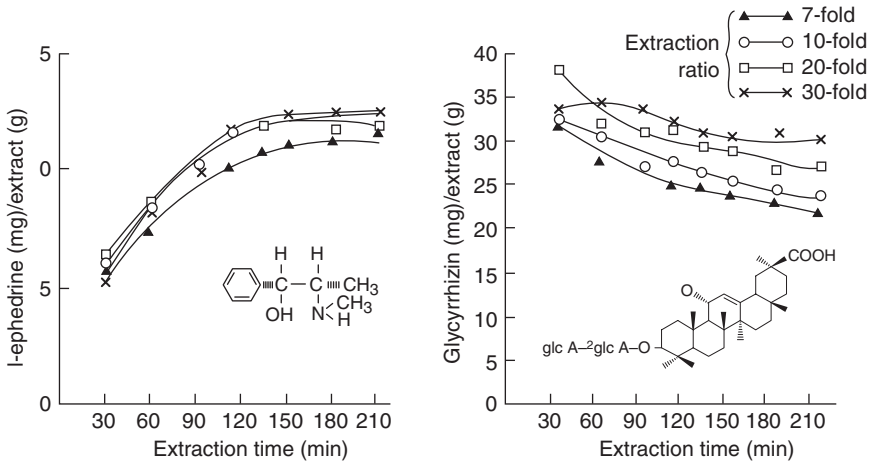

Figure 4.2 Monitoring of quality of Kampo extract formulations for medical use.

Notes
Studies on manufacturing conditions of Mao-to extract formulation. Behaviour of l-ephedrine and glycyrrhizin at extraction step (extraction time, extraction ratio).

quality of the extract powder obtained can be maintained and the quality of the objective product can be controlled (Figure 4.2).

Here we will discuss the details of Sho-saiko-to based on the data of Mao-to[1] as conducted by the Ministry of Health and Welfare study.

Figure 4.2 shows the amounts extracted of l-ephedrine and glycyrrhizin (glycyrrhizic acid) from extract solid every 30 min when Mao-to is heat-extracted with 7–30 volumes of water.

Mao-to: 5 g Mao (Ephedrae herba);
5 g Kyonin (Armeniacae semen);
4 g Keihi (Cinnamomi cortex);
1.5 g Kanzo (Glycyrrhizae radix).

l-Ephedrine was gradually extracted initially and remained constant beyond 120 min (including ascending heating time), and even with the volume of extraction agent of 10 or greater, there was no significant difference in the extractability.

On the other hand, glycyrrhizin was already extracted by 30–60 min after the start of the extraction and was constant thereafter, although differences in the extractability were seen depending on the ratio of the extraction agent.

Thus, the behaviour of the extraction differs depending on the properties of the components of the raw herb contained in the Kampo medicine prescription. It is, therefore, necessary to have the extraction agent ratio and extraction time be controlled such that the quality is similar to the decoction standard based on experimental data.

Glycyrrhizae radix is also a raw herb component in the Sho-saiko-to, and behaviour of the Glycyrrhizae radix component (glycyrrhizin) is similar to that shown in Figure 4.2. Therefore, controlling the extraction ratio and extraction volume is important.

It is known that some raw herb components are hydrolysed by enzymes present in the raw herbs. As indicated in Figure 4.3, the baicalin found in Scutellaria baicalensis is hydrolysed by

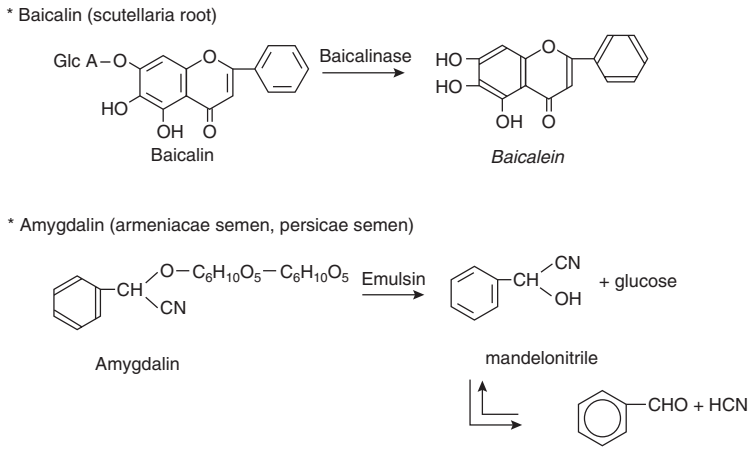

Figure 4.3 Components in raw herbs degraded by enzymes (enzymatic hydrolysis).

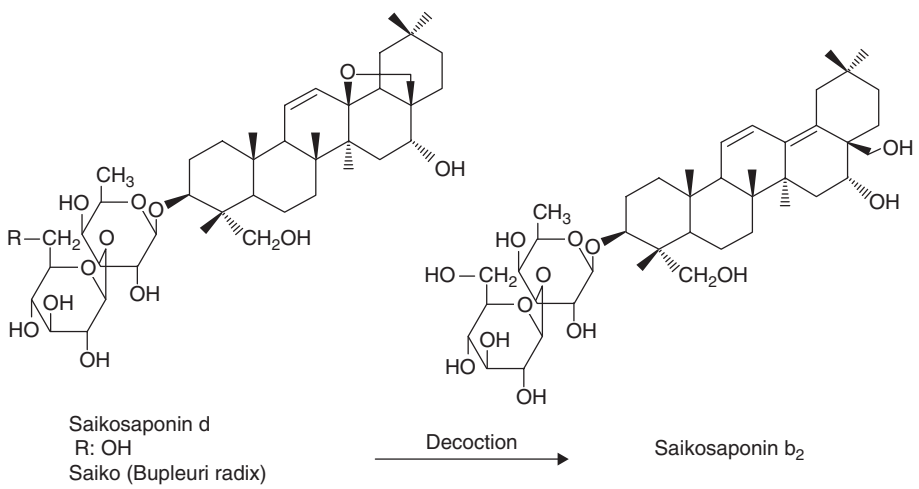

Figure 4.4 Behaviour of components during decoction of Bupleuri radix (saikosaponin).

the enzyme baicalinase into baicalein, while the amygdalin contained in almond and peach kernels is hydrolysed by the enzyme emulsin to mandelonitrile and benzaldehyde.

When the raw herbs are mixed with water and decocted, the enzymes in the raw herbs carry out hydrolysis during the time period from when the water temperature is low until the water reaches the boiling point. By altering the heating conditions, the proportion undergoing hydrolysis varies, and changes in the size or the scale of the extraction tank used for the extraction of the Kampo extract can affect the heating time. To ensure that there is no difference in the extent of hydrolysis, it is necessary to control the heating unit, facility and heating conditions.

Scutellaria baicalensis is contained as a component raw herb in Sho-saiko-to. During the manufacturing of Sho-saiko-to extract, it is important to control and maintain the ascending heating time in reference to the data from the decoction standard.

The saikosaponins contained in the Saiko Bupleuri Radix after decoction are also hydrolysed, and saikosaponin d (a) is converted into saikosaponin b_2 (b_1) (Figure 4.4). If the extraction temperature and time are not maintained, the proper ratio of these compounds cannot be maintained.

Thus, properly controlling the ascending heating time for the water to reach the boiling point to 100°C and the infusion time thereafter is necessary to properly control this process.

Methods for manufacturing Kampo extract formulations for medicinal use

Consistent production, from selection of raw herbs to manufacture of Kampo extract formulation (extract granule formulation)

The manufacturing process for Kampo extract formulations (Figure 4.5) (extract granule formulations) can be broadly divided into the extract powder manufacturing step, the formulation step and the packaging step. The major points for each step are now described.

The manufacturing process for extract granules is composed of the raw herb selection, chopping process, weighing of raw herb, extraction and separation step, concentration step and spray drying to convert the concentrate into an extract powder form.

Among these steps, the one that particularly affects the quality of the extract powder is the extraction step. In this step, the important factors (variables) are the heating time and extraction time, and extraction ratio (volume of water relative to the raw herbs). The factors important for the concentration step and drying step are the heating temperature. It is important that the conditions be appropriately selected.

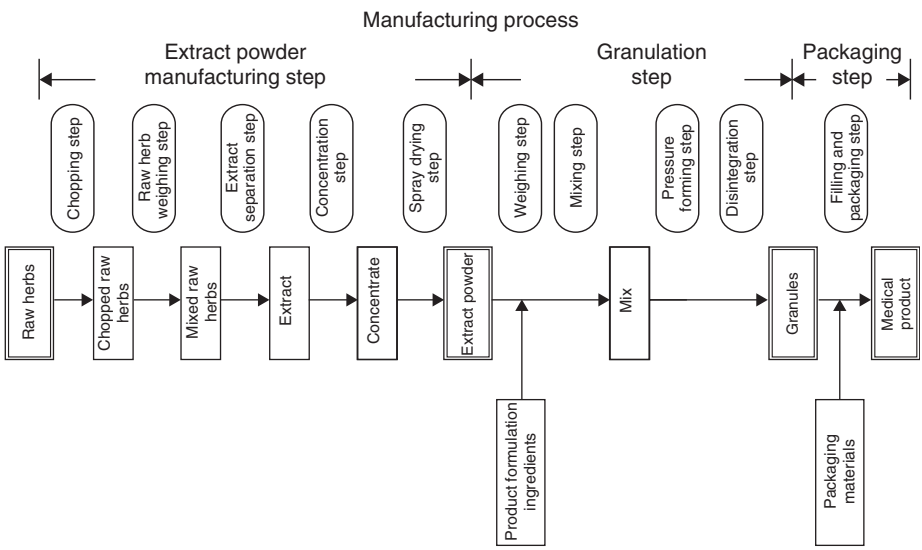

Figure 4.5 Manufacturing process for Kampo extract granules (for medical use).

The formulation step involves the weighing and mixing of the extract powder and raw materials (filler) needed in the formulation, pressure moulding step using the dry powder method, and homogenizing step to form the powder formulation.

As indicated in Figure 4.5, conditions have been established for the manufacturing step, formulation step, packaging step for the extract granules and consistent manufacturing has been achieved by the appropriate selection of raw herbs, manufacturing of extract granules, and foil packaging and packaging into 500-g bottles.

Manufacturing of Kampo extract granules: spray drying method

If one can obtain Kampo extracts and concentrates that are of equivalent quality to the decoction standard, then they can be dried and made into an extract powder without any loss of the quality.

After concentrating the Kampo extract solution, the extract powder can be obtained by the spray dry method or the freeze dry method (Figure 4.6).

In the spray dry method, hot air is introduced as the sprayed concentrate falls through the spray dry chamber, and the concentrate droplets are immediately dried into an extract powder. In this method, there is concern that the hot air may degrade the components in the concentrate.

Thus, an experiment was conducted on the extract obtained from the Rhei rhizoma. If an extract powder is prepared from a Rhei rhizoma extract with the inlet air temperature in the spray dryer at 120–250°C, the sennoside A content decreases somewhat at 200°C or greater, while drying is possible without decomposition of the contents in the extract at less than 200°C (Figure 4.7). The stability of the components of the extract powder is due to the fact that during the evaporation of water in the extract droplets, the vaporization heat is lost, so that the magnitude of the temperature increase in the extract powder is less than expected.

Thus, through precise control of the spray dry conditions, the spray dry process permits the preparation of extract powder without affecting the quality of the Kampo extract and without decomposition of those Kampo prescriptions containing heat-unstable components.

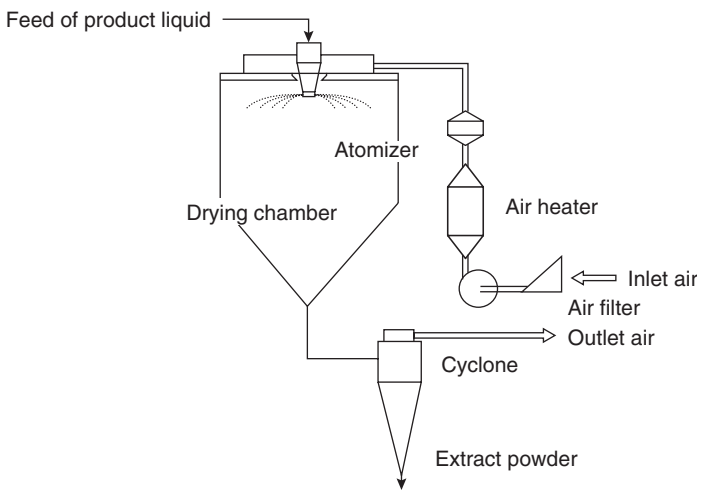

Figure 4.6 Process flow diagram of spray drying apparatus (Kampo extract powder manufacturing).

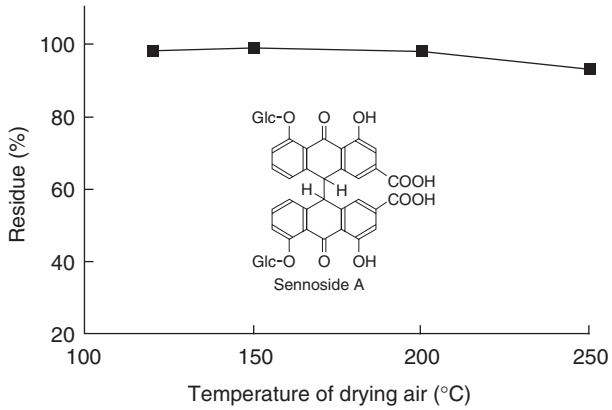

Figure 4.7 Stability of sennoside A in Rhei rhizoma extract fluid with respect to various inlet air temperatures in spray drying.

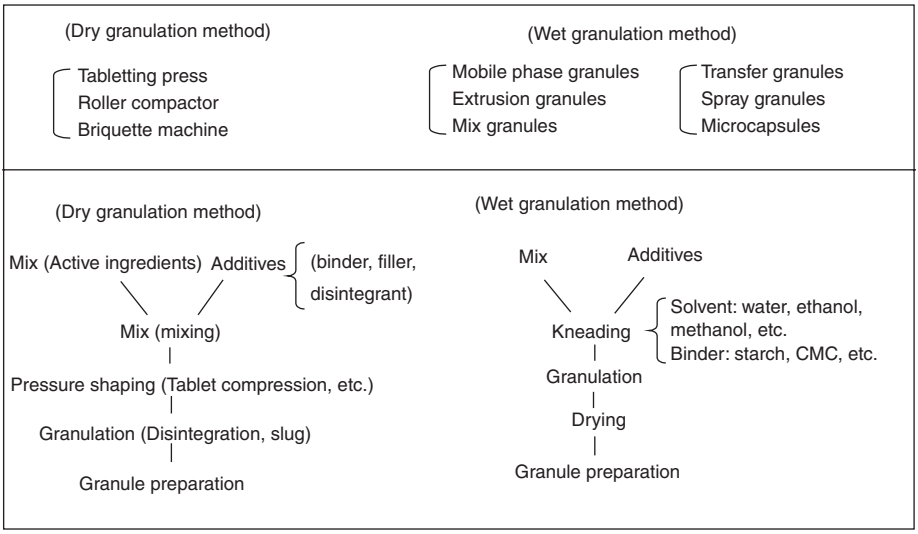

Figure 4.8 Manufacturing of granules (dry granulation method, wet granulation method).

Manufacture of Kampo extract formulation: granule formulation by dry granulation method

The Kampo extract powder has multiple components and is extremely hygroscopic, so if it is left standing at room temperature, it undergoes colour changes, clumping and becomes sticky. This can lead to poor stability and difficulties in the formulation and administration. Therefore, formulation requires the use of fillers.

Making granules from extract powder uses primarily either the dry granulation method or the wet granulation method (Figure 4.8).

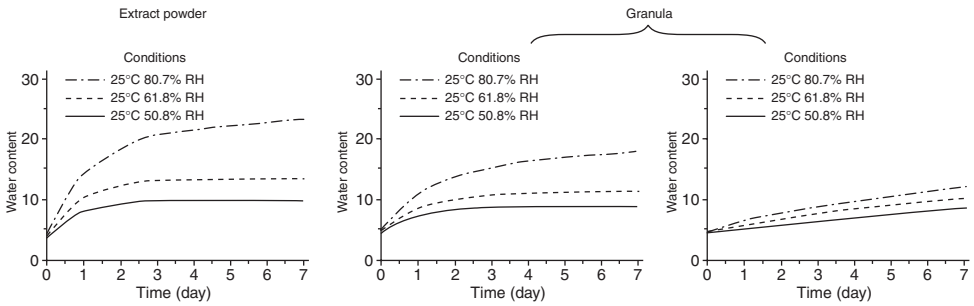

Figure 4.9 Stability of extract granule formulation (hygroscopicity) (Sho-saiko-to extraction powder and extract granules).

In the dry granulation method, water or organic solvents are not used, and there is also no heat drying step. This is considered to be ideal for the formulation of medical products, such as Kampo medicines, containing multiple components, some of which may be heat-unstable components.

In this way, the dry granular formulation prepared by adding a designated amount of filler to the extract powder can greatly reduce the highly hygroscopic nature of the extract powder (Figure 4.9).

Quality control of Kampo extract formulation for medicinal use

The most important characteristic of the Kampo formulations for medical use is that unlike synthetic medical products, it is made of raw herbs. Ensuring the quality of the raw herbs is extremely important in the preservation of the quality of the Kampo formulation. To ensure the preservation and maintenance of the product quality of the Kampo extract formulation for medical use, the important factors are: (1) use of raw herb materials with a clear source of origin and uniform quality, (2) detailed quality check from the raw herb source to the final product, namely (a) monitoring by personnel responsible for raw herb handling and by those responsible for sample checks; (b) checking of product quality of intermediate products at each step and (c) understanding and overall management of all manufacturing conditions, (3) establishment of appropriate quality control items, namely (a) establishment of standards for those with two or more components in a single prescription; (b) establishment of inspection items such as index composition and others and (4) use of a production engineering information system to ensure comprehensive product management and process monitoring.

Assurance of raw herb and quality control

We now discuss the assurance of quality of the raw herbs that are the source of the Kampo extract formulation (Figure 4.10). To preserve the efficacy and safety of Kampo extract formulation with uniform quality, it is obvious that monitoring of the manufacturing process is important, but as stated above, it is also important to assure the product quality of the raw herbs at the source.

To accomplish this task, it is necessary to study the factors which affect the product quality, such as origin, producer region, properties and preparation of the raw herb, and to establish and maintain appropriate conditions. Maintaining these quality control factors will produce uniform components and ensures that the raw herb will be of high efficacy.

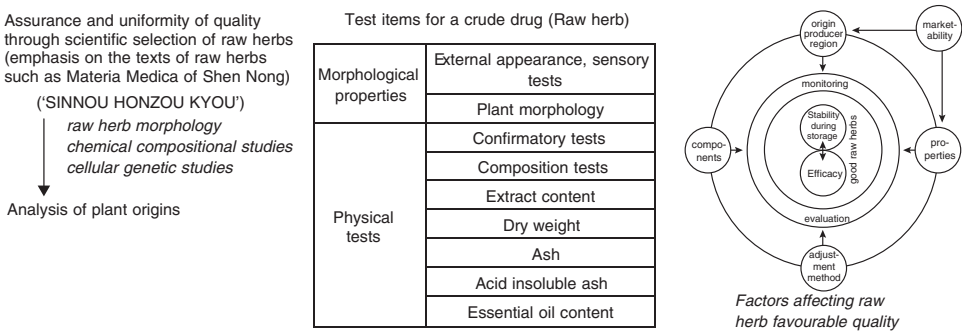

Figure 4.10 Assurance of raw herb materials and quality control.

Table 4.2 Safety assurance of raw herbs

1 Regulation of residual pesticides
Methods for testing impurities in raw herbs [13th revision, Japan Pharmacopoeia, addendum (effective October 1997)]

- Applicable raw herbs: ginseng, senna leaf
- Applicable pesticides: total BHC, total DDT (0.2 ppm or less)
- Measures taken: restriction of type of pesticides used, restriction of timing of use

2 Control of microbiological contamination
(1) Conduct of microbiological tests
['Microbiological limitation tests' 13th revision, Japan Pharmacopoeia, general test methods]
(2) Prevention of mycotoxin contamination

- Tests for aflatoxin-producing *Aspergillus flavus*
- Hygienic control during harvesting and preparation of raw herbs
- Monitoring of temperature and humidity during shipment and storage of raw herbs

Use of raw herbs with assured quality and manufacturing processes that are controlled and monitored permits the manufacturing of extract and formulation of the desired quality (Table 4.2).

To manufacture Kampo extracts with a high degree of safety, one needs to ensure that the raw herbs are safe. It is especially necessary to regulate residual pesticides and control microbiological contamination.

It is necessary to regulate or prohibit the use of various pesticides during the cultivation and storage of raw herbs and to restrict the distribution period. The 13th revision of Japan Pharmacopoeia addendum includes regulations regarding the residual BHC and DDT in ginseng and senna by conducting contamination tests (purity tests).

For the prevention of microbiological contamination, it is important to conduct tests for aflatoxin-producing organisms (Aspergillus flavus) to prevent mycotoxin contamination, which is known to be carcinogenic, and to monitor the temperature and humidity during storage.

Quality control items in Kampo extract formulation

Next we discuss the monitoring of quality of the Kampo extract formulation. Table 4.3 shows the quality control test items of the manufactured Kampo extract formulations (granular

Table 4.3 Test item for Kampo extract formulations for medicinal use (granules)

Test item	*Test objective and method*
Physical properties Colour, properties, taste, odour, pH	Measurement of properties and pH specific to a prescription ensures product quality
Identifications	TLC method, Qualitative tests for characteristic
Confirmatory tests (Quantitative assay)	• Test for 'Indicator ingredients' (index components) to ensure equivalence to standard decoction (Pharmaceutical Affairs Council, notice 120) • Index components for assurance of product quality (establish two or more components) (Monitoring of process and product quality through tests of specific components in raw herb) • HPLC method
Tests for extract content	Measurement of the soluble components in an appropriate solvent ensures comprehensive quality (e.g. Dehydrated ethanol extract)
Purity tests Heavy metals, arsenic, acid-insoluble ash, ash	No foreign matter is introduced during the manufacturing process. Confirms that the manufacturing was conducted properly (ash contains also the non-volatile inorganic material derived from the raw herbs contained in the prescription)
Water content determination	Measurement of the water content (by Karl Fisher method) to confirm that it is below a certain content is useful as an index of product stability
Formulation tests (Pharmaceutical preparation tests) Degree of disintegration, degree of granulation, weight variations	Confirms that the granular formulation is appropriate for a medical product
Microbiological limit tests	Confirms that industrial hygiene was properly considered during manufacturing of medical product (Bacteria, Fungi, Specified microbial species)

formulation package). Quality control is conducted by confirmatory tests of physical properties and TLC, quantitative tests of index components by monitoring of 2–3 components (indicator ingredients) by HPLC, purity tests, microbiological tests and formulation tests.

Table 4.4 shows monitored components of Kampo extract formulations. Two to three components for indicator ingredients are monitored in combination as a part of the quality control monitoring of the formulations. These are also used for quality control of the raw herbs (Figure 4.11).

Dissolution tests of Sho-saiko-to extract granules (TJ-9) are shown. The glycyrrhizin and baicalin (and saikosaponin b2), which are the index components monitored in Sho-saiko-to, are eluted to 100% in 10–15 min (80% for the case of saikosaponin b2 because of its solubility in water), with little lot-to-lot variation.

Manufacturing facility and monitoring of manufacturing hygiene in reference to prevention of microbiological contamination

The microbiological tests of the Kampo extract formulations that are independently conducted by a Kampo extract formulation maker are shown (Table 4.5). The tests are conducted in accordance

Table 4.4 Indicator ingredients (index components) for quality control in Kampo extract formulations for medicinal use

Crude drug (raw herb)	*Indicator ingredients (index components) for quality control {example}*
Glycyrrhizae radix	Glycyrrhizin (Glycyrrhizic acid)
Paeoniae radix, Moutan cortex	Paeoniflorin, Paeonol
Coptidis rhizoma, Phellodendri cortex	Berberine
Scutellariae radix	Baicalin, Baicalein, Wogonin
Ephedrae herba	l-Ephedrine, pseudo-Ephedrine
Ginseng radix	Ginsenoside Rb_1, G-Rb_2, G-Re
Bupleuri radix	Saikosaponin a, S-d
(saiko formulation)	(Saikosaponin b_1, S-b_2)
Puerariae radix	Puerarin, Daidzein
Pinelliae tuber	Adenine
Alismatis rhizoma	Alisol A(B,C) monoacetate
Asiasari radix	l-Asarinin
Cinnamomi cortex	Cinnamic acid, Cinnamic aldehyde
Zizyphi fructus	Cyclic AMP, Zyzyphus saponin
Corydalis tuber	Dehydrocorydaline
Artemisiae capillarI spica	6,7-Dimethylescletin, Capillarisin
Aurantii fructus immaturus	Naringin, Hesperidin
Evodiae fructus	Evodiamine, Rutaecarpine
Cnidii rhizoma	Ligustilide, Ferulic acid
Gardeniae fructus	Geniposide, Genipin
Asini corri collas	Glycine
Corni fructus	Loganin, Morroniside
Aurantii nobilis pericarpium	Hesperidin, Naringin
Magnoliae cortex	Magnolol, Honokiol
Anemarrhenae rhizoma	Mangiferin
Saposhnikoviae radix	5-methylvisaminol glucoside
Platycodi radix	Platycodin D
Rhei rhizoma	Sennoside A, Sennoside B
Sinomeni caulis et rhizoma	Sinomenine
Zingiberis rhizoma, Z. siccatum r.	Shogaol, Zingerol
Rehmanniae radix	Catalpol, Stachyose
Schisandrae fructus	Schizandrin, Gomisin A
Aconiti tuber	Aconite alkaloids (aconitine, jesaconitine, mesaconitine, hypaconitine) (Processed Aconiti tuber)

with the microbiological testing of Japan Pharmacopoeia, with highly strict standards, thus assuring the safety of the medical product.

To achieve these standards, it is important that the personnel wash their hands before entry into the production facility, detailed hygienic monitoring by the use of air shower and uniforms, the use of total stainless steel systems for the entire manufacturing equipment, the use of totally closed systems in the manufacturing line to prevent the introduction of microbiological agents from the outside, and a wash system that allows washing of the entire manufacturing line.

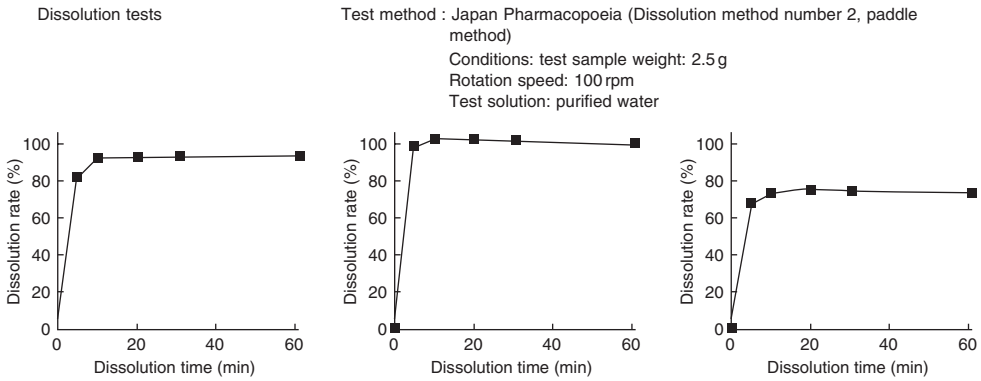

Figure 4.11 Dissolution curve of Sho-saiko-to extract granules (in purified water).

Notes
1 Dissolution behaviour of glycyrrhizin.
2 Dissolution behaviour of baicalin.
3 Dissolution behaviour of saikosaponin b_2.

Table 4.5 Microbiological tests for Kampo extract granules (for medical use)

			Standard of self-determined limit
Microbiological tests	Total viable aerobic count	Bacteria	≦100 cfu/g*
		Fungi	≦100 cfu/g
	Detection test of specified bacteria	*Escherichia coli*	Negative
		Staphylococcus aureus	Negative
		Pseudomonas aeruginosa	Negative
		Salmonella	Negative

Note
* cfu/g: Colony forming unit per gram of Kampo extract powder, granules and raw herbs.

Conclusions

We have discussed quality control of the Kampo extract formulations for medical use (establishment of quality standards of Kampo extract formulation, important factors in manufacturing methods and quality control). Today, Kampo extract formulations for medicinal use are used in a wide range of diseases, along with Western medicines (synthetic medical products), and their clinical utility is well recognized. On the basis of re-confirmation by the Ministry of Health and Welfare, double-blind clinical trials are in progress, and the efficacy and safety are being reconfirmed.

For the Kampo extract formulation to be widely accepted by physicians and patients as medical products and to be useful in medical therapy, one must continue to place great efforts in the quality assurance and stability of the Kampo extract formulation for medical use, with demonstration of efficacy, preservation of safety and development of formulations that are easy for patient administration.

Acknowledgement

The author wishes to acknowledge Jiho, Inc. for permission to use figures and tables presented in The Pharmaceuticals Monthly. The figures and tabular presentations used in this chapter are a translation of those in The Pharmaceuticals Monthly 39(11), 69–78 (1997).

Note

1 Mao-to is obtained by decocting the following four crude drugs at the same time; Apricot Kernel, Ephedra Herb, Cinnamon Bark and Glycyrrhiza Root.

Formulation II

Pharmacokinetics, toxicology and pharmacology of Sho-saiko-to

Sakae Amagaya

Sho-saiko-to is widely used in the treatment of patients with type B or C active hepatitis in Japan. Fujiwara and Mochida (1992) reported that Sho-saiko-to decreased the serum transaminase activities and induced seroconversion from HB antigen positive to HB antibody positive in type B active hepatitis patients. In type C active hepatitis patients, Gibo *et al*. (1994) and Kumada (1998) reported that Sho-saiko-to decreased serum transaminase activities, and Sekinaga *et al*. (1999) reported that Sho-saiko-to decreased serum markers of fibrosis, procollagen III peptide and 7S collagen. Oka *et al*. (1995) reported that Sho-saiko-to clinically inhibited the development of hepatocyte cancer from cirrhosis. In addition, Sho-saiko-to is used to treat many inflammatory diseases like cold, pulmonary inflammation, ulcer etc.

This chapter summarizes the pharmacokinetic, toxicological and pharmacological properties of Sho-saiko-to.

Pharmacokinetic study

Kampo medicine has many ingredients and most of them are unknown, although many researchers have reported the structural properties of the ingredients and their pharmacological activities. Some of the ingredients in Kampo medicine are glycosides, which are typical for plant components, and they are commonly metabolized to their aglycones in the intestine by the actions of several glycosidase secreted from intestinal flora. These characteristics complicate the pharmacokinetic and pharmacological studies of Kampo medicine.

Sho-saiko-to, one of the Kampo medicines, is an extracted mixture of seven crude drugs and its ingredients seem to be numerous. The main ingredients of Sho-saiko-to are shown in Figure 4.12, which is a three-dimensional HPLC profile. The quality of Sho-saiko-to is regulated by the quantity of two main components, baicalin (BG) and glycyrrhizin (GL). Both compounds are glucuronides, and Hattori *et al*. (1983) and Akao *et al*. (1994) reported that they were easily metabolized to their aglycones in intestine. When GL is administered orally, sugar moiety, di-glucronic acids, is separated by glucuronidase in intestine, and the reaction compound, which is an aglycone, glycyrrhetinic acid (GA), is transfered to the blood flow (Figure 4.13). When BG is administered orally, sugar moiety, glucuronic acid, is separated in intestine as well as GL and the aglycone, baicalein, is absorbed from intestine. However, this absorbed baicalein is rapidly glucuronized to baicalin, a starting compound, and other glucuronides in the intestinal epitherial cells, and most of baicalein is absorbed in the blood flow as transformed glucuronides (Figure 4.13). Kobashi (1998) reported the transformation of glycosides using germ-free and gonotobiotic rats.

Uchida *et al*. (1995) reported the plasma GA and BG concentrations, which are transformed in intestine, by the oral administration of Sho-saiko-to, 5 and 10 g/kg, to healthy volunteers.

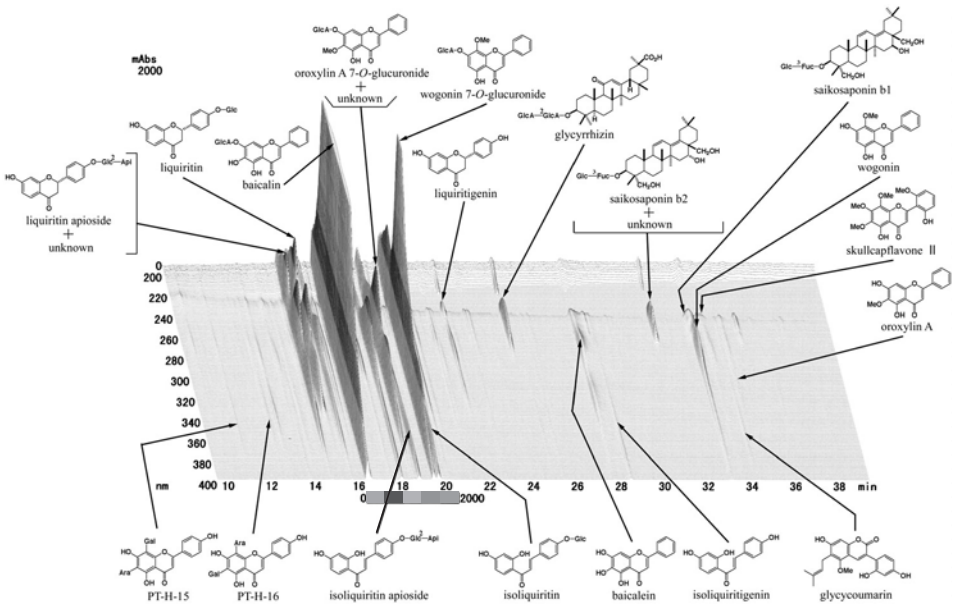

Figure 4.12 3D-HPLC profile of Sho-saiko-to extract.

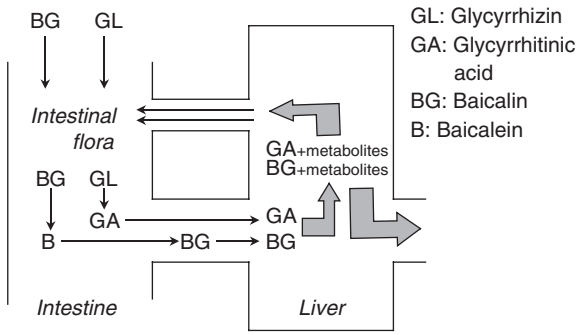

Figure 4.13 Intestinal transformation of GL and BG.

Plasma GA increases in a dose-dependent manner and reaches a maximum 12 h after the oral treatment of Sho-saiko-to. In this study, GL did not appear in the plasma (Figure 4.14). Plasma BG concentration reached to maximum level 6–8 h after the Sho-saiko-to treatment (Figure 4.15). The delay of the appearance of the peaks of the plasma GA and BG, and their disappearance from blood was due to the mild hydrolization process of sugar bond in the intestine by glucuronidase. These results suggest that these glycosides, GL and BG, should be the natural pro-drugs for long-lasting clinical effects by maintaining the high blood concentration of active metabolites.

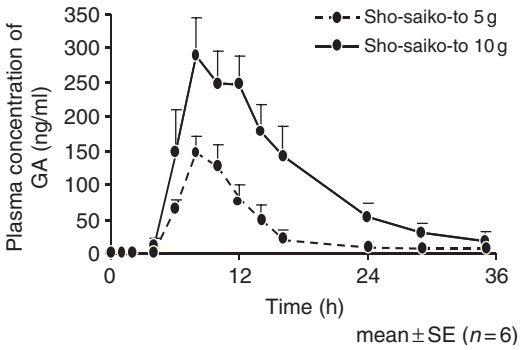

Figure 4.14 Plasma GA concentration after the oral administration of Sho-saiko-to to healthy volunteer.

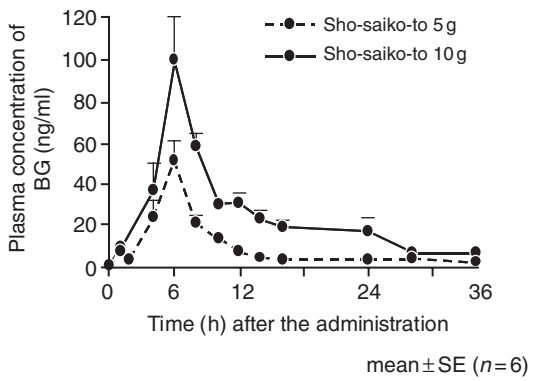

Figure 4.15 Plasma BG concentration after the oral administration of Sho-saiko-to to healthy volunteer.

Besides GL and BG, Sho-saiko-to has many active compounds like polysaccharides, proteins, flavonoids, saikosaponins, ginsenosides, gingerols, shogaols, cAMP, etc. Therefore, the pharmacological activities of Sho-saiko-to can not be explained by the activity of only two ingredients. The additive and synergistic actions of the ingredients are essential for an understanding of Sho-saiko-to's remarkable pharmacological and clinical effects. Therefore, the effect of extract itself on safety and pharmacological study should be examined for the evaluation of Sho-saiko-to.

Toxicological properties

General toxicological studies

A single oral dose toxicity study

Minematsu *et al*. (1995) reported a single dose toxicity study using SD rats of both sexes to assess the potential acute toxicity of Sho-saiko-to. Sho-saiko-to was orally administered to rats at dose

levels of 2000 and 6400 mg/kg after overnight fasting. There were no deaths, and the oral approximate lethal dose was estimated to be over 6400 mg/kg. Clinical signs and terminal autopsy showed no treatment-related abnormalities. These results show that Sho-saiko-to has low acute toxicity potential under the experimental conditions.

A 13-week repeated dose toxicity study

Minematsu *et al*. (1992) reported a 13-week repeated dose toxicity study using SD rats of both sexes to assess the potential sub-chronic toxicity of Sho-saiko-to. Sho-saiko-to was orally administered at dose levels of 125, 500 and 2000 mg/kg/day for a period of 3 months followed by a 4-week recovery period. The general conditions, mortality, ophthalmology, body weight, food consumption, clinical laboratory studies (urinalysis, haematology, biochemistry), organ weight and pathology showed no toxicologically significant changes. These results show that a dose of Sho-saiko-to up to 2000 mg/kg/day produces no toxicologically significant effects.

Toxicokinetics in 13-week repeated dose toxicity study

The purpose of a toxicokinetic study conducted in a long-term safety study is to assure that the animals are being continuously exposed to the drug in a dose-related manner throughout the safety study and to find any unusual drug accumulation that might be associated with unexpected toxicity. Yanagisawa *et al*. (1992) studied the absorption of two main active ingredients, BG and GA contained in Sho-saiko-to, in male rats.

Judging from their plasma concentration profile, the absorption after single oral administration is dose-dependent. The toxicokinetics of Sho-saiko-to was studied in rats by giving the drug at 125, 500 and 2000 mg/kg/day orally for over 13 weeks. The plasma concentration of BG is kept at almost the same level during the treatment period and its level increases dependently to the administered dose. Through the 13 weeks, the concentration remained at their respective levels. All doses were well tolerated and the maximum non-toxic dose was estimated to be more than 2000 mg/kg/day. There was a significant correlation between the dose and the AUC (ng. weeks/ml); $r = 0.9998$, $P < 0.004$. After the withdrawal of Sho-saiko-to at the end of the 13-week period, the concentration of BG decreased rapidly. Plasma concentration of GA showed a dose-dependent increase, but there was no linear regression relationship; $r = 0.9814$, $P < 0.121$. This result showed an insufficient absorption of GA at the 2000 mg/kg dose, and could be explained by the delay of the absorption of GA at the 2000 mg/kg dose as seen in the single dose study. The total absorption estimated from the AUC increased dose-dependently. This study clearly demonstrates that dose-dependent exposure of Sho-saiko-to occurs in rats when BG and GA are used as marker compounds.

Mutagenicity studies

Kuboniwa *et al*. (1999) reported the mutagenic potential of Sho-saiko-to by reversed mutation, chromosomal aberration, micronucleus tests and unscheduled DNA synthesis (UDS) assay.

Reverse mutation test (in vitro)

Four strains (TA100, TA1535, TA98, TA1537) of *Salmonella typhimurium* and one strain (WP2uvrA) of *Escherichia coli* were used. The S9 mixture was used for the metabolic activation. A plate incorporation method was used.

Table 4.6 Bacterial reverse mutation test of Sho-saiko-to

Compound	*Concentration* (μg/plate)	*S9Mix*	*No. of revertant colonies (mean of 2 plates)*				
			TA100	*TA15535*	*WP2uvrA*	*TA98*	*TA1537*
DW	0	−	132	14	28	11	6
ST	313	−	117	10	21	18	7
	625	−	115	19	21	16	7
	1250	−	147	13	24	14	8
	2500	−	145	17	27	15	9
	5000	−	167	10	29	14	10
AF2	0.01	−	814	NT	161	NT	NT
	0.1	−	NT	NT	NT	398	NT
NaN3	0.5	−	NT	395	NT	NT	NT
9AA	80	−	NT	NT	NT	NT	824
DW	0	+	118	14	27	17	12
ST	16	+	107	NT	NT	NT	NT
	31	+	140	NT	NT	NT	NT
	63	+	114	NT	NT	NT	NT
	125	+	108	NT	NT	NT	NT
	156	+	NT	NT	NT	NT	14
	250	+	117	NT	NT	NT	NT
	313	+	NT	6	29	27	16
	500	+	156	NT	NT	NT	NT
	625	+	NT	11	31	28	21
	1250	+	NT	8	34	27	39
	2500	+	NT	8	29	20	50
	5000	+	NT	10	42	18	55
2AA	0.5	+	NT	NT	NT	205	NT
	1	+	2103	NT	NT	NT	NT
	2	+	NT	340	NT	NT	416
	10	+	NT	NT	1518	NT	NT

Notes
DW: Distilled water, AF2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, NaN_3: Sodium azide, 9AA: 9-aminoacridine, 2AA: 2-aminoanthracene, NT: Not detected.

By the addition of higher concentration of Sho-saiko-to, 1250, 2500 and 5000 μg/plate, the number of colonies increased by more than twice only in the TA1537 strain with the metabolic activations (Table 4.6).

Chromosomal aberration test (in vitro)

The cultured Chinese hamster lung cells (CHL) were used. The number of aberrant cells did not increase after the treatment with Sho-saiko-to (1.25, 2.5 and 5 mg/ml) with or without metabolic activation (Tables 4.7 and 4.8).

Micronucleus test (in vivo)

In vitro tests are not suitable for the mixtures like herbal drugs or Kampo medicines. The non-selective actions that are not shown in *in vivo* assay systems often occurs by the physical and/or chemical reactions of low and high molecular compounds, which have no pharmacological activity, with enzymes and/or cell membrane receptors. For these reasons, *in vivo* tests are necessary to

Table 4.7 Chromosomal aberration test of Sho-saiko-to treated for 24 and 48 h without metabolic activation

Compound	*Concentration* (mg/ml)	*S9Mix*	*Time schedule* (h)	*No. of cells*	*Structural aberrations (%)*						*TA* (%)	*Poly* (%)
					g	*ctb*	*cte*	*csb*	*cse*	*o*		
DW	0	−	24	100	1	0	0	0	0	0	1	0
		−	48	100	0	0	0	0	0	0	0	1
Sho-saiko-to	1.25	−	24	100	0	0	0	0	0	0	0	1
		−	48	100	1	0	0	0	0	0	1	1
	2.6	−	24	100	1	1	0	0	0	0	2	1
		−	48	100	1	0	0	0	0	0	1	0
	5	−	24	100	1	0	0	0	0	0	1	1
		−	48	100	1	1	0	0	0	0	2	1
ENNG	0.005	−	24	100	23	29	36	0	0	0	62	1
		−	48	100	18	25	19	0	0	0	39	0

Notes
g: gao, ctb: Chromatid break, cte: Chromatid exchange, csb: Chromosome break, cse: Chromosome, exchange, o: Others, TA: Total aberrant cells, Poly: Polyploid, DW: Distilled water, ENNG: N-ethyl-N′-nitro-N-nitrosoguanidine (positive control).

Table 4.8 Chromosomal aberration test of Sho-saiko-to treated for 6 h with or without metabolic activation

Compound	*Concentration* (mg/ml)	*S9Mix*	*Time schedule* (h)	*No. of cells*	*Structural aberrations (%)*						*TA* (%)	*Poly* (%)
					g	*ctb*	*cte*	*csb*	*cse*	*o*		
DW	0.00	−	6–18	100	1	0	0	0	0	0	1	1
		+	6–18	100	0	0	0	0	0	0	0	1
Sho-saiko-to	1.25	−	6–18	100	1	0	0	0	0	0	1	1
		+	6–18	100	1	0	0	0	0	0	1	0
	2.50	−	6–18	100	0	1	0	0	0	0	1	0
		+	6–18	100	1	0	0	0	0	0	1	1
	5.00	−	6–18	100	1	1	0	0	0	0	2	1
		+	6–18	100	1	1	0	0	0	0	2	0
B[a]P	0.02	−	6–18	100	2	1	0	0	0	0	3	1
		+	6–18	100	4	7	29	0	0	0	38	1

Notes
g: Gap, ctb: Chromatid break, cte: Chromatid exchange, csb: Chromosome break, cse: Chromosome exchange, o: Others, TA: Total aberrant cells, Poly: Polyploid, DW: Distilled water, B[a]P: bexzo[a]pyrene (positive control).

estimate the quality of the medicinal mixtures. The micronucleus test is the *in vivo* evaluation system for chromosomal aberration. Sho-saiko-to (2500, 5000 and 10,000 mg/kg/day) was orally administered for two days. Twenty four hours after the final treatment, the smears of bone marrow were prepared. Sho-saiko-to showed no significant increase in micronucleated polychromatic erythrocytes (Table 4.9).

Unscheduled DNA synthesis (UDS) assay (in vivo)

UDS assay is the *in vivo* evaluation system for reverse mutation. Sho-saiko-to (2000 mg/kg) is orally administered to rats. After the removal of the liver, the ^{3}H thymidine uptake of primary

Table 4.9 Micronucleus test using bone marrow erythrocytes from ICR male mice after oral administration of Sho-saiko-to

Compound	*Dose* (mg/kg/day)	*Sampling time* (h)	*No. of mice*	*No. of PCE scored for MNPCE*	*No. of MNPCE*	*Frequency of MNPCE (%)*[a]	*Frequency of PCE (%)*[a]
DW	0	24	5	5000	9	0.18 ± 0.04	57.4 ± 1.39
Sho-saiko-to	2500	24	5	5000	4	0.08 ± 0.08	57.4 ± 1.71
	5000	24	5	5000	7	0.14 ± 0.09	57.1 ± 1.80
	10 000	24	5	5000	8	0.16 ± 0.05	57.9 ± 1.02
MMC	2[b]	24	5	5000	331[c]	6.62 ± 0.33	45.4 ± 0.91[d]

Notes
MNPC: Micronucleated polychromatic erythrocytes, PCE: Polychromatic erythrocytes, DW: Distilled water, MMC: Mitomycin C (positive control).
a mean ± SD.
b mg/kg (single i.p. injection).
c $P < 0.05$ vs DW group.
d $P < 0.01$ vs DW group.

Table 4.10 Unscheduled DNA synthesis assay using the liver of male rats after oral administration of Sho-saiko-to

Compound	*Dose* (mg/kg)	*Sampling time* (h)	*No. of rats*	*Nuclear grains mean ± SD*	*Cytoplasmic grains mean ± SD*	*Net nuclear grains mean ± SD*
DW	0	2	3	4.6 ± 0.5	7.0 ± 0.4	−2.4 ± 0.3
		12	3	6.6 ± 1.2	9.7 ± 0.1	−3.1 ± 1.2
Sho-saiko-to	2000	2	3	6.8 ± 1.2	9.3 ± 1.9	−2.5 ± 0.9
		12	3	7.6 ± 1.1	11.0 ± 1.0	−3.3 ± 0.3
DMN	5	2	3	35.5 ± 1.9	5.7 ± 0.6	29.8 ± 2.1
		12	1	45.0 ± 6.7	8.0 ± 0.4	37.0 ± 7.0

Notes
DW: Distilled water, DMN: Dimethylnitrosamine (positive control).

cultured hepatocyte was examined. The frequency of unscheduled DNA synthesis did not increase as a result of treatment of Sho-saiko-to (Table 4.10).

The negative results in UDS assay after the oral administration of Sho-saiko-to suggest that the positive result in reverse mutation test in TA1537 strains with S9 mixture are due to the non-selective influence by the unknown substances in the extracted mixture *in vitro*.

Reproductive and development toxicity studies

Shimazu *et al*. (1997) studied the reproductive and development toxicity of Sho-saiko-to. Sho-saiko-to at dose levels of 1000 and 2000 mg/kg was administered orally once daily to groups of 20 male and 40 female (20 for embryo/foetuses observation and 20 for F1 pups observation) CD rats. Males were treated for 4 weeks before the mating period, during the two mating periods and after copulation until the day before necropsy. Females were treated for 2 weeks before the mating period, during the mating period and after successful copulation until day 19 of gestation (embryo/foetuses observation groups) or until day 21 after delivery (F1 pups observation groups). The general toxicological effects and reproductive effects on male/female parent rats and the effect on the next generation (foetuses and pups) were examined.

No treatment-related effects were found in either male or female parent rats in their general condition, body weights, food consumption or necropsy observation. There were no treatment-related effects on the reproductive ability of either male or female parent rats. There were no treatment-related effects and malformations/variety one in the external, visceral or skeletal examinations of foetuses. There were no treatment-related abnormalities in the growth or development of F1 pups, sensual and reflex function, behaviour, nor in the reproductive ability of F1 generation and F2 foetuses.

Based on the above results and under the conditions of this study, the non-toxic dose level of Sho-saiko-to for general toxicity and reproductivity of F0 parents are seemed to be above 2000 mg/kg/day, and the non-toxic dose level of Sho-saiko-to for the next generation is also above 2000 mg/kg/day.

These toxicological properties suggest that Sho-saiko-to has no significant toxicity at pharmacologically effective doses.

Pharmacological study

General pharmacology

Amagaya *et al*. (1998) reported the general pharmacological profile (central nervous system, autonomic nervous system, respiratory and cardiovascular system, gastrointestinal system, renal system and coaguration amd fibrinolysis system) of Sho-saiko-to using normal animals. The doses of Sho-saiko-to were 0.3, 1.0 and 3.0 g/kg. In this study, Sho-saiko-to showed no effect on spontaneous locomotor activity, hexobarbital-induced sleeping time, electroshock-, strychnine-, and pentylenetetrazol-induced convulsions, frequency of acetic acid-induced writhing, body temperature and skeletal muscle coordination in mice. In anaesthetized dogs, Sho-saiko-to had no effect on the frequency of respiration, blood pressure, heart rate and ECG. Sho-saiko-to at 10^{-4}, 10^{-5}, 10^{-6} g/ml also had no effect on acetylcholine or barium chloride-induced contraction of guinea-pig ileum. Sho-saiko-to at 10^{-4} g/ml, however, increased histamine-induced contractions, and the spontaneous motility of the guinea-pig ileum. Sho-saiko-to had no effect on blood coagulation and platelet aggregation in rats. However, Sho-saiko-to at the lowest dose of 0.3 g/kg inhibited gastric juice secretion, gastric pH and gastric acid output, and at 1.0 g/kg it inhibited the gastric acidity in pylorus ligated rats. Sho-saiko-to at a dose of 1.0 g/kg further inhibited bile secretion in rats. Sho-saiko-to at all doses, however, showed no effect on the intestinal transport of charcoal meal in rats. Sho-saiko-to at a dose of 3.0 g/kg produced a decrease in urine volume, but did not decrease the urine electrolyte, Na^+, K^+ and Cl^- concentrations. These data suggest that Sho-saiko-to should have anti-ulcer action and that it shows no notable pharmacological effect on the central nervous system, autonomic nervous system or smooth muscle function, respiratory and cardiovascular system, and blood coagulation and fibrinolysis function. Therefore, Sho-saiko-to should have no significant adverse effect at pharmacologically effective doses.

Anti-inflammatory and anti-allergic actions

Amagaya *et al*. (1986) reported that Sho-saiko-to inhibited the platelet aggregation *in vivo* (Figure 4.16). In this study oral administration of Sho-saiko-to, 1.1 g/kg, improves the intravenous injection of collagen-induced decrease of plasma platelet with two inhibitory peaks at 1 and 5 h after the Sho-saiko-to treatment in a significant manner. Furthermore Shimizu *et al*. (1984) reported that Sho-saiko-to, 1.1 g/kg, inhibited the carrageenan-induced oedema and

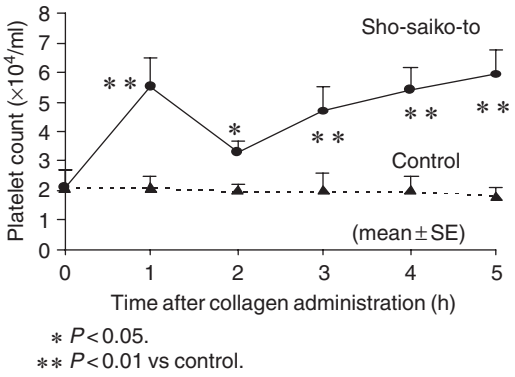

Figure 4.16 Time course in the manifestation of Sho-saiko-to against collagen-induced thrombocytopenia in mice. A single oral administration of Sho-saiko-to was given at time 0. Blood samples for platelet counts were taken 2 min after an intravenous injection of collagen, 1 mg/kg.

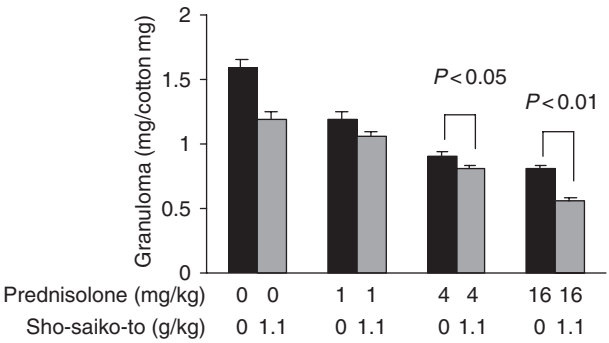

Figure 4.17 Effects of the combination of prednisolone and Sho-saiko-to on the formation of cotton pellet granuloma in rats.

granuloma formation, and increased the prednisolone, 4 or 16 mg/kg, -induced anti-inflammatory actions (Figure 4.17). At the same time, the adrenal atrophy induced by prednisolone is also inhibited by the treatment of Sho-saiko-to. The improvement in prednisolone-induced adrenal atropy is explained by the stimulative effect of Sho-saiko-to on the pituitary–adrenal axis system. These data suggest that Sho-saiko-to, in the combination with glucocorticoid, shows synergistic anti-inflammatory action with the reduction of adverse steroidal effects. For these reasons Sho-saiko-to can decrease the dose of steroid when used in the combination with steroidal anti-inflammatory drugs.

Anti-inflammatory actions of Sho-saiko-to are explained by its multiple action mechanisms, steroidal and non-steroidal anti-inflammatory actions and anti-allergic actins. Amagaya *et al*. (1984) reported the steroidal action of Sho-saiko-to as follows. The anti-inflammatory action of Sho-saiko-to is blocked by progesterone or 17 α-methyltestosterone, anti-glucocorticoids, actinomycin D, a blocker of mRNA synthesis, and by cycloheximide, a protein synthesis inhibitor (Figures 4.18 and 4.19). These results suggest that Sho-saiko-to has glucocorticoidal action by inducing the steroid specific protein, and some flavonoids proved to be the active components.

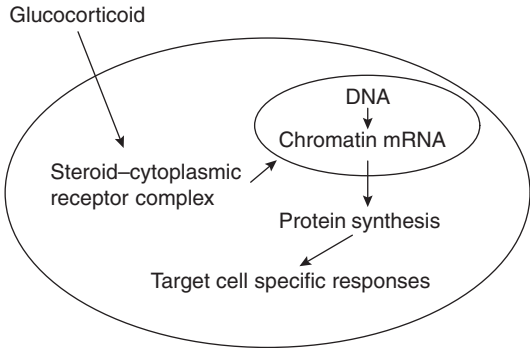

Figure 4.18 Anti-inflammatory mechanism of glucocorticoid.

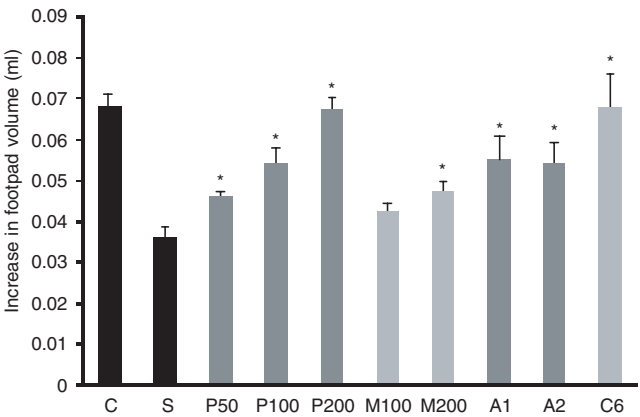

Figure 4.19 Effects of anti-glucocorticoids on anti-inflammatory action of Sho-saiko-to.

Notes
S: Sho-saiko-to 1.3 g/kg, P50: Progesterone 2 × 50 mg/kgs, P100: Progesterone 2 × 100 mg/kgs, P200: Progesterone 2 × 200 mg/kgs, M100: 17 α-Methyltestosterone 2 × 100 mg/kgs, M200: 17 α-Methyltestosterone 2 × 200 mg/kgs, A1: Actinomycin D 3 × 1 mg/kgs, A2: Actinomycin D 3 × 2 mg/kgs, C6: Cycloheximide 3 × 6 mg/kgs.
Each bar indicates the mean ± SE.

Furthermore, Tamura *et al*. (1979) reported that GA, an aglycone of GL, which is one of the main ingredients of Glycyrrhiza Radix, blocked the metabolism of the endogenous glucocorticoid hormone by antagonizing the 5α-reductase receptor. Thus, GA increase the blood concentration of endogenous glucocorticoid.

Furthermore, Inoue *et al*. (1990) reported that Sho-saiko-to made the liver sensitive to glucocorticoid. In particular, the oral administration of Sho-saiko-to enhances the dexamethasone-induced tyrosine aminotransferase (TAT) activity. Further, TAT activity in mice treated with Sho-saiko-to is effectively induced by a small dose of dexamethasone, as compared with that in control mice. However, Sho-saiko-to itself does not induce TAT activity in the liver in both *in vivo* oral administration and *in vitro* cultured hepatocytes experiments. Moreover, Sho-saiko-to does not affect the induction of TAT activity by butyryl adenosine 3′,5′-cyclic

monophosphate. The amplifying effect of Sho-saiko-to seems to be specific for the induction by dexamethasone. Kuriyama and Akiyama (1988) reported the inhibition of PAF activity in human neutrophile.

In the anti-allergic actions, Tanizaki *et al*. (1991) reported the inhibition of histamine release from the house-dust or anti-human IgE-stimulated neutrophile, and Okada *et al*. (1995) reported the inhibitions of compound 48/80-induced histamine release by Sho-saiko-to. Furthermore, Eda (1990) reported that Sho-saiko-to inhibited the 48 h homologous PCA, and its active crude drugs were Scutellaria Radix and Zizyphus Fruit. Watanabe *et al*. (1991) reported the inhibitions of cyclooxygenase and 5-lipoxygenase activities by Sho-saiko-to. And, Kimura *et al*. (1987) reported Baicalin and baicalein, an aglycone of baicalin, were the active ingredients, and baicalin and baicalein, the main ingredients of Scutellaria Radix, showed anti-allergic activity by inhibiting the 5-lipooxygenase activity. These ingredients are further reported to show anti-oxidative and anti-virus activities. Ono *et al*. (1989) reported that baicalein showed the inhibition on reverse transcriptase of HIV virus. In the experiment using HIV-infected human Molt 4 cells, anti-virus effects have also been reported in Scutellaria Radix and its component, baicalein.

Anti-oxidant action

Sakaguchi *et al*. (1993) reported the preventive effects of Sho-saiko-to on oxygen toxicity and membrane damage in liver during endotoxemia. The liver lipid peroxide level and xanthin oxidase activity 18 h after the administration of endotoxin (6 mg/kg i.p.) to Sho-saiko-to (500 mg/kg p.o.)-pre-treated mice were markedly lower than that in endotoxin-treated control mice, whereas the administration of Sho-saiko-to significantly increased superoxide dismutase and glutathione peroxide activities in liver of endotoxin-injected mice. In the mice pre-treated with Sho-saiko-to, the levels of alpha-tocopherol and non-protein SH in liver tissue 18 h after endotoxin injection were markedly increased as compared to those in endotoxin-treated mice. Leakage of acid phosphatase and lactate dehydrogenase isozyme in serum were markedly lower in endotoxin and Sho-saiko-to-treated mice than those in mice treated endotoxin. The administration of Sho-saiko-to clearly prevents the membrane protein damage arising from endotoxin challenge. Sho-saiko-to thus appears to protect the liver plasma membrane from injury by free radicals which occur in a tissue ischaemic state during endotoxemia.

Sakaguchi *et al*. (1994) further reported the preventive effects of Sho-saiko-to against various metabolic disorders during endotoxemia. In this study the cytosolic-free Ca^{2+} concentration ($[Ca^{2+}]i$) in liver single cell was examined using a photonic microscope system. The $[Ca^{2+}]i$ in a single liver cell of endotoxin (6 mg/kg i.p.)-injected mice was greater at 18 h post-intoxication than that in the control, whereas the administration of endotoxin to Sho-saiko-to (500 mg/kg p.o.)-pre-treated mice results in a markedly lower level of $[Ca^{2+}]i$ than that in endotoxin-treated mice. In the mice pretreated with Sho-saiko-to, the CA^{2+}-ATPase activity in liver plasma membrane 18 h after endotoxin injection was markedly increased as compared to that in the endotoxin-treated mice. In contrast, the Ca^{2+}-ATPase activity in liver mitochondria was lower in endotoxaemic mice pre-treated with Sho-saiko-to than in mice given endotoxin alone. State 3 respiration and the respiratory control index (RCI), which are the parameters of mitochondrial function, were 41% and 35% lower, respectively, in the liver of mice that were given endotoxin than those levels in the control. However the levels of state 3 and RCI in endotoxin and Sho-saiko-to treated mice were markedly increased as compared to those of the endotoxin-treated mice. These findings suggest the protective effect of Sho-saiko-to in the damage of liver mitochondrial function in endotoxin-poisoned mice.

Sakaguchi *et al*. (1995) further investigated whether or not Sho-saiko-to can suppress nitric oxide (NO) generation by endotoxin-activated J774A.1 cells. In this experiment, the NO_2 in the murine macrophage cell line J774A.1 using the Griess method were studied. J774A.1 cells stimulated with endotoxin (0.01–10 μg/ml) could effectively produce NO, and its production was dependent on the dose of endotoxin. On the other hand, the NO level when the cells were incubated with endotoxin (0.1 μg/ml) and Sho-saiko-to (10–20 μg/ml) was slightly lower than that in cells treated with endotoxin alone. In contrast, treatment with Sho-saiko-to (50–100 μg/ml) significantly inhibited endotoxin-activated NO generation in J774A.1 cells, whereas the treatment with Sho-saiko-to (10–100 μg/ml) alone was ineffective in inducing NO formation and in inhibiting cell viability in the J774A.1 cells. These findings suggest that Sho-saiko-to shows a suppressive effect on NO generation in macrophages stimulated with endotoxin, and that it may be useful in improving endotoxin-shock symptoms.

Miyahara and Tatsumi (1990) reported the suppression of lipid peroxidation by Sho-saiko-to in rat liver subcellular membranes using guinea-pig neutrophils, rat liver mitochondria and microsomes. Sho-saiko-to had no effect on arachidonate- and PMA-induced NADPH-O_2-generation in neutrophils though it produced a weak LPS-like effect on the cells. No inhibition on reduced NADPH oxidation was found in liver microsomes. However, it markedly inhibited iron-induced lipid peroxidation in microsomes and mitochondria. The active components are baicalein, ginsenoside Rf_1 and baicalin, of which ED_{50} values are 0.2, 0.2 and 1 nmol/mg prot, respectively.

Yoshida *et al*. (1994) reported the effect of Sho-saiko-to on the concentration of vitamin E in serum in carrageenan cotton pellet-induced granuloma rats. As a result, in the granuloma rats of Sho-saiko-to-treated group, a significant inhibitory effect on granuloma formation, and a higher concentration of vitamin E in serum was observed compared to the control group. Higher concentrations of cholesterol and phospholipid in serum were also observed in Sho-saiko-to treated group. Despite the lipid-increasing action of Sho-saiko-to, the concentration of serum lipid peroxide was significantly lower than in the control group. Furthermore, a significant negative correlation between the concentration of vitamin E and granuloma weight was observed. These results suggest that vitamin E plays an important role in promoting the anti-inflammatory effect of Sho-saiko-to.

Immunomodulation

Sho-saiko-to has been shown to have multiple immunological effects and can regulate the host-defence system. Many pharmacological studies on the immunomodulation have been reported.

Maruyama *et al*. (1985) reported that Sho-saiko-to enhanced the phagocytic activity of peritoneal exudate cells (PEC), when Sho-saiko-to, 0.3 g/kg or 2 g/kg, was given to mice either intraperitoneally or orally. But Sho-saiko-to showed no stimulation of spleen cells and bone marrow cells. Ito and Shimura (1985) reported that the peritoneal macrophages increased, and the phagocytic activity of macrophages also increased, when Sho-saiko-to, 0.6 g/kg, was orally administered in mice. In this case, the complement C3 on macrophages was activated.

Further, Kumazawa *et al*. (1988) reported that intraperitoneal administration of Sho-saiko-to into F1 mice (BALB/c × DBA2) resulted in marked activation of macrophages with respect to phagocytic and lysosomal enzyme activities (acid phosphatase and N-acetyl-β-D-glucosaminidase) compared with the control. The maximal responses were induced by an i.p. injection of 3 mg Sho-saiko-to 4 days previously. Enhanced activities induced by Sho-saiko-to were also seen in C3H/HeJ mice, which was a non-responder strain to bacterial lipopolysaccharide (LPS). Significant macrophage accumulation in the peritoneal cavity and increased lysosomal

enzyme activities were observed in mice injected with Sho-saiko-to. Sho-saiko-to exhibited significant cytostasis-inducing. In addition, the administration of Sho-saiko-to led to a moderate expression of Ia antigen on the surface of peritoneal macrophages. These results suggest that Sho-saiko-to is a potent macrophage activator.

Chin *et al*. (1992) administered Sho-saiko-to, 7.5 g/day/head, orally 3 months before the total hysterectomy to 5 patients with myoma of the uterus, benign ovarian tumour, and the number of peritoneal macrophages, leukocytes and lymphocytes were counted. At the same time, the IL-1β and prostaglandin E_2 production from the adherent cells were measured. After the operation, the treatment of Sho-saiko-to did not increase the number of peritoneal leukocytes and lymphocytes, but it increased the number of macrophages in a significant manner; 1 day after the operation the number of peritoneal macrophages increased by 6 times over that of the Sho-saiko-to non-treated control patients. IL-1β production was also increased by the Sho-saiko-to treatment.

In addition, Kaneko *et al*. (1994) and Kaga *et al*. (1991) reported that oral Sho-saiko-to stimulated the natural killer cell activity. And Sho-saiko-to exhibited a stronger mitogenic activity on B cell-rich population than on T cell-rich population, suggesting that Sho-saiko-to acts as B-cell mitogen. Treatment of mice with Sho-saiko-to by oral administration once a day for 13 days at a dose of 250 mg/kg increased the number of splenic antibody-producing cells against sheep erythrocytes, accompanied with an increase in serum haemagglutinin and haemolysin titres. Treatment with carrageenan, an inhibitor of macrophage, inhibited the antibody production, and Sho-saiko-to improved the inhibition of antibody production by carrageenan. Further Iwama *et al*. (1987) reported that Sho-saiko-to improved the steroid-induced inhibition of antibody production. Fujiwara *et al*. (1995) reported the regulation of hepatic macrophage function by the oral administration of Sho-saiko-to in rats. In the partial hepatectomy rats, Sho-saiko-to has been reported to improve the blocked function of hepatic macrophages in activation.

Kawakita *et al*. (1987b, 1988) reported the protective effect of Sho-saiko-to on Listeria monocytogenes or Pseudomonas aeraginom infections. The numbers of bacteria (Listeria monocytogenes) in the peritoneal cavity and liver were smaller in Sho-saiko-to-treated mice 4 days before intraperitoneal bacterial infection. Macrophage accumulation in the peritoneal cavity after intraperitoneal inoculation of Listeria monocytogenes was observed in both untreated and Sho-saiko-to treated mice. Although rates of such increases were almost the same between both groups, the absolute number of macrophages was larger in Sho-saiko-to-treated mice. In the untreated mice, the bactericidal activity of peritoneal macrophages decreased from 1 to 3 days after the intraperitoneal injection of killed Listeria monocytogenes, and such a level of activity was maintained from 1 to 3 days in Sho-saiko-to-treated mice. However, 4 days after the Sho-saiko-to treatment, the absolute number of macrophages was larger in Sho-saiko-to-treated than untreated mice. Augmented accumulation of macrophages and maintenance of their bactericidal activity may be the main mechanisms of the augmented resistance in Sho-saiko-to-treated mice. The effect of Sho-saiko-to observed at an early stage of infection may be T-cell independent, since such an effect is observed in athymic nude mice and delayed footpad reaction could not be detected at such a timing in euthymic normal mice. In the study of Pseudomonas aeruginosa infection in mice, survival of mice after intraperitoneal or intravenous infection with bacteria was augmented in the mice pre-treated intraperitoneally with Sho-saiko-to before 6 h or 4 days. The pre-treatment of Sho-saiko-to before 6 h induced an accumulation of polymorphonuclear leucocytes (PMN) in the peritoneal cavity, and its protective effect against intraperitoneal infection was not impaired by the treatment with carrageenan, a macrophage blocking agent. These results suggest that the protective effects of Sho-saiko-to against Pseudomonas aeruginosa infection depends mainly on PMN in mice pre-treated at this timing.

The pre-treatment with Sho-saiko-to for 4 days induced an accumulation of macrophages showing an augmented phagocytosis of Pseudomonas aeruginosa *in vitro* in the presence of immune serum, and its protective effect against Pseudomonas aeruginosa was impaired by the treatment with carrageenan. In addition, the pre-treatment with Sho-saiko-to accelerated the bacterial clearance from the blood. The sera obtained from mice treated with Sho-saiko-to 4 days previously showed a high titre of antibody specific to Pseudmonas aeruginosa. When this sera was transferred to native mice, these recipients showed an accelerated bacterial clearance and an increased survival to challenge infection with Pseudomonas aeruginosa. These results suggest that protective effects of Sho-saiko-to against Pseudomonas aeruginosa infection at this timing depend on the cooperation of macrophages and antibody, which produced by the stimulation of Sho-saiko-to, a polyclonal B-cell activator. Such polyclonal antibodies are also effective in protecting against encapsulated Klebsiella pneumoneae for which the antibody is essential in the expression of resistance. These results suggest that Sho-saiko-to could augment non-specific resistance to a variety of bacteria in which antibodies play an effective role.

Interferons (IFNs) are known to show anti-viral activity, and Nakajima *et al*. (1983) reported that the oral administration of Sho-saiko-to induced IFNα in human and Kawakita *et al*. (1990) reported that Sho-saiko-to induced IFNα/β in mice, independent of the effect of LPS. In this experiment, a maximum activity (105 units/ml) of IFN appears in the serum of mice 16 h after intraperitoneal treatment with 250 mg/kg of Sho-saiko-to. Addition of polymyxin B dose not abrogate the ability of Sho-saiko-to to induce IFN. The IFN was identified as IFNα/β by neutralizing test using IFNα/β antibodies. Pre-treatment of mice with carrageenan suppresses the IFN induction by Sho-saiko-to, whereas the IFN induction by Sho-saiko-to is impaired neither in mice treated with anti-asialo-GM1 antibody nor in the T-cell deficient athymic nude mouse. IFN was produced *in vitro* by spleen cells obtained from Sho-saiko-to-treated mice. Moreover, spleen cells from untreated mice could also produce IFN when they were cultured with Sho-saiko-to. Additionally, serum IFN is also induced by the adoptive transfer of spleen cells from Sho-saiko-to treated mice to normal mice. On the other hand, peroral administration of Sho-saiko-to also induced IFNα/β in the serum. While IFN activity induced by intraperitoneal administration of Sho-saiko-to declined after repeated treatments, the activity induced by its peroral administration did not decline during a long-term treatment. These results showed that Sho-saiko-to was an IFNα/β inducer capable of repeated peroral administration and suggest the possibility that splenic B cells are the cellular origin of the IFNs produced in response to Sho-saiko-to.

Furthermore, Kawakita *et al*. (1987a) reported that Sho-saiko-to augmented the production and maturation of B cells and act as a B-cell mitogen, which act as a polyclonal B-cell activator. Namely an intraperitoneal injection of Sho-saiko-to induces accumulation of B lymphocytes (sIgM+) in the peritoneal cavity and spleen. Cell surface marker analysis by a fluorescence-activated cell sorter (FACS) demonstrated that the accumulated B cells on day 4 or 7 after Sho-saiko-to administration (early phase) were composed mainly of sIgM+IgD− cells and suggested that these B cells maturated into sIgM+IgD+ cells on days 10 or 14 (late phase). The relative decrease of IgM+IgD+ cells in the early phase is more profound in peritoneal cells (PC) than in spleen cells. With respect to spleen lymphocytes, antibody responses to a thymus-independent (TI) antigen of type 2 (trinitrophenylated Ficoll) and a thymus-dependent (TD) antigen (sheep erythrocyte) were enhanced during the late phase but not during the early phase. In contrast, responses to trinitrophenylated lipopolysaccharide (TNP-LPS) as a TI-1 antigen and LPS as a B-cell mitogen or a polyclonal B-cell activator were enhanced markedly during the early phase

but declined during the late phase. With respect to peritoneal lymphocytes, responses to LPS are suppressed during the early phase but recovered during the late phase. Enhanced responses to TI and TD antigen during the late phase in spleen lymphocytes and suppressed response to LPS during the early phase in peritoneal lymphocytes may be explained by increases of IgM+IgD+ mature B cells and IgM+IgD− immature B cells, respectively. Enhanced responses to TI-1 or LPS in spleen lymphocytes during the early phase may be explained by elevated sensitivity of IgM+IgD+ cells, which reside in the spleen before Sho-saiko-to administration and receive the direct stimulation by Sho-saiko-to, or by acquired responsiveness of IgM+IgD− cells, which migrate after stimulation with Sho-saiko-to. These reports suggest that B cells play important roles in the immunopharmacological effects of Sho-saiko-to.

Matsuura *et al*. (1993) reported the role of B-lymphocytes in the immunopharmacological effects of Sho-saiko-to. In this study, murine spleen cells were cultured *in vitro* with Sho-saiko-to, and B cells isolated by anti-immunoglobulin-coated plates were confirmed to generate IFN in response to Sho-saiko-to stimulation. IFN activity was induced in the serum after intraperitoneal administration of Sho-saiko-to and its constituents, Glycyrrhizae Radix, Scutellariae Radix, Bupleuri Radix and Pinelliae Tuber. Sho-saiko-to and these four extracts of constituents induced IL-6 in the serum after their administration. Further Sho-saiko-to and the extracts of Glycyrrhizae Radix, Bupleuri Radix and Pinelliae Tuber showed mitogenic activities. These results indicate that B cells participate in the immunopharmacological effects of Sho-saiko-to through mitogenic activity, IFN induction and the effect of IL-6.

The Peyer's patches contain a large number of precursor cells committed to the production of immunoglobulin A (IgA) and play an important role in IgA responses in the mucosal immune system. Tauchi *et al*. (1993) reported the induction of IgA producing cells in Peyer's patches by plaque forming cell assay after oral administration of Sho-saiko-to. The number of total IgA producing cells in the Peyer's patches detected by the protein-A plaque assay increased by about two times as a result of Sho-saiko-to administration, and the numbers of anti-SRBC and anti-HRBC IgA producing cells also increased after such a treatment. On the other hand, when SRBC alone was administered orally, only the number of anti-SRBC IgA producing cells increased. When T-cell subpopulations in the gut-associated lymphoid tissues after oral administration of Sho-saiko-to were studied by flowcytometry, marked alterations in T-cell subpopulations were not detected in the Peyer's patches, though TcR$\gamma\delta^+$T cells in the intraepithelial lymphocytes and Thy1.2$^-$TcR$\alpha\beta^+$T cells in the mesenteric lymph nodes were slightly increased. These results show that orally administered Sho-saiko-to acts as a polyclonal B-cell activator, which induces IgA production in the mucosal immune system.

The regulation of cytokines by the treatment of Sho-saiko-to is mainly studied in hepatitis conditions, and has often been reported. The increase of IL-1β production is parallelled with the activation of macrophages by Sho-saiko-to. Terada *et al*. (1989) reported IL-2 reactivity, production and the receptor of the lymphocytes obtained from the patients with chronic non-A, non-B hepatitis affected by Sho-saiko-to. The patients were divided into three groups by the values of the reactivity of the lymphocytes against IL-2 before Sho-saiko-to administration. The IL-2 reactivity and its production significantly decreased to the normal range according to the Sho-saiko-to administration in the higher reactivity group. In lower reactivity group, they significantly increased to the normal range during the administration of Sho-saiko-to. The serum GOT and GPT levels were reduced in the higher reactivity group in response to the reduced tendency of the reactivity during Sho-saiko-to administration. On the other hand, in the groups of both higher and lower values of the IL-2 receptor, treatment with Sho-saiko-to improved the value of receptor to the normal range. These data suggest that Sho-saiko-to has an immunomodulatory effect on lymphocytes with chronic non-A and non-B hepatitis. Furthermore, the effect of

Sho-saiko-to on the soluble IL-2 receptor production was investigated. When Sho-saiko-to was administered to the chronic hepatitis patients for 1 month or 2 months, blood-soluble IL-2 receptor production increased. In *in vitro* experiment, Sho-saiko-to also increased the soluble IL-2 receptor production in peripheral mononuclear cells.

Nakajima *et al*. (1993) reported that the increase of the IFN receptor by oral Sho-saiko-to administration. This study was performed to determine the action mechanisms of Sho-saiko-to for its inhibition of virus-induced chronic hepatitis, cirrhosis and hepatocyte tumour. The number of IFN receptor on peripheral blood mononuclear cells, and serum GOT and serum GPT were measured after Sho-saiko-to was orally administered to the 11 patients with type C chronic hepatitis. The number of receptor increased by 140% at 2 weeks, 130% at 6 weeks, 160% at 11 weeks and 170% at 25 weeks during the Sho-saiko-to administration. The patients were divided into two groups, responder and non-responder, according to the improvement in the ALT value. The responder group whose ALT value was below 50% of the initial value had 3 patients and the non-responder group whose ALT value was above 70% of the initial value had 7 patients. In the responder group, a significant increase in the IFN receptor and a significant decrease in the ALT value were observed. On the other hand, in the non-responder group no increase in the IFN receptor was observed. In *in vitro* experiment using mononuclear cells isolated from both healthy volunteer and patients with type C chronic hepatitis, the number of IFN receptor did not increase as a result of the addition of Sho-saiko-to to the cultured mononuclear cell plate. This result could be explained by the down-regulation by the increase of the concentration of IFNα.

To examine whether Sho-saiko-to can modulate the immune response of immunocompetent cells to hepatitis B virus-associated antigens, Kakumu *et al*. (1990) studied *in vitro* IFNγ and antibody (antibody to HB core and e antigens; anti-HBc and anti-Hbe) production by peripheral blood mononuclear cells from 8 patients with chronic active hepatitis (4 with HBeAg and 4 with anti-Hbe) in the presence of recombinant HBcAg and purified HBeAg. IFNγ and antibody production were measured using ELISA and RIA, respectively. Peripheral mononuclear cells from both HBeAg and anti-HBe positive patients generated significant increases in IFNγ and antibody (anti-HBc and anti-HBe) production in the culture containing Sho-saiko-to in a dose-dependent manner in comparison with those of medium alone culture. Similarly, when various concentrations of Sho-saiko-to were added to the HBV antigen-stimulated cultures, Sho-saiko-to was found to enhance both IFNγ and antibody production dose-dependently. These results indicate that Sho-saiko-to is able to modulate both cellular and humoral immune responses specific for HBV-associated antigens. These findings also may account for, at least in part, the efficacy of Sho-saiko-to treatment for type B chronic hepatitis.

Tanaka *et al*. (1991) reported that liver sinusoidal large granular lymphocytes, pit cells, had natural killer (NK) activity as well as NK cells, and pit cells had anti-tumour effects. In this study, pit cells were shown to be a liver-associated NK cells, and has killer activity against YAC-1 cells, which is sensitive to the NK cell. Furthermore, pit cell showed the anti-tumour effects on P815 and AH109 cells, which are not sensitive to the NK cell. This activity decreased according to the ageing. When Sho-saiko-to is orally administered to 5-weeks old rats for 5 days, NK activity of pit cells isolated from Sho-saiko-to-treated rats increased by 37%. In the study of 8-weeks old rats, treatment of Sho-saiko-to increased the NK activity by 45.5%, and the activities against P815 cell and AH109 cells also increased. These data indicate that Sho-saiko-to increases the NK activity and anti-tumour activity in pit cells and demonstrate Sho-saiko-to's protective effect on both infection and tumour formation as biological response modifier.

It is said that sex hormones are important factors in regulating the immune system. Butterworth *et al*. (1967) reported that serum immunoglobulin levels of mammals including humans were higher in females than in males, Terres *et al*. (1968) reported that antibody

production induced by stimulation with specific antigen was also higher in females than in males, and Kenny *et al*. (1967) reported that autoimmune diseases were generally more frequently seen in females. Castro (1974) also reported that in male mice, antibody production increased with the administration of oestradiol and decreased with the administration of male hormones. Similarly, in hepatitis B-virus-infected patients, virus elimination and induction of the immune responses to viral antigens seem to differ between male and female. Mizoguchi *et al*. (1988) reported the studies on various virus markers in the serum of chronic hepatitis B virus carriers to show that seroconversion is more frequently seen in females, especially in sexually mature females. For these reasons Mizoguchi *et al*. (1991) studied the effect of Sho-saiko-to on oestradiol receptors on the cytosol of hepatic sinusoidal endothelial cells. In this study, oestradiol receptors were found in the cytosol of hepatic sinusoidal endothelial cells from rats. Moreover, when these cells were incubated with Sho-saiko-to, the level of cytosol oestradiol receptors increased. These results suggest that Sho-saiko-to acts on hepatic sinusoidal endothelial cells to increase the level of estradiol receptors, thereby affecting the immune reactions in the liver.

Yamashiki *et al*. (1992a) reported that Sho-saiko-to showed to increase in the production of granulocyte colony-stimulating factor (G-CS) in a dose-dependent manner in an *in vitro* study using the culture of peripheral blood mononuclear cells (PBMC) obtained from healthy volunteers. Yonekura *et al*. (1990) reported the colony stimulating factor (CSF) induction in an *in vivo* study. CSF rich serum was obtained from mice injected intraperitoneally with Sho-saiko-to. Transfer of the CSF-rich serum into native mice augmented the resistance against the Listeria monocytogenes. A dose-dependent induction of CSF was observed in mice given Sho-saiko-to *via* intravenous route as well as *via* intraperitoneal route. Since the serum CSF induction was observed in both LPS-responder C3H/He mice and LPS-non-responder C3H/HeJ mice, the effect of Sho-saiko-to seems to be free from possibly contaminating LPS. The level of serum CSF induced by Sho-saiko-to in athymic nude mice was similar to that in control athymic mice, and the induction of CSF was completely blocked by the previous administration of carrageenan, a selective macrophage blocker. In mice treated intraperitoneally with Sho-saiko-to, CSF activity was detected in the peritoneal cavity, the site of injection, and the time course was similar to that of serum CSF induction. In a bone marrow culture system, the composition of colonies formed by Sho-saiko-to-induced CSF was similar to that formed by standard GM-CSF. The CSF activity was scarcely affected by treatment of the sera with anti-M-CSF antibody. These results suggest that Sho-saiko-to augments the host defence by inducing mainly GM-CSF, and that CSF is produced by cells of macrophage lineage. In addition, it was shown that CSF could be induced even after oral administration of Sho-saiko-to. In another study reported by Yamashiki *et al*. (1992b), peripheral blood samples were collected from normal subjects and chronic viral hepatitis patients, and the *in vitro* capability of the peripheral blood mononuclear cells to produce various cytokine were analysed by adding pokeweed mitogen. The production levels of 4 cytokines (IL-1β, IL-6, IFNγ and GM-CSF) were significantly lower in the peripheral blood mononuclear cells of the patients than in those of the normal subjects. The addition of Sho-saiko-to to the patients group resulted in improved productions of these cytokines, as well as remarkable improvement of IL-1β production. These results demonstrate that Sho-saiko-to acts to improve immunological abnormalities such as decreased cytokine productions. Administration of Sho-saiko-to to chronic viral hepatitis patients is also expected to have immunological benefits.

Furthermore, Yamashiki *et al*. (1997a) reported the G-CSF production in the absence of any stimulation on peripheral blood mononuclear cells. Further Yamashiki *et al*. (1996) reported that the G-CSF production was significantly lower than in healthy controls in patients with

hepatitis B or C. The addition of Sho-saiko-to increased G-CSF production. Dose-dependent increases in production levels of TNFα and G-CSF by adding Sho-saiko-to in peripheral mononuclear cells of patients with hepatocellular carcinoma accompanied by liver cirrhosis has also been reported.

Yamashiki *et al*. (1997b) also reported the IL-10, IL-4 and IL-5 productions by PBMC of patients with chronic hepatitis B and C. Results showed that without stimulants, IL-10 production in mononuclear cells of hepatitis B and C patients was significantly lower than that of healthy subjects. IL-10 production induced by either phytohaemagglutinin (PHA) or pokeweed mitogen (PMN) in mononuclear cells of hepatitis C patients was significantly lower than in patients with hepatitis B and healthy subjects. IL-10 production induced by anti-CD3 or LPS is significantly lower than in healthy subjects. The addition of Sho-saiko-to to the cultures strongly induced IL-10, and this induction is mainly attributable to the effects of two components, scutellalia root and glycyrrhiza root, on the monocyte/macrophage fraction. The production of IL-4 and IL-5 in cultures with concanavalin A (conA) is significantly higher in patients with hepatitis C than in the healthy subjects, but the addition of Sho-saiko-to suppresses these increases by 25–33%. Therefore, Sho-saiko-to can normalize both the decreased IL-10 production and the increased IL-4 and IL-5 productions of mononuclear cells from patients with hepatitis C. Moderate regulation of cytokine production system in patients with hepatitis C using Sho-saiko-to may be useful in the prevention of disease progression.

Fukuda *et al*. (1995) reported the effect of Sho-saiko-to on cytokine induction in the murine lung. Examination was made of the effects of Sho-saiko-to and IFNα/β on the production of TNFα and IL-1β in lung tissue. Sho-saiko-to, 0.92 g/kg, was orally administered to mice for 3 weeks with and without intraperitoneal injection of IFNα/β (10^5 unit/kg, twice a week). Sho-saiko-to and IFNα/β each increased TNFα and IL-1β in the lungs and additively increased cytokines in the tissue. The results suggest the effectiveness of Sho-saiko-to in treating infectious lung diseases, since TNFα and IL-1β are major pro-inflammatory cytokines essential for the host defence system. The additive effect may possibly be related to the occurrence of interstitial pneumonia, which has been reported to occasionally occur clinically when Sho-saiko-to

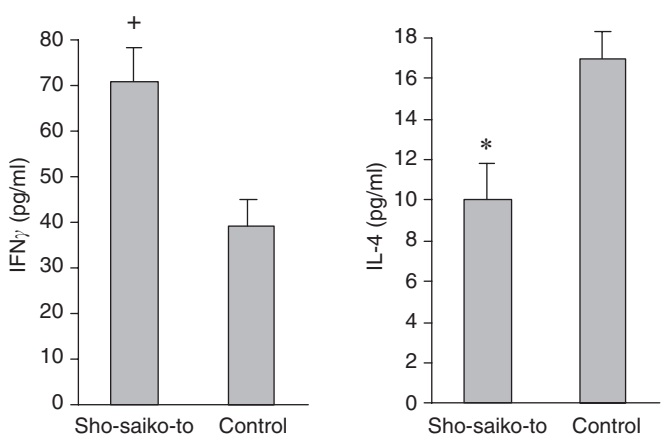

Figure 4.20 Cytokines in lung tissues of Balb/c mice treated with Sho-saiko-to.

and IFNα/β were used in combination. Further, Sho-saiko-to was proved to shift the Th2 constituent to Th1 one in Balb/c mice by increasing the IFNγ and decreasing IL-4 in lung tissues (Figure 4.20). This result also suggests that Sho-saiko-to stimulates the host defence system, and shifts the allergic constituent to a healthy one.

Matsuda *et al*. (1988) reported the study on peripheral lymphocytes from patients with human immunodeficiency virus (HIV) infection. The effect of monocyte culture supernatants (conditioned medium) derived from haemophiliacs and male homosexuals infected with HIV cultured with Sho-saiko-to and various fractionated components on the T-cell colony formation test, which has been proved to reflect the helper T-lymphocyte function, was studied. Monocyte culture supernatants derived from Sho-saiko-to enhanced T-cell colony formation of persons infected with HIV. This is assumed to be caused by IL-1 derived from the monocytes in the supernatant. However, typical IL-1 properties were not necessarily demonstrated when the supernatant was treated with heat and enzymes. It is therefore hypothesized that a colony potentiating factor other than IL-1 is also present. On the other hand, the monocyte culture supernatant of various fractionated components suppress colony formation. It is assumed that this is caused by suppressive factors induced to override IL-1 and IL-2 activity or that there is a possibility that HIV-infected lymphocytes have increased sensitivity to the suppressive factors. The above findings confirm that Sho-saiko-to is a potent biological response modifier (BRM).

Protection of hepatocyte necrosis

Amagaya *et al*. (1988a) reported the effect of the oral treatment of Sho-saiko-to on carbon tetrachloride (CCl_4)-induced hepatic necrosis and functional disorder. Treatment of Sho-saiko-to, 600 mg/kg, restores the CCl_4-induced increase of serum GOT and serum GPT activities 24 h after the CCl_4 treatment (Figure 4.21). Furthermore, Sho-saiko-to reduced the CCl_4-induced elongation of prothrombin time (Figure 4.22) and inhibited the CCl_4-induced increase of cytochrome P-450 activity 24 and 48 h after the CCl_4 treatment. These results indicate that Sho-saiko-to reduced the hepatocyte necrosis and functional disorder. Eybl *et al*. (1992) reported that pre-medication of Sho-saiko-to protected the hepatotoxic effects of CCl_4 and T1-acetate, which were manifested by increased peroxidation of lipids and increased depletion of reduced glutathione in liver homogenates.

Yamamoto *et al*. (1985) and Ohta *et al*. (1997) reported the preventive effect of Sho-saiko-to on the progression of D-galactosamine-induced liver injury in male rats by oral and intraperitoneal administration. A single intraperitoneal injection of 800 mg/kg or 1500 mg/kg D-galactosamine HCl induces remarkable histo- and cytrogic changes in the rat liver. Microscopically, the liver shows diffuse parenchymal damage, in which hepatic cell cords were disorganized and a marked accumulation of lipid droplets were found in the hepatocytes.

Disruption of the lamellar arrangement of the rough endoplasmic reticulum, dissociation of intrahepatic cell space and an increase in the number of autophagic vacuoles were observed in the control groups after the administration of D-galactosamine HCl. Histo- and cytochemical detection of 5′-nucleotidase and alkaline phosphatase activities revealed a disruption of the bile canaliculi system and a disturbance in the plasma membrane. However, no conspicuous pathological and histocytochemical changes were found in the liver pre-administered with Sho-saiko-to, 20 mg/kg i.p. for four consecutive days. Biochemical assay revealed that much higher enzyme activities were preserved in the groups pre-administered with Sho-saiko-to. From these results, it can be seen that the pre-administration of Sho-saiko-to is undoubtedly effective in preventing changes induced by D-galactosamine HCl. Effects of oral and i.p. treatment of Sho-saiko-to were then compared. Rats treated once with D-galactosamine (500 mg/kg i.p.) and received

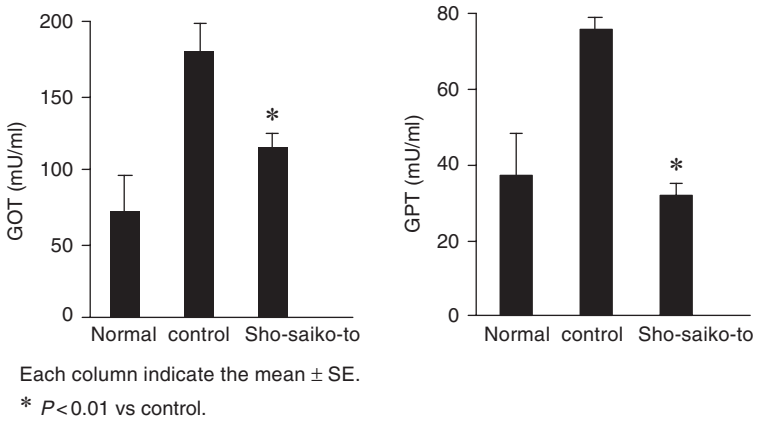

Figure 4.21 Effects of Sho-saiko-to on serum GOT and GPT activities in rats treated with CCl_4.

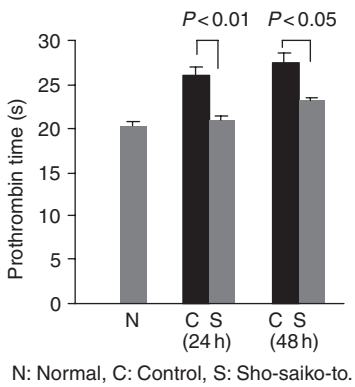

Figure 4.22 Effects of Sho-saiko-to on prothrombin time in rats treated with CCl_4.

Sho-saiko-to at a dose of 1.0 g/kg (p.o. or i.p.) 2 h after D-galactosamine treatment at the time an apparent liver injury occurred. Both p.o. and i.p. administration of Sho-saiko-to significantly inhibited the progress of liver injury 24 h after D-galactosamine injection. Although the total protein and albumin concentration in serum and the protein concentration in the liver decreased with the progression of D-galactosamine-induced liver injury, oral or i.p. administration of Sho-saiko-to prevented the decreases of these parameters. However, decreases in serum and liver triglyceride concentration with the progression of liver injury were not attenuated after p.o. or i.p. administration of Sho-saiko-to. The activities of liver 5′-nucleotidase and glucose-6-phosphatase, marker enzymes of liver plasma and microsomal membranes, respectively, decreased during the progression of liver injury. A similar preventive effect on the decrease of both enzyme activities was found after p.o. or i.p. administration of Sho-saiko-to. These results indicate that the oral or i.p. administration of Sho-saiko-to have similar preventive effect on the progression of D-galactosamine-induced liver injury, and that the effect of Sho-saiko-to may be due to the

improvement on both the impaired liver protein synthesis and disrupted liver plasma and microsomal membranes.

Mitsukawa *et al*. (1991) reported the effect of oral administration of Sho-saiko-to on hepatic injury induced by exposure to 1.1% halotane under hypoxic condition for 2 h in rats. The aetiology of halotane induced hepatitis is unknown. Serum transaminase, histological score and area of necrosis were examined in rats treated with Sho-saiko-to, 900 mg/kg, before and after halotane exposure. Twenty-four hours following halotane exposure, serum transaminase levels were significantly depressed. The level of serum GPT was significantly lower in the rats that received Sho-saiko-to treatment than in rats that did not receive Sho-saiko-to treatment. Histological score in rats treated with Sho-saiko-to was significantly lower than in the untreated rats. The area of centrilobular necrosis was significantly lower in the treated rats than in the untreated rats. These results indicate that Sho-saiko-to inhibits the hepatic necrosis and functional disorder following exposure to halothane.

Further Mizoguchi *et al*. (1990) reported the protective effects of Sho-saiko-to in an immunologically induced allergic hepatic cell injury. When trinitrophenylated hepatocytes were intravenously injected into trinitrophenylated liver protein-sensitized guinea-pigs through mesenteric vein, hepatic cell necrosis was seen to a large extent in all of the guinea pigs and the values of serum GOT and GPT increased to 1067 ± 265 and 101 ± 24 U/l 24 h later. However, when trinitrophenylated hepatocytes were injected into trinitrophenylated liver protein-sensitized guinea-pigs which has been administered with Sho-saiko-to 30, 24 and 6 h before, only spotty necrosis could be seen and massive hepatic cell necrosis was not seen in any of the guinea-pigs. In addition, the values of GOT and GPT are significantly improved. These results suggest that Sho-saiko-to is effective in the immunologically induced allergic hepatic cell injury.

In other reports by Toda *et al*., fatty liver model induced by the liquid alchohol diet is examined. The increased fat spots in liver is inhibited. The decreased hepatic glutathione (GSH) and the increased hepatic lipid peroxide (LPO) were also recovered by the treatment of Sho-saiko-to.

Further, in the bile acid metabolic disorder induced by lithocholic acid reported by Kobayashi *et al*., Sho-saiko-to decreased the abnormal bile acids, lithocholic acid and deoxycholic acid, and improved the bile acid metabolism. At the same time, Sho-saiko-to inhibited the fibrosis in the area of portal vein.

Stimulation of liver regeneration

The compensatory function of liver is important for the protection of hepatic injury. Amagaya *et al*. (1988c) reported the stimulative effect of Sho-saiko-to on liver regeneration *in vivo*. In partially hepatectomized (70% hepatic parenthyma was removed) rats, Sho-saiko-to at a dose of 1.2 g/kg, facilitated the gain of liver weight (Figure 4.23). At the same time, Sho-saiko-to increased the liver RNA content, liver DNA content and liver protein content. The mitosis of the hepatocytes as a parameter of the cell proliferation was evaluated as a mitotic index (the number of mitosis/100 nucleis). The peak of mitotic index was achieved on the first day after partial hepatectomy, and the pre-treatment of Sho-saiko-to from 3 days before the partial hepatectomy increased the mitosis on the first day in a significant manner (Figure 4.24). Furthermore, the regeneration of the hepatocyte collected from the partially hepatectomized rats pre-treated with Sho-saiko-to increased by comparison with that of hepatocyte collected from rats non-treated with Sho-saiko-to. However, Sho-saiko-to did not inhibit the decrease of mitotic index induced by streptozotocin. These results suggest that the acceleration of Sho-saiko-to on liver regeneration is due to the increase of pancreatic hormonal secretion. Therefore, the action on hormonal secretion from pancreas was investigated. Sho-saiko-to was

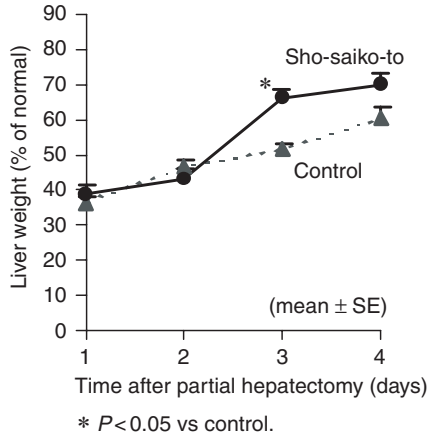

Figure 4.23 Liver weight after partial hepatectomy.

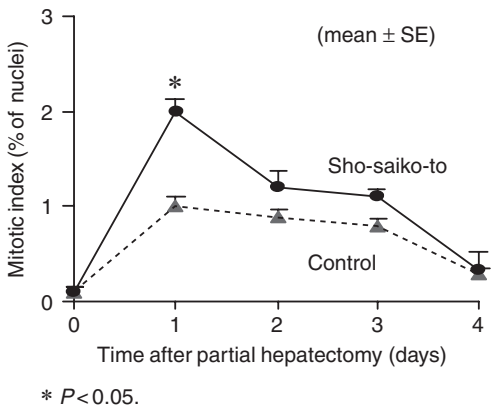

Figure 4.24 Mitotic index of liver after partial hepatectomy.

found to stimulate the glucagon secretion, and the plasma glucose was also increased with the constant secretion of insulin.

Protective effect on hepatic fibrosis

The development of cirrhosis and hepatic tumour is a severe problem in the type C chronic hepatitis patients. About 70% of type C hepatitis patients in Japan are not sensitive to IFN. Therefore, it is important to treat the non-sensitive patients against IFN to protect the development of cirrhosis. Sho-saiko-to has recently been orally administered to patients with chronic liver disease in Japan and has been reported to inhibit the development of cirrhosis and hepatocellular carcinoma. One of the aims of study is to investigate the inhibitory effect of Sho-saiko-to on the development of liver fibrosis.

Preventive effects of Sho-saiko-to on hepatic fibrosis are reported. Amagaya *et al*. (1989) reported that the hepatic hydroxyproline content gradually increased to approximately 2.5 times

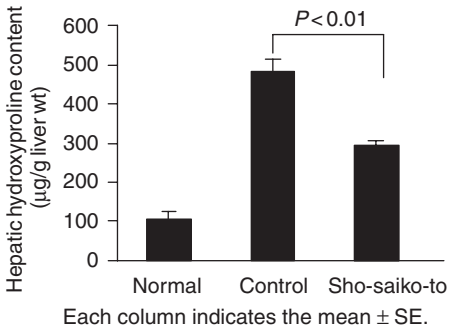

Figure 4.25 Effect of Sho-saiko-to on hepatic hydroxyproline content in mice with repeated doses of CCl_4.

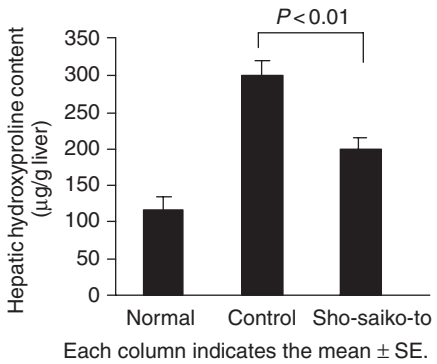

Figure 4.26 Effect of Sho-saiko-to on hepatic hydroxyproline content in DMA-treated rats at 4 weeks.

the normal levels 3 months after the injection in the chronic liver injury induced by the repeated twice-weekly injection of CCl_4 for 6 months. The maximum levels remain almost unchanged for the following 3 months. In the Sho-saiko-to-treated group, the levels of hepatic hydroxyproline at 1, 3, 5 and 6 months were significantly lower than those in the control group (Figure 4.25). In the histological findings, Sho-saiko-to significantly inhibited the hepatic fibrosis formation.

Another experiment reported by Amagaya *et al*. (1988b) showed the preventive effect of Sho-saiko-to on hepatic fibrosis induced by dimethylnitrosamine (DMA) or porcin serum (PS). DMA, 30 mg/kg, was i.p. injected once in rats and hepatic fibrosis is induced 2 weeks after the DMA treatment. On the other hand, PS, 0.5 ml, was i.p. injected twice a week in rats, and hepatic fibrosis was induced 2 months after the PS treatment. Serum GPT activity was not increased in PS treated mice throughout the experiment, which indicates that PS-induced fibrosis formation did not originate from the hepatocyte necroinflammation, but it increased to twice that of the normal group at 2 weeks after the DMA treatment. In both models, prothrombin time was also prolonged. DMA- or PS-induced increase of hydroxyproline content/g liver was inhibited (Figures 4.26 and 4.27) by the administration of Sho-saiko-to. This increase of

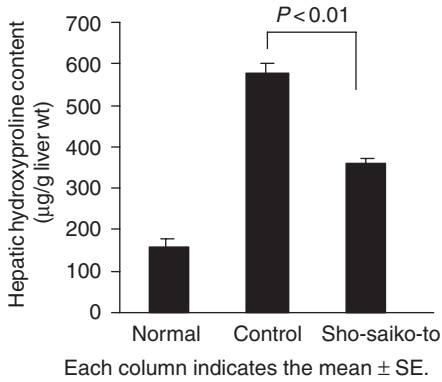

Figure 4.27 Effect of Sho-saiko-to on hepatic hydroxyproline content in PS-treated rats at 3 weeks.

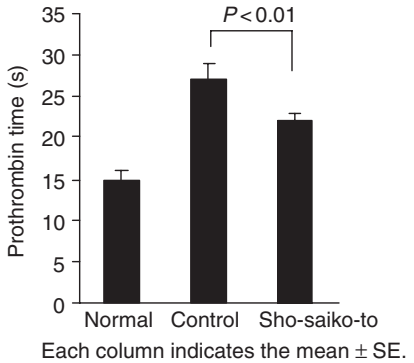

Figure 4.28 Effect of Sho-saiko-to on prothrombin time in DMA-treated rats at 4 weeks.

hydroxyproline content was rather strongly inhibited by the pre-treatment of Sho-saiko-to. In addition, Sho-saiko-to restored the elongation of prothrombin time in both models (Figures 4.28 and 4.29). These results suggest that Sho-saiko-to directly inhibits hepatic fibrosis formation, and Sho-saiko-to is beneficial for the treatment of hepatic fibrosis or cirrhosis.

It has been shown that lipid peroxidation is associated with hepatic fibrosis and stellate cell activation. Shimizu *et al*. (1999) reported the action mechanism of Sho-saiko-to against hepatic fibrosis using DMA- or PS-induced hepatic fibrosis models. Male Wistar rats were given a single i.p. injection of DMA, 40 mg/kg, or 0.5 ml PS twice weekly for 10 weeks. In each model, rats were fed a diet which contained 1.5% Sho-saiko-to extract throughout. Sho-saiko-to suppressed the induction of hepatic fibrosis, increased hepatic retinoids, and reduced the hepatic collagen and malondialdehyde (MDA), a product of lipid peroxidation. Immunohistochemical examination showed that Sho-saiko-to reduced the deposition of type I collagen and the number of α-smooth muscle actin (α-SMA) positive stellate cells and inhibited, not only lipid peroxidation in cultured rat hepatocytes that are undergoing oxidative stress, but also the production of type I collagen, α-SMA expression, cell proliferation and oxidative burst in cultured rat stellate

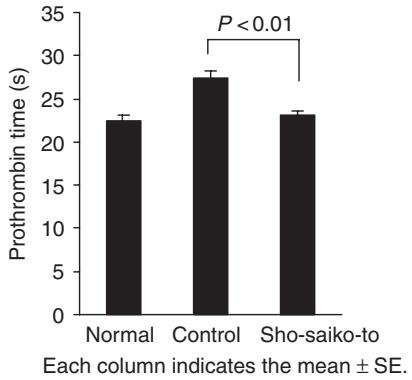

Figure 4.29 Effect of Sho-saiko-to on prothrombin time in PS-treated rats at 3 months.

cells. In addition, Sho-saiko-to inhibited Fe^{2+}/adenosine 5′-diphosphate-induced lipid peroxidation in rat liver mitochondria in a dose-dependent manner and showed a radical scavenging activity. Among the active components of Sho-saiko-to, baicalin and baicalein contained in Scutellariae Radix, one of the composed crude drugs of Sho-saiko-to, were found to be mainly responsible for the anti-oxidative activity. These findings suggest that Sho-saiko-to functions as a potent anti-fibrosuppressant by inhibition of lipid peroxidation in hepatocytes and stellate cells *in vivo*.

The effect of Sho-saiko-to on the process of liver fibrosis induced by DMA and accelerating liver regeneration was further investigated, especially its effect on Ito cells by Miyamura *et al*. (1998). Sho-saiko-to improved the hepatic function, histopathological findings and retinoid levels. When Sho-saiko-to was administered to 70% hepatectomized normal and liver-injured rats, liver weight, the number of S-phase cells and retinoid levels increased.

There have been studies on whether Sho-saiko-to has an inhibitory effect on the development of pre-neoplastic lesions in addition to the liver fibrosis. Sakaida *et al*. (1998) reported the effect of Sho-saiko-to using the choline-deficient L-amino acid-defined (CDAA) diet-induced liver fibrosis model in male Wistar rats. Rats were fed a CDAA diet or a CDAA diet containing 1% Sho-saiko-to extract for 16 weeks. Sho-saiko-to prevented fibrosis formation, as indicated by reduced hydroxyproline content in the liver and by the inhibition of the increase in a serum marker of fibrosis, hyaluronic acid, without reducing the increase in serum alanine aminotransferase and aspartate aminotransferase. Sho-saiko-to also reduced the expression of type III procollagen alpha 1 mRNA in the liver, and inhibited the proliferation of myofibroblast-like cells, which was an activated and α-SMA positive-stellate cells. Furthermore, Sho-saiko-to reduced the number of pre-neoplastic lesions, detected as enzyme-altered (glutathione S-transferase placental form-positive) lesions, in the liver. These results indicate that Sho-saiko-to prevents the formation of both fibrosis and pre-neoplastic lesions, not by inhibiting hepatocyte cell death but by inhibiting the activation of stellate cells. It is of extreme importance to prevent liver fibrosis and subsequent progression of cirrhosis and hepatocyte tumour by the treatment of Sho-saiko-to.

Kayano *et al*. (1998) reported the inhibitory action of Sho-saiko-to on hepatic stellate cell. Hepatic stellate cells were isolated from male Wistar rats. Water-soluble ingredients of Sho-saiko-to were obtained at concentrations of 10, 100, 250, 500 and 1000 μg/ml. The morphological transformation was observed under a phase-contrast microscope. The cell cycle of the

stellate cells were analyzed using flow cytometry on day 4 after the culture to evaluate the activities of the proliferation of the stellate cells. Northern blot analysis was carried out on day 3 after culture to determine the expression of type I and type III pro-collagen mRNAs. As a result, Sho-saiko-to, 500 and 1000 μg/ml, inhibited morphological transformation of stellate cells to myofibroblast-like cells. Further, Sho-saiko-to, 500 and 1000 μg/ml, significantly accumulated the cells in the G0/G1 phase (118.8 ± 0.7%, 119.2 ± 0.4%, respectively as compared with control) and significantly decrease cell numbers subsequently in G2/M phase (47.5 ± 8.1%, 48.9 ± 2.0%, respectively). Sho-saiko-to, 500 and 1000 μg/ml, also significantly suppressed pro-collagen mRNA expression of type I to 51.5 ± 6.4%, 34.9 ± 3.7%, respectively, and type III to 51.3 ± 12.3%, 46.7 ± 11.4%, respectively. From these data Sho-saiko-to was found to inhibit hepatic stellate cells. Sho-saiko-to could be a potent inhibitor of liver fibrosis.

Yano *et al*. (1994) reported that Sho-saiko-to inhibited tumour cell proliferation and possessed at least two different mechanisms of action. Water-soluble ingredients of Sho-saiko-to dose-dependently inhibited the proliferation of a human hepatocellular carcinoma cell line, KIM-1, and a cholangiocarcinoma cell line, KMC-1. ED50 value on day 3 of exposure to Sho-saiko-to was 353.5 ± 32.4 μg/ml for KIM-1 and 236.3 ± 26.5 μg/ml for KMC-1. However, almost no suppressive effects were detected in normal human peripheral blood lymphocytes or normal rat hepatocytes. Sho-saiko-to suppressed the proliferation of the carcinoma cell lines more strongly than do each of its major ingredients. Morphological, DNA and cell cycle analyses revealed two possible modes of action of Sho-saiko-to to suppress the proliferation of carcinoma cells. One was to induce apoptosis in actively proliferating tumour cells and the other is to induce arrest at the G0/G1 phase and to decrease DNA synthesis. The active fractions of Sho-saiko-to on inducing apoptosis was reported to be flavonoids, and the active component of Sho-saiko-to on inducing arrest at the G0/G1 phase and decreasing DNA synthesis was reported to be GL involved in Glycyrrhizae Radix. This report says that there are synergistic and additive effects of various ingredients in Sho-saiko-to and that there might be other unknown substances in Sho-saiko-to possessing anti-tumour effects. These data suggests that Sho-saiko-to can inhibit the development of cirrhosis and liver cancer in type C chronic hepatitis patients.

Protective effect on lung injury

Osanai and Takahashi (1990) reported the effects of Sho-saiko-to on diffuse interstitial pneumonia and pulmonary fibrosis in bleomycin (BLM)-induced experimental fibrosis. BLM, 0.5 units, was endotracheally administered once to hamsters that had been given feed mixed with Sho-saiko-to at 6.3 mg/g feed (estimated amount of daily intake, 63 mg) from 7 days before BLM administration. In Sho-saiko-to treated group, the suppression of body weight gain was smaller than that in control group. The suppression of the body weight gain might have been caused by poor intake due to respiratory failure. Four days after BLM administration, Sho-saiko-to did not inhibit the increase of the total cell count or neutrophil count in the bronchoalveolar lavage (BAL) fluid, but inhibited the counts of alveolar macrophages and lymphocytes in a significant manner. However, the total protein level in the BAL was rather higher in the Sho-saiko-to treated group. Thirty days after BLM administration, the lung hydroxyproline amount tended to be lower in the Sho-saiko-to treated group than in the control group. In the optical microscopic findings in the lung tissue 30 days after the BLM administration, the control group showed uniform distribution of fibrogenesis over the whole lung, however, the Sho-saiko-to treated group had fibrogenesis centering around the hilus of the lung, and most of the peripheral lung area was almost normal. The above results suggest that Sho-saiko-to suppresses BLM-induced lung damage and pulmonary fibrosis.

The radiation to lung tissue is often tried in the treatment of various tumours. Radiation, however, induces lung pneumonia and following fibrosis. Kure (1992) reported the effects of methylprednisolone and Sho-saiko-to on the radiation damage in lung tissue in four main groups of female ICR mice, one control group and three groups irradiated with single doses (6 Gy, 12 Gy, 18 Gy) of ^{60}Co gamma rays. Methylprednisolone and/or Sho-saiko-to were administered. Direct quantitative measurements of collagen accumulation in lung (lung fibrosis) were made analysing digitally processed microscopic images of Azan–Mallory stained sections 24 weeks after irradiation. Administration of methylprednisolone suppressed the expected development of fibrotic lung tissue in each of the irradiated groups. In a further study, peplomycin, a lung fibrosis enhancing agent, was administered to all four groups in addition to methylprednisolone and Sho-saiko-to. Methylprednisolone was demonstrated to be effective only in the 12 Gy group. Overall, Sho-saiko-to showed a lesser degree of effect in the prevention of the fibrosis than methylprednisolone, but the administration of both was demonstrated to be more effective than the administration of methylprednisolone alone.

Protective effect on gastric injury

The effect of Sho-saiko-to on gastric function was examined by Kase *et al*. (1997). Oral administration of Sho-saiko-to, 125–500 mg/kg, suppressed both water-immersion stress-induced gastric lesions and ethanol-induced gastric injury in a dose-dependent manner. Intraduodenal administration of Sho-saiko-to at 125–500 mg/kg suppressed gastric juice secretion dose-dependently. Sho-saiko-to, however, showed no effect on gastric emptying in normal rats. These results suggest that Sho-saiko-to has anti-ulcer effects, probably based on its ability to suppress gastric secretion and to protect the gastric mucosa.

Protective effect on renal injury

The anti-nephritic effect of Sho-saiko-to on crescentic type anti-glomerular basement membrane (GBM) nephritis in rats was studied by Hattori *et al*. (1988). The nephritis was induced by an i.v. injection of anti-GBM serum followed by an i.d. injection of 6.5 mg rabbit-gamma globulin in Freund's complete adjuvant into their hind foot pads and Sho-saiko-to was given to the rats from day 1 to day 39. In the treatment with 1.8 g/kg of Sho-saiko-to, urinary protein, plasma cholesterol and plasma urea nitrogen were significantly reduced during the experimental period. Additionally, crescent formation and adhesion of capillary walls Bowman's capsule were significantly inhibited by the administration of 0.45 and 1.8 g/kg of Sho-saiko-to. The index of glomerular lesions was significantly reduced in the 0.45 and 1.8 g/kg groups. In the next experiment, Sho-saiko-to at the dose of 4.5 g/kg was given to the rats from day 23 to day 53. Sho-saiko-to at the dose of 4.5 g/kg significantly reduced urinary protein excretion and tended to inhibit the glomerular lesions. Therefore, Sho-saiko-to has pronounced effects on biochemical and histological parameters for crescentic-type anti-GBM nephritis in rats.

Anti-tumour effect

Ito and Shimura (1986) reported the anti-tumour effects of Sho-saiko-to with or without 5-fluorouracil (5-FU) or cyclophosphamide (CY) in an experimental system of lung metastasis induced by Lewis lung carcinoma in C57BL/6crSlc mice. Lewis lung carcinoma cells were implanted into the footpads of the mice. Ten days later, the implanted tumours were surgically removed. The effects of Sho-saiko-to were evaluated by the number of lung surface nodules

present 14 days after removal of the implanted tumour. The oral administration of Sho-saiko-to, 300 mg/kg × 2/day, for 10 days causes the anti-metastatic effect. Therapy with Sho-saiko-to and 5-FU or CY significantly inhibits the development of lung metastases. The number of peritoneal macrophages and the degree of the binding of C3 cleavage products (C3b) to macrophages were enhanced in the mice treated with Sho-saiko-to. Lung metastases were inhibited by the i.v. administration of peritoneal macrophages activated with oral Sho-saiko-to. These findings raise the possibility that Sho-saiko-to may have clinical value in the prevention of cancer metastasis.

The anti-tumour and anti-metastatic effects of Sho-saiko-to were further studied by Kato *et al*. (1998). At the same time, its chemically defined ingredients on the primary skin melanoma that develops in a metallothionein-I/ret transgenic mouse line and on a melanoma cell line, which is derived from a primary tumour developed in a metallothionein-I/ret transgenic mouse, were examined. *In vitro*, Sho-saiko-to suppressed the growth of a melanoma cell more strongly than any single ingredient of Sho-saiko-to, although baicalin as one of the ingredients tested also suppressed it significantly. In *in vivo*, Sho-saiko-to significantly prolonged the onset of tumour development (1.5 mo), definitely retarded the transition of malignancy, significantly decreased the incidence of distant metastasis to brain, kidney and liver at the malignant stage, and significantly prolongs life span (2.6 mo). Moreover, Sho-saiko-to and baicalin down-regulate the matrix metaroproteinase-2 and -9 expression levels, and up-regulate their inhibitor expression level in both the primary tumours and melanoma cells. In conclusion, Sho-saiko-to displays anti-tumour and anti-metastatic effects on melanoma with regulation of the balance of matrix metalloproteinase and tissue inhibitor of the matrix metalloproteinase levels.

Liu *et al*. (1994) reported the effect of Sho-saiko-to on formation and growth of squamous cell papillomas induced by 7,12-dimethylbenz(a)anthracene application in mouse skin. Chronic oral administration of Sho-saiko-to reduced the incidence and growth of papillomas with the reduction of activities of succinate-dehydrogenase and thymidylate synthetase, which were evaluated as the cell viability and DNA synthesis *via* the *de novo* pathway, respectively.

Tatsuta *et al*. (1991) reported the effect of Sho-saiko-to on hepatocarcinogenesis induced by N-nitrosomorpholine in male Sprague–Dawley rats. Rats were given normal chow pellets containing 0.5% or 1.0% Sho-saiko-to until the end of the experiment and drinking water containing N-nitrosomorpholine for 8 weeks. Pre-neoplastic and neoplastic legions staining for gamma-glutamyl transpeptidase (GGT) or the placental type of glutathione-S-transferase (GST-P) were examined histologically. In week 15, quantitative histological analysis showed that prolonged treatment with 0.5% Sho-saiko-to significantly reduced the number and volume of GGT-positive and GST-P positive hepatic lesions. Treatment with 1.0% Sho-saiko-to inhibited the development of GGT positive and GST-P positive lesions, but was less effective than 0.5% Sho-saiko-to. Administration of 0.5% Sho-saiko-to also caused a significant increase in the proportion of helper T lymphocytes and a significant decrease in the labelling index of pre-neoplastic lesions. These findings indicate that Sho-saiko-to inhibits the development of hepatic foci.

Okita *et al*. (1993) reported the anti-growth effects of components of Sho-saiko-to on cultured human hepatoma cells (HuH-7). Cell cycle analysis was carried out using the flow cytometry and the bromodeoxyuridine (BrdU)-labelling method. The results showed that baicalin, baicalein and saikosaponin-a inhibited cell proliferation dose-dependently but independently of the cell cycle. Furthermore, it was found that saikosaponin-a possessed a strong cell-killing effect. On the other hand, ginsenoside-Rb1, saikosaponin-c and ginsenoside-Rg1 had no effect on cell proliferation.

It is known that the hyperplastic alveolar nodule is a representative pre-neoplastic state in the mammary glands of mice. Therefore, the effects of Sho-saiko-to on the formation and growth of

hyperplastic alveolar nodule in a high mammmary tumour strain of SHN virgin mice was studied by Sakamoto *et al*. (1993b). Oral administration of Sho-saiko-to for 60 days beginning at 90 days of age reduced the number and area of hyperplastic alveolar nodules and mammary thymidylate synthetase activity with a reduction of serum prolactin level. These results indicate that Sho-saiko-to may have a preventive effect on malignant mammary transformations.

Sakamoto *et al*. (1993a) further reported the effect of Sho-saiko-to on DNA-synthesizing enzyme activity in 1,2-dimethylhydrazine-induced colonic carcinomas. Six-week administration of Sho-saiko-to prevented nearly 100% of the body weight loss and decreased the final number of the colonic carcinomas. Sho-saiko-to, further, suppressed the enhanced activities of thymidylate synthetase and thymidine kinase, which were involved in the *de novo* and salvage pathway of pyrimidine synthesis, respectively, in 1,2-dimethylhydrazine-induced colonic carcinomas. These results indicate that Sho-saiko-to may have directly and/or indirectly inhibitory effects on the development of colonic carcinomas.

Conventional therapy for renal carcinoma using IL-2 has shown limited anti-tumour action. Huang *et al*. (1997) studied the synergistic anti-tumour effect of IL-2 and Sho-saiko-to using the murine renal cell, Renca, *in vivo*. The treatment was started 5 days after subcutaneous transplantation of Renca tumour. Sho-saiko-to was given orally at a dose of 2.5 g/kg daily for 30 days. IL-2 was given by subcutaneous injection every other day eight times at a dose of 10^4 U/mouse. The combination of Sho-saiko-to and IL-2 inhibited the growth of the tumour and prolonged the survival significantly compared with the untreated mice. Increased cellular infiltration was observed in tumour tissue and the lungs of mice treated with Sho-saiko-to alone and in combination with IL-2, but there were no histological changes in the liver and kidney. Elevation of serum IL-6 was observed in tumour-bearing mice, but IL-6 was significantly suppressed by administration of Sho-saiko-to. The results suggest that the combination of Sho-saiko-to and IL-2 induces enhanced immunological reaction in specific organs and tissues, and IL-6 may have a role in the synergistic effect of these two agents. The results show that a combination of Sho-saiko-to and IL-2 is useful in the treatment of patients with renal cell carcinoma.

Sakaguchi *et al*. (1993) reported the effect of Sho-saiko-to on the various metabolic disorders and on anti-tumour activity of recombinant human necrosis factor (rhTNF) administered to mice. The glycogen level in liver of rhTNF (5×10^4 units/mouse, i.v.)-injected mice was markedly lower 4 h after intoxication than that in control, whereas the administration of rhTNF to Sho-saiko-to (0.5 g/kg, p.o.)-pre-treated mice results in a greater level of glycogen than that in rhTNF alone-treated mice. In mice pre-treated with Sho-saiko-to, the level of fibrinogen 4 h after rhTNF injection markedly increases as compared to that in mice treated with rhTNF alone. The NO_2 in murine macrophage cell line J774A.1 using mouse serum after administration of Sho-saiko-to was also estimated. J774A.1 cells stimulated with endotoxin (1 μg/ml) and rhTNF ($1 \times 10(4)$ units/ml) can effectively produce NO, and the suppressive effect of Sho-saiko-to (0.5 g/kg, p.o.)-pre-treated serum on NO generation by endotoxin/TNF-activated J774A.1 cells is ascertained. When the cells were incubated with endotoxin/TNF and Sho-saiko-to-pre-treated serum (10–100 μλ), the NO level was significantly lower than that in control serum incubated with endotoxin/TNF alone. The inhibitory effect of Sho-saiko-to (1 and 10 μg/l) on *in vitro* cytotoxicity by rhTNF in Meth-A sarcoma cells was dose-dependent. In addition, there was a significant enhancement in anti-tumour activity of rhTNF in mice pre-treated with Sho-saiko-to. These findings suggest that Sho-saiko-to may protect mice from severe shock syndrome induced by rhTNF, and that it may enhance rhTNF-induced anti-tumour activity.

Anti-HIV effect

Piras *et al*. (1997) reported the Sho-saiko-to's inhibitory effect on human immunodeficiency virus type 1 (HIV-1) replication in PBMC. Sho-saiko-to alone at concentration of 25 μg/ml moderately inhibited HIV-1 replication. When Sho-saiko-to was combined with zidovudine (AZT), lamivudine (3TC) or AZT + 3TC, Sho-saiko-to enhanced the anti-HIV-1 activity of 3TC. In contrast, Sho-saiko-to slightly enhanced the anti-HIV activity of AZT + 3TC, but did not enhance the activity of AZT alone. These results suggest that the combination of Sho-saiko-to and 3TC has potential as a chemotherapeutic modality of HIV-1 infection.

Miyamoto *et al*. (1996) reported the effects of Sho-saiko-to on the production of PGE_2 from monocytes, LTB_4 and superoxide from PMN cells in HIV-infected individuals. Sho-saiko-to inhibited the production of PGE_2 from monocytes stimulated by opsonized zymosan in all groups including the health control group and also inhibited the production of superoxide from PMN cells after stimulation with FMLP. On the other hand, Sho-saiko-to enhanced the production of LTB_4 when PMN cells were stimulated by the calcium ionophore A23187. These results suggest that Sho-saiko-to has different effects on the production of prostanoids or superoxide from monocytes and PMN cells. Furthermore, the inhibition of PGE_2 or superoxide production will lead to indirect suppression of HIV, and enhancement of LTB_4 will contribute to the up-regulation of the immune reaction in HIV infected individuals.

Baicalin and baicalein are components of Sho-saiko-to, which are claimed to be therapeutically effective in treating HIV-infected patients. Wu *et al*. (1995) reported their apoptotic effect. Although 20 μg/ml of baicalin is not cytotoxic to a cultured T-cell line, it proved to be cytotoxic to a HIV-infected cultured T-cell line with a higher HIV-releasing capacity, and DNA fragmentation was detected within 24 h of incubation. However, after incubation of the HIV-infected cultured T cell with a lower dose of baicalin (0.1, 0.3 and 2 μg/ml) for 24 h and 48 h, the viable cell number increased by about 25% and the p24 released into the medium was 25% lower than that of the control. After further incubation in the presence of the agent for 6 and 9 days, only cells with a lower HIV-releasing capacity survived. Baicalin might selectively induce apoptosis of HIV-infected cultured T cells which have a high virus-releasing capacity and stimulate proliferation of HIV-infected cultured T cells which have a relatively lower capacity of HIV-production.

Sho-saiko-to was further tested for its ability to inhibit the production of HIV by Buimovici *et al*. (1990). The testing was done with cultures of human lymphocytes obtained from HIV-positive asymptomatic subjects and patients with ARC or AIDS. The replication of the virus was monitored by quantitative assay of the reverse transcriptase activity and of the synthesis of antigen p24. The lymphocyte cultures were maintained in the absence and in the presence of 25, 50 or 100 μg/ml of Sho-saiko-to, and monitored for up to 5 weeks. The results showed that in lymphocyte cultures from asymptomatic subjects reverse transcriptase activity and synthesis of p24 were completely inhibited by low concentrations of Sho-saiko-to. A high concentration of Sho-saiko-to inhibited virus replication in 80% of the lymphocyte cultures from ARC patients, but was completely ineffective in lymphocyte cultures from AIDS patients. The reverse transcriptase activity was more sensitive to inhibition by Sho-saiko-to than the synthesis of p24, and the anti-viral effect was dependent on the virus load of the lymphocyte cultures.

Ono *et al*. (1990) reported that Sho-saiko-to differentially inhibited the activities of reverse transcriptase and human cellular DNA polymerase alpha and beta. Reverse transcriptases from murine leukaemia virus and HIV were inhibited by over 80% and 50%, respectively, in the presence of 100 μg/ml Sho-saiko-to, whereas DNA polymerase alpha was much less sensitive to inhibition by this drug at concentrations of up to 500 μg/ml. DNA polymerase gamma was not

inhibited by this drug at concentrations of up to 500 μg/ml. Only DNA polymerase beta was moderately inhibited by Sho-saiko-to. Thus, it has been shown that the inhibition by Sho-saiko-to is relatively specific for reverse transcriptase.

Protective effect on atherosclerosis and lipid metabolism

Umeda *et al*. (1988) reported the effects of the oral treatment of Sho-saiko-to on the experimental atherosclerosis in rabbits fed a 0.75% cholesterol diet for 6 months. Non-invasive optical measurement of volume elastic modulus was done for indirect evaluation of atherosclerosis. Non-invasive measurement of atherosclerotic changes in the arterial system reported by Shimazu *et al*. (1985) and Kamiya *et al*. (1983) was performed as follows. A non-traumatic technique to measure the *in vivo* pressure–volume relationship between the infra-red light absorption through the tissue and the blood volume contained in it was used. The system designed for this purpose consisted of a light source, a photodetector, a compressing cuff driven by a micro-roller pump and an amplifier for the phototransistor output and for the cuff compression pressure. The light source and detector chips were directly fixed on the rabbit forearm skin in mutually opposing positions, and the compression cuff was placed around the forearm so as to cover the tips at the middle portion. As the compression pressure of the cuff was gradually increased, the transmural pressure of the vessels under the cuff was reduced, and the associated decrease in blood volume according to the overall vascular compliance was detected by the change in the transmitted light intensity proportional to the transistor output. The relative pressure–volume relationship normalized with the unstressed vascular volume was obtained for the arterial system, as well as elastic modulus volume for various levels of the transmural pressure. The control group was fed a 0.75% cholesterol diet, and Sho-saiko-to treated group is fed a 0.75% cholesterol diet containing powdered extracts of Sho-saiko-to, 0.9 g/kg/day. Although the volume elastic modulus level was not changed up to 12 weeks, it increased drastically in the cholesterol-treated control group after 20 weeks at 40 and 60 mm Hg of transmural pressure. The cholesterol-induced increase in the volume elastic modulus value was inhibited by the treatment of Sho-saiko-to. However, Sho-saiko-to showed no effects on the increased serum cholesterol and phospholipid concentration. After the removal of thoracic aorta of all rabbits at 6 months, atherosclerotic lesions were studied histopathologically. The hydroxyproline obtained by the hydrolysis of aortic collagen and lipid content in both serum and thoracic aorta were also determined. In Sho-saiko-to treated group, atherosclerotic regions and atherosclerotic index as a ratio of area of atherosclerotic regions against that of thoracic intimal surface were inhibited compared to that in control group. The histopathological findings showed that the cholesterol-treated control group had a very thick intima, an accumulation of foam cells, serious hyperplasia of collagen, serious calcium deposit and numerous necrotic substances. In the Sho-saiko-to treated group, the intima histopathological findings were remarkably improved. In particular, the decrease in hyperplasia of collagen, calcium deposit and necrotic substances were attractive. In media, elastic lamina tears were also repaired by Sho-saiko-to treatment. Cholesterol feeding increased the aorta weight about 3-fold over that of the normal group, and aorta lipid content is also drastically increased. Sho-saiko-to treatment showed no significant effect on the increased aorta weight, but it inhibited the total aortic cholesterol and phospholipid content. The hydroxyproline which increased 2-fold in the cholesterol-treated control group compared with that in normal group was significantly inhibited by the treatment of Sho-saiko-to. These results suggest that Sho-saiko-to improves the hyperlipidemia-induced injury of aortic endothelial and/or smooth muscle cells.

To determine the anti-atherosclerotic activity of Sho-saiko-to, Nagatsu *et al*. (1992) studied the effect of Sho-saiko-to on cholesterol metabolism in macrophages. Although macrophages, harvested from mice treated with Sho-saiko-to, took up a small amount of control low density lipoprotein (LDL) (thiobarbituric acid-reactive substance (TBA-RS) value is 0.27 pmol/mg of protein) as control macrophages, they took up more LDL modified with $CuSO_4$ (TBA-RS value is 6.12 pmol/mg of protein) than control macrophages. Degradation of both control LDL and oxidized LDL was enhanced in Sho-saiko-to-treated macrophages. In the presence of control LDL, or in the absence of LDL, incorporation of [3H] oleic acid into cholesteryl oleate was significantly reduced in Sho-saiko-to treated macrophages. This suggests that acyl-coenzyme A: cholesterol acyltransferase (ACAT) activity in macrophages is partly inhibited by Sho-saiko-to treatment. On the other hand, in the presence of oxidized LDL, cholesteryl ester accumulated in Sho-saiko-to treated macrophages as much as in the controls. However, cholesteryl oleate efflux from macrophages in the presence of high density lipoprotein (HDL) was enhanced in the Sho-saiko-to treated macrophages. These results indicate that Sho-saiko-to facilitates oxidized LDL catabolism in macrophages, resulting in the augmentation of oxidized LDL uptake and the elimination of cholesterol from macrophages by HDL. These effects of Sho-saiko-to may prevent foam cell formation and the progression of atherosclerotic lesions.

Anti-atherosclerotic action of Sho-saiko-to was further investigated by Shen *et al*. (1996) using hypercholesterolemic mice. Oral administration of Sho-saiko-to significantly suppressed the elevation of serum cholesterol in C57BL/6 mice fed a 1.25% cholesterol-enriched diet for 4 weeks and improved the T-cell ratio in peripheral blood, which decreased with the increase of the serum cholesterol level. In addition, Sho-saiko-to reduced the accumulation of cholesteryl oleate, which alters macrophages into foam cells, after the treatment of macrophages with oxidized or acetylated LDL. The enzymatic study revealed that the treatment of macrophages with oxidized LDL enhanced ACAT activity and markedly reduced neutral cholesteryl ester hydrolase (NCEase) activity. Sho-saiko-to treatment prevented the decrease in the NCEase activity induced by the oxidized LDL treatment, although it slightly augmented ACAT activity. Thus, Sho-saiko-to, which is known to modulate the immune system, improves macrophage and lymphocyte functions diminished by hypercholesterolaemia, resulting in an anti-atherosclerotic action.

Macrophages play important roles both in immune responses and in lipid metabolism and contribute to the development of atherosclerosis. To clarify the mechanism by which Sho-saiko-to shows anti-atherosclerotic action, its effect on macrophage function was studied by Inoue *et al*. (1996). The production of NO, prostaglandin E_2 and IL-1 by macrophages in mice was reduced by feeding of cholesterol-enriched diet, and the reduced production was observed 1 week after the beginning of cholesterol feeding. Furthermore, although oxidized LDL and lysophosphatidylcholine reduce NO production, macrophages prepared from mice treated with Sho-saiko-to at a dose of 1.2 g/kg/day restored the reduced NO production. When the content of lysophosphatidylcholine was measured, no difference was observed between mice fed a cholesterol-enriched diet in the presence or in the absence of Sho-saiko-to treatment, suggesting that the restorative effect of Sho-saiko-to is not due to the inhibition of lysophosphatidylcholine production or accumulation. These results conclusively show that Sho-saiko-to prevents the modification of macrophage function induced by atherogenic factors, which is probably linked to its displayed anti-atherosclerotic action.

Discussion

Sho-saiko-to has multiple pharmacological actions. Among them, the immunological actions, especially on macrophage functions which are important for the host-defence system by regulating

the cytokine and prostaglandin productions etc., are neccessary to explain the pharmacological actions of Sho-saiko-to. Clinically, Sho-saiko-to is used to treat type B or type C chronic active hepatitis in order to inhibit the development of chronic hepatitis to cirrhosis and liver cancer. In the clinical study Sho-saiko-to prevented the appearance of liver cancer and prolonged the survival time of patients. Although, it is unknown whether Sho-saiko-to directly prevents the fibrosis formation and/or hepatocyte tumour, its immunomodulately actions might be the important factors for explaining its clinical effects. In recent data, anti-oxidant activities are also important for proving the multiple pharmacological and clinical actions of Sho-saiko-to. The active components of Sho-saiko-to is mostly unknown and the efficacy of Sho-saiko-to cannot be explained from the purified active ingredients. Synergistic actions of active ingredients are very important to estimate the remarkable pharmacological and clinical actions of Sho-saiko-to, which are shown in several chapters described in this book. Conclusively, the remarkable effects of Sho-saiko-to could be explained only by its characteristic property as mixture.

Clinical efficacy of a mixture must be evaluated in clinical trials based on EBM. The characteristic actions of Sho-saiko-to is rather mild in efficacy, and it has few adverse effects and a wide pharmacological spectrum. It will be useful clinically and should have advantages over Western medicines in terms of adverse effects, medical cost and compliency, especially in aged patients who have multiple problems.

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5 Clinical studies

Hiroko Oka

Introduction

Chinese herbal medicine originated in China 3000 years ago on the basis of clinical experience and practice, and was introduced in Japan in the fifth century. There are many prescriptions that have been handed down since then, but the effective ingredients have usually not been identified. Sho-saiko-to (Xiao-Chai-Hu-tang in Chinese) is one of the most popular prescriptions and has been widely used in Japan.

The traditional diagnostic method of Chinese medicine is quite different from that of Western medicine, and the same method was used in Japan. After Western medicine was introduced in Japan and having become popular in the last 100 years, both methods of diagnosis have been used. Sho-saiko-to is known to be an effective medicine for the treatment of acute and chronic inflammatory disease, pneumonia, tuberculosis and chronic liver disorder, etc.

There are many papers about clinical studies and experimental studies concerning Sho-saiko-to. Their diagnosis techniques and methods of analysis were mainly based on the Western medicine.

This chapter summarizes the main clinical studies and experimental research using Sho-saiko-to concerning about liver disease.

Chronic hepatitis

Clinical studies

There are many case reports in Japanese journals on the effectiveness of Sho-saiko-to for the treatment of chronic hepatitis. However, most of them are written in Japanese, and the number of patients was relatively small.

In 1987, Fujiwara *et al*. reported on the effectiveness of a combination of Sho-saiko-to and Keishi-bukuryou-gan (Gui-Zhi-Fu-Ling-Wan in Chinese) which is one of the prescriptions of traditional herbal medicine in the treatment of chronic viral hepatitis by analysing the results of liver function tests in 102 patients receiving this combination. The aetiology was of viral origin in all; 25 patients with hepatitis type-B (HBV) infection and 77 patients with type non-B. The patients were divided into low- and high-dose groups, which received 2.5 g of each drug twice or three times a day before meals, respectively. All patients were treated for at least 6 months; 17 patients continued for 6 months, 14 for 12 months, 23 for 18 months, 3 for 24 months, 9 for 30 months, 8 for 36 months, 8 for 42 months and the remaining 20 for 48 months. The period of observation before this treatment ranged between 2 and 6 months. The drugs reduced high serum alanine transaminase (ALT) and serum aspartate transaminase (AST) values without affecting serum total protein, albumin and total cholesterol concentrations. The improvement occurred sooner at the

Table 5.1 Score for physical constitution

Item	*Score*		
	+1	0*	−1
1 Physique	Sturdy		Lean
2 Face colour	Ruddy		Pale
3 Bowel habit	Hard stools, constipation		Loose stools, diarrhoea
4 Epigastric discomfort	Frequent, severe		Rare, mild or none
5 Appetite loss	Mild or none		Severe
6 Easy fatigability	Rare, mild, or none		Frequent, severe

Note
* Intermediate groups were scored as 0.

higher dosage and became more evident with the duration of treatment. Patients aged more than 50 years old required the higher dosage to obtain the same effect as that seen in those younger than 50 years old. The higher dosage caused a greater reduction in serum ALT activities.

Hasegawa *et al*. (1987) also evaluated the effect of the same combination of Sho-saiko-to and Keishi-bukuryou-gan on 55 patients with chronic liver disease; 43 patients with chronic hepatitis and 12 with cirrhosis of the liver. As the efficacy of herbal medicine has been said to be related to the patient's physical constitution that is known as *Sho* in oriental medicine, they designed the score system for physical constitution for every patients to evaluate their *Sho* as shown in Table 5.1.

The effect of the combination of the two herbal preparations on changes in serum ALT were studied in relation to the *Sho* scores in the patients who were given these medicines for more than 6 months. A high rate of improvement was found in the high-physical constitution-score group. To clarify the mechanism of action of these medicines on patients with chronic liver diseases, the T-cell function of these patients was studied. T-cell responsiveness to several mitogens, such as phytohaemagglutin and concanavalin A, increased after administration of these medicines. Examination of the proportion of various T-cell subsets showed that the number of suppressor T cells decreased. The authors suggested that these herbal medicines affect suppressor T cells.

Hirayama *et al*. (1989) did a double-blind multicentre randomized controlled trial of Sho-saiko-to in chronic active hepatitis to evaluate the effect Sho-saiko-to using the method of Western medicine. One hundred and sixteen patients (52 patients were positive for HBs antigen) received Sho-saiko-to in a daily dose of 5.4 g for 12 weeks, followed by the same dose for a further 12 weeks. One hundred and six patients (47 patients were positive for HBs antigen) received a placebo containing 0.5 g of Sho-saiko-to for 12 weeks, followed by a cross-over to Sho-saiko-to for a further 12 weeks. Of the liver tests, the serum ALT values decreased significantly with the administration of Sho-saiko-to. The difference in the mean value between the Sho-saiko-to group and the placebo group was significant after 12 weeks. In patients with chronic active type-B hepatitis, a tendency towards a decrease of HBe antigen (HBeAg) and an increase of Anti-HBe antibodies (HBeAb) was also observed. No remarkable side-effects were noticed.

Tajiri *et al*. (1991) reported on the effect of Sho-saiko-to on HBeAg clearance in children with chronic hepatitis B virus infection and with sustained liver disease. The average age of the patients was 7.8 years (range, 1.0–14.5 years). The dosage of Sho-saiko-to was determined according to Augsberger's table with 7.5 g as a daily standard dose for an adult. Out of 14, 7 patients became HBeAg negative in the average observation period of 0.47 years. Out of the

7 patients 4 developed HBeAb. Sho-saiko-to induced seroconversion in HBeAg/HBeAb system at a higher rate of 50.0% than the 22.7% in 22 patients who were observed during the same period as the control group. The comparison of predisposing factors between responders and non-responders revealed that although statistically not significant, serum ALT was higher, and both HBeAg and DNA-polymerase were lower in responders than in non-responders. This implied that HBeAg clearance by Sho-saiko-to might occur in a patient with a great degree of hepatitis and with a lesser degree of HBV replication.

Gibo *et al*. (1994) did a controlled study on Sho-saiko-to's effect on patients with chronic hepatitis type C. They enrolled 80 patients with chronic hepatitis. HBV infection, hepatotoxic drugs, autoimmune hepatitis and alcoholics were excluded from the study. The patients were divided into two groups. The trial group was given Sho-saiko-to 7.5 g/day in addition to conventional medicine for at least 6 months. The control group was given only conventional medicine. The number of patients, histological background of the two groups were matched. These patients were followed up for approximately 7 years. Serum AST and ALT levels were markedly decreased and normalized in 5 of 40 patients (12.5%) in the trial group, and 1 of 40 patients (2.5%) in the control group. In 1 of the 5 patients whose ALT improved in the trial group, anti-HCV antibody converted to negative and HCV-RNA was also negative at the end of the observation period. However, no patient became negative for the anti-HCV antibody in the control group. One patient in the trial group developed hepatocellular carcinoma (HCC), whereas 5 patients developed it in the control group.

Yamamoto *et al*. (1995) did a follow-up study of 98 patients with chronic hepatitis (59 patients had type-B hepatitis) using Sho-saiko-to for 5 years. The mean age was 52.4 ± 1.2 years. The serum levels of AST, ALT, glutamyl transpeptidase (γ-GTP), ALP and TTT were significantly reduced with administration of Sho-saiko-to. Seroconversion was observed in 3 of 7 patients who were positive for HBeAg. An improvement of liver function was observed in 78% of the group of type-B hepatitis and in 67% of the group of non-A non-B hepatitis.

On the basis of Chinese traditional diagnosis, Itoh *et al*. (1997) evaluated the effect of some prescriptions of Chinese herbal medicine on 47 patients with chronic hepatitis. The number of patients with hepatitis B and C were 8 and 39, respectively. The prescriptions given to each patient were selected according to the traditional diagnosis based on *Sho*. Twelve prescriptions including Sho-saiko-to were used in this study. An improvement of serum ALT was observed in 75% of hepatitis B patients and in 44% of hepatitis C patients after 6 months of treatment. Symptoms such as easy fatigability and general malaise were brought under control in 90% of the patients. Comparing the prognosis of 18 patients with HCV, treated for more than 3 years to the patients who were not treated, the rate of liver cirrhosis was lower, but the rate of HCC and the rate of normalization of liver function were similar. Out of the 5 patients with HBeAg 3 achieved seroconversion within the initial 3 years of treatment. They suggested that the traditional diagnosis based on *Sho* is useful for correct selection of prescription of Chinese herbal medicine.

Experimental studies

To elucidate the mechanism of the effect of Sho-saiko-to on chronic liver disease, Yamashiki *et al*. (1997) examined the effect of Sho-saiko-to on *in vitro* interleukin-10 (IL-10) production using peripheral blood mononuclear cells of patients with chronic hepatitis C. They investigated (1) cytokine production levels, mainly IL-10, in peripheral blood mononuclear cell of chronic active hepatitis B and C patients, and healthy volunteers (2) the effects of Sho-saiko-to on these levels and (3) the effects of each of its herb components on cytokine production in cell fractions. As IL-10 was for the first time identified as a factor which is generated by helper-T type 2-cells (Th2) and which suppresses cytokine production in helper-T type-1 cells (Th1), so IL-10 was previously

termed as the cytokine synthesis inhibitory factor. Later, when it was determined that it possessed various biological activities, the name was changed to IL-10. One of the biological activities of IL-10 is to stimulate to such cells as B cell, cytotoxic T lymphocyte, thymocyte and mast cell, and another is to suppress to helper-T cell and monocyte/macrophage. IL-10 is expected to be an effective therapy for progressive systemic sclerosis, inflammatory bowel disease, endotoxin shock and development of diabetes. This anti-inflammatory effect has raised expectation for IL-10 as an efficacious drug against chronic hepatitis. On the other hand, IL-4 and IL-5 are known to be produced by Th2 cells and to be involved in antibody production.

The subjects were 30 patients with chronic active hepatitis C, 10 patients with chronic active hepatitis B as disease controls, and 37 healthy volunteers who were the normal controls. The IL-10 production index, after a 48-h culture with each herb component was examined using peripheral blood mononuclear cells of five healthy volunteers. The results showed that without stimulant, IL-10 production in mononuclear cells of hepatitis B and C patients was significantly lower than that of healthy subjects. The addition of Sho-saiko-to to the cultures strongly induced IL-10, and the induction was significantly higher in cultures with glycyrrhiza root or scutellaria root. The major chemical component of scutellaria root is baicalin and that of glycyrrhiza root is glycyrrhizin. They investigated *in vitro* IL-10 production in mononuclear cell cultures using baicalin and glycyrrhizin by using the same procedure for the Sho-saiko-to cultures, but the IL-10 induction was not confirmed. They supposed that the IL-10 induction observed for the two herb components might be attributable to unknown chemical components.

The production of IL-4 and IL-5 in cultures with concanavalin A was significantly higher in patients with hepatitis C than in the healthy subjects, but the addition of Sho-saiko-to suppressed these increases from 25% to 33% ($P < 0.01$). Therefore, Sho-saiko-to could adjust the decreased IL-10 production and the increased IL-4 and IL-5 production of mononuclear cells from patients with hepatitis C, and then repair abnormalities in the cytokine cascade.

From a different viewpoint, Sakaida *et al*. (1998) examined *in vivo* the effects of Sho-saiko-to on the development of liver fibrosis and pre-neoplastic lesions using the choline-deficient L-amino acid defined (CDAA) diet-induced liver fibrosis model in 16-week-old male Wistar rats. Sho-saiko-to in powdered form was mixed uniformly into the CDAA diet at 1% (w/w) concentration. Sho-saiko-to prevented fibrosis, as indicated by reduced hydroxyproline content in the liver and inhibition of the increase in a serum marker fibrosis (hyaluronic acid), without reducing the increase in serum ALT and AST. Sho-saiko-to also reduced the expression of type-III procollagen alpha I mRNA in the liver, as well as the proliferation of myofibroblast-like cells (activated stellate cells, activated Ito cells). Furthermore, Sho-saiko-to reduced the number of pre-neoplastic lesions, detected as enzyme altered (glutathione S-transferase placental form-positive) lesions, in the liver.

Kayano *et al*. (1998) also did *in vitro* study to determine whether Sho-saiko-to exerted inhibitory effects on hepatic stellate cells which have now been clearly identified as the primary cellular source involved in the pathogenesis of liver fibrosis. Hepatic stellate cells were isolated from male Wistar rats. Water-soluble ingredients of Sho-saiko-to were obtained at concentrations of 10, 100, 250, 500 and 1000 g/ml. They used the most widely accepted *in vitro* model, in which stellate cell activation was induced by growth on uncoated plastic dishes. Morphological transformation was observed under a phase-contrast microscope. Flow cytometric analysis was performed on day 4 after culture to evaluate the potential to proliferate of the stellate cells by analysing cell cycles. Northern blot analysis was carried out on day 3 after culture to determine the expressions of type I and type III procollagen mRNAs.

The results were as follows: Sho-saiko-to 500 and 1000 μg/ml inhibited morphological transformation of the stellate cells to myofibroblast-like cells. Sho-saiko-to 500 and 1000 μg/ml

significantly accumulated the cells in the G_0/G_1 phase as compared with control and significantly decreased cell numbers subsequently in G_2/M phase. Sho-saiko-to 500 and 1000 μg/ml also significantly suppressed procollagen mRNA expression of type I and type III. They suggested that Sho-saiko-to could be a potent inhibitor in the pathogenesis of liver fibrosis.

In 1999, Shimizu *et al*. assayed the preventive and therapeutic effects of Sho-saiko-to on experimental hepatic fibrosis, induced in rats by dimethylnitrosamine (DMN) or pig serum (PS), and on rat stellate cells and hepatocytes in primary culture, and assessed the anti-oxidative activities and the active components of Sho-saiko-to. Male Wistar rats were given a single intraperitoneal injection of 40 mg/kg DMN or 0.5 mL PS twice weekly for 10 weeks. In each model, rats were fed a basal diet throughout, or the same diet, which also contained 1.5% Sho-saiko-to, for 2 weeks before treatment or for the last 2 weeks of treatment. Sho-saiko-to suppressed the induction of hepatic fibrosis, increased hepatic retinoids and reduced the hepatic levels of collagen and malondialdehyde, a production of lipid peroxidation. Immuno-histochemical examination showed that Sho-saiko-to reduced the deposition of type-I collagen and the number of α-smooth muscle actin (α-SMA) positive-stellate cells in the liver and inhibited, not only lipid peroxidation in cultured rat hepatocytes that were undergoing oxidative stress but also the production of type-I collagen, α-SMA expression, cell proliferation and oxidative burst in cultured rat stellate cells. Sho-saiko-to inhibited Fe^{2+}/adenodine 5′-diphosphate-induced lipid peroxidation in rat liver mitochondria in a dose-dependent manner and showed radical scavenging activity. Among the active components of Sho-saiko-to, baicalin and baicalein were found to be mainly responsible for the anti-oxidative activity. These findings suggest that Sho-saiko-to functions as a potent anti-fibrosupppressant by inhibition of lipid peroxidation in hepatocytes and stellate cells *in vivo*.

Summary

This chapter described many clinical studies on the effectiveness of Sho-saiko-to in patients with chronic hepatitis. Significant improvement was observed remarkably in the patients with type-B hepatitis and more than type-C hepatitis patients in some of the reports. Sho-saiko-to improved the serum level of AST and ALT, although no histological improvement has been observed yet.

In the experimental studies, Sho-saiko-to increased the IL-10 production from macrophage and suppressed the activity of stellate cells and prevented hepatic fibrosis.

Geerts and Rogiers (1999) evaluated the work of Shimizu *et al*. (1999) because they showed the mechanism of protective effect of Sho-saiko-to against hepatic fibrosis, and they identified the active components of Sho-saiko-to as two flavonoids present in the root of *Scutellaria baicalensis Georgi*, baicalein and baicalin.

Geerts pointed out the similarity of chemical structures of baicalin and baicalein to quercetin (Kawada *et al*., 1998) and silybin (Boigk *et al*., 1997), which are flavonoids also reported to have anti-fibrogenic properties. He suggested that the striking similarity between the chemical structures of baicalein, quercetin and silybin is an important lead to develop novel anti-fibrogenetics, and that the longstanding clinical experience with Sho-saiko-to in Japan and with silymarin in Europe (Ferenci *et al*., 1989) showed the safety of these medicines.

Cirrhosis of the liver, hepatocellular carcinoma and chemoprevention

Clinical study

The term ‘cancer chemoprevention’ refers to prevention or prolongation of the steps leading to carcinogenesis by intervention with drugs before the malignant (invasive) stage of carcinogenesis

(Sporn *et al*., 1976). The difficulty in the testing of chemopreventive drugs intervention trials lies in the long observation period and large study population needed to examine whether cancer incidence has been reduced.

Hepatocellular carcinoma is one of the most common cancers in the world, with an estimated 500 000 −1 million new cases diagnosed yearly. More than 80% of patients with HCC have underlying cirrhosis, and the yearly incidence of HCC being diagnosed in cirrhotic patients was 7% in Japan in our study (Oka *et al*., 1990). Recently, it became possible to detect HCC in the early stage by ultrasonography and the assay of alpha-fetoprotein (AFP) and des-γ-carboxy prothorombin (PIVKA-II). So, HCC is suitable for investigation of the efficacy of potential chemopreventive drugs. First, the liver can be easily and non-invasively observed by ultrasonography, and HCC can be histologically diagnosed by biopsy; second, patients with cirrhosis are at high risk for HCC; and third, the follow-up period before HCC can be detected is shorter than in cancers of other organs. Nevertheless, we are not aware of any reports of chemopreventive studies of HCC. We did a prospective randomized non-blind controlled study in an evaluation of the chemopreventive effect of Sho-saiko-to (Oka *et al*., 1995).

Patients and methods

Two-hundred and sixty outpatients with cirrhosis were monitored for 60 months beginning June 1985, at the Third Department of Internal Medicine, Osaka City University Medical School and affiliated hospitals. The patients were 164 men and 96 women, with a mean age of 58 years (range, 31–84 years). On entry into the study, the patients were examined by ultrasonography to rule out the presence of HCC. The patients all had AFP levels below 200 ng/ml 3 months or less before entry. About 37 patients had HBsAg and 223 patients did not. About 54 patients without HbsAg had had blood transfusions and 57 patients without HBsAg had a history of heavy drinking, having consumed 75 g of alcohol or more per day for at least 10 years. There was some overlap among these two groups. Tests for anti-C100–3 antibodies gave positive results for 76 of the 94 patients whose stocked sera were tested.

Patients were stratified by age, sex, presence of HBsAg and liver dysfunction score, which was based on the levels of serum albumin, bilirubin and cholinesterase (Table 5.2).

The score was significantly related to the incidence of HCC in our previous study (Oka, *et al*., 1994). Patients of the same sex, age (classified as 30–39, 40–49, 50–59, 60–69 and > 70 years), liver dysfuncton score (classified into three grades; 0 or 1; 2 or 3; and 4,5 or 6), and presence of HBsAg were classified into subgroups. In the same subgroup, patients were randomly assigned to the control group or the trial group. The patients in the control group received conventional medicine only, and the patients in the trial group were given Sho-saiko-to at the daily dose of 7.5 g orally in addition to the conventional medicine. All patients started conventional therapy,

Table 5.2 Liver dysfunction score

Score	*Serum bilirubin* (mg/dl)	*Serum albumin* (g/dl)	*Serum cholinesterase (pH change)*
0	< 1.5	> 4.0	> 0.60
1	1.5–3.0	3.0–4.0	0.40–0.60
2	> 3.0	< 3.0	< 0.40

Source: Reproduced from Oka *et al*. (1995), Cancer (1995) with permission from Wiley Publishers.

Table 5.3 Profiles and laboratory data of patients at the time of entry into the study

Data	*Trial group*	*Control group*
Patient characteristics		
Sex (M/F)†	82 : 48	82 : 48
Age (year)†	58 ± 9	59 ± 9
HBsAg detected†	16/130	21/130
History of blood transfusion	24/130	30/130
History of heavy drinking	23/130	34/130
Laboratory data		
AFP (ng/ml)	29.2 ± 48.6	23.1 ± 35.0
AST	104.4 ± 61.6	91.8 ± 60.2
ALT	130.3 ± 103.5	107.4 ± 86.9
γ-GTP	46.4 ± 27.7	45.0 ± 51.2
Cholinesterase	0.50 ± 0.18	0.48 ± 0.18
Total bilirubin	1.24 ± 0.63	1.26 ± 0.77
Albumin	3.6 ± 0.5	3.5 ± 0.6
Liver dysfunction score*	2.0 ± 1.5	1.9 ± 1.4

Source: Reproduced from Cancer (1995) with permission from Wiley Publishers.

Notes
HBsAg: hepatitis B surface antigen; AFP: alpha-fetoprotein; AST: aspartate transaminase; ALT: alanine transaminase; GTP: glutamly transpeptidase.
* Results are expressed as ratios or as means ± standard deviation.
† Matched items.

and when a matched patient became available, randomization to treatment took place. Informed consent was obtained from the patients before they entered the study. All patients were prospectively followed up by monitoring of the serum level of AFP every 2 months and by ultrasonography scanning every 3 months. The mean length and the standard deviation of the follow-up period was 38.4 ± 22.3 months (median, 41 months).

Table 5.3 shows the backgroud of the patients at entry into the study. There was no significant difference in any test results between the two groups.

Evaluation of chemopreventive effect

The chemopreventive effect of Sho-saiko-to was evaluated by the cumulative incidence of HCC and survival rates. Patients in whom HCC was detected within 6 months after entry were excluded to rule out patients who had a large number of HCC cells but not enough to be detectable at the time of entry into the study.

The cumulative incidence of HCC was computed by the method of Gaynor *et al*. (1993). The differences in the cumulative incidences were analysed by the test proposed by Gray (1988), because some patients died without HCC. For comparison of the survival between the two treatment arms, the log–rank test was used. Differences with a P value < 0.05 were considered to be significant.

Results

HCC was found in 63 of the 260 patients during the follow-up period. Seven patients were found to have HCC within 6 months from the entry and were excluded. The treatment pertaining to HCC status and survival status are shown in Table 5.4.

Forty-seven of the patients not found to have HCC died 6–60 months after entering the study. The most common causes of death were liver failure and variceal bleeding. One hundred and

Table 5.4 Patient outcomes

Category	*Trial group*	*Control group*
Total randomized	130	130
HCC occurred in first 6 months	3	4
Total analysed	127	126
Patients with HCC	23	33
Alive with HCC	17	24
Dead with HCC	6	9
Dead without HCC	19	28
Alive without HCC	85	65
Patients surviving follow-up period	102	89

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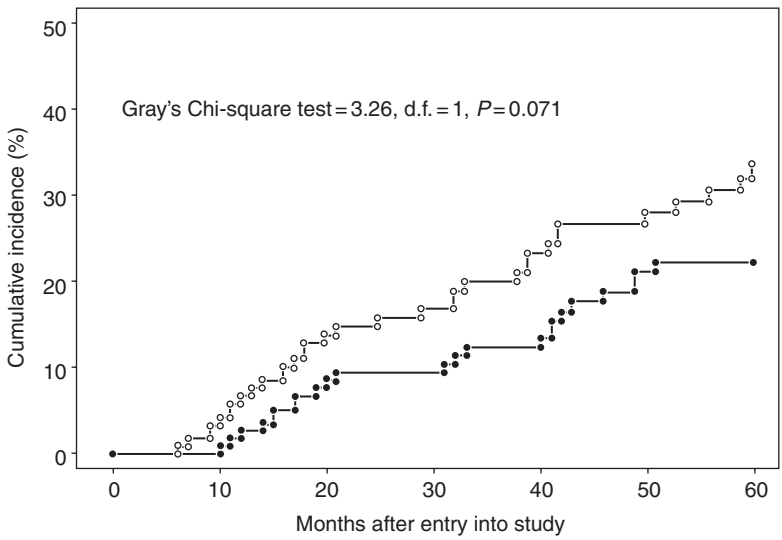

Figure 5.1 Cumulative incidence of HCC detected 6 months or more after entry into the study. ●: trial group ($n = 127$); ○: control group ($n = 126$). Reproduced from Cancer (1995) with permission from Wiley Publishers.

ninety-one patients were alive until the end of the follow-up period. Sho-saiko-to had no undesired side-effects. None of the patients in the trial group refused to continue the drug during the study.

Figure 5.1 shows the cumulative incidence of HCC in the treated and control groups during the trial. There were 23 (23%) patients with HCC in the trial group and 33 (34%) patients with HCC in the control group ($P = 0.071$).

In the control group, the tumour was 2 cm or smaller in diameter at the time of detection in 24 patients. In the trial group, the greatest dimension of the tumour when detected was 2 cm or less in 11 patients.

At the time of entry by patients not later excluded, 184 patients had AFP levels below 20 ng/ml. Among the remaining 69 patients who had AFP levels between 20 and 199 ng/ml, only 11 patients had AFP levels of 100 ng/ml or more. Figure 5.2 shows the cumulative incidence of HCC during the 5-year follow-up study in the 184 patients who had AFP levels below

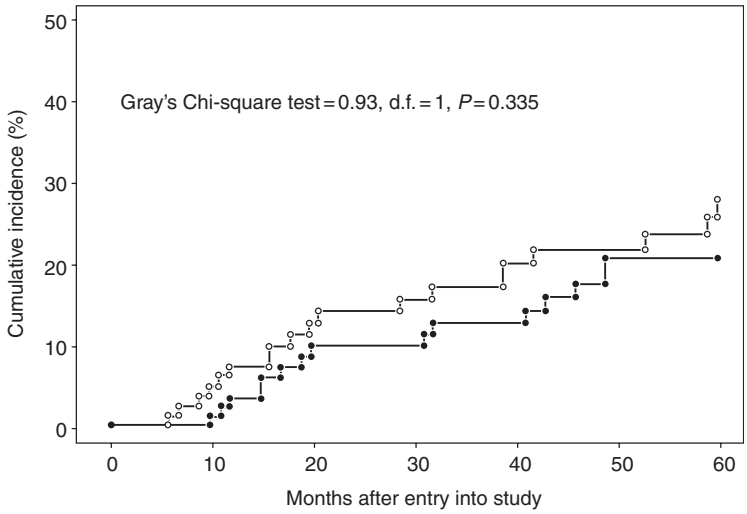

Figure 5.2 Cumulative incidence of HCC in the 184 patients with AFP levels below 20 ng/ml at entry. ●: trial group ($n = 93$); ○: control group ($n = 91$). Reproduced from Cancer (1995) with permission from Wiley Publishers.

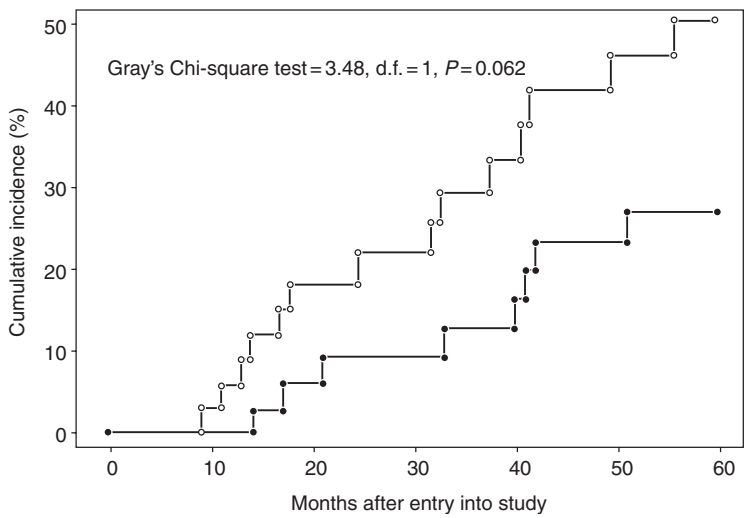

Figure 5.3 Cumulative incidence of HCC in the 69 patients who had AFP levels between 20 and 200 ng/ml at entry. ●: trial group ($n = 34$); ○: control group ($n = 35$). Reproduced from Cancer (1995) with permission from Wiley Publishers.

20 ng/ml at entry. The incidence of HCC was 21% (15 cases) in the trial group and 28% (19 cases) in the control group ($P = 0.335$). Figure 5.3 shows the cumulative incidence of HCC in the 69 patients who had AFP levels of 20 ng/ml or higher but below 200. The incidence of HCC was 27% (8 cases) in the trial group and 50% (14 cases) in the control group ($P = 0.062$).

Figure 5.4 shows the cumulative incidence of HCC in the patients without HBsAg. The incidence was significantly lower in the trial group (22%) than in the control group (39%) ($P = 0.024$).

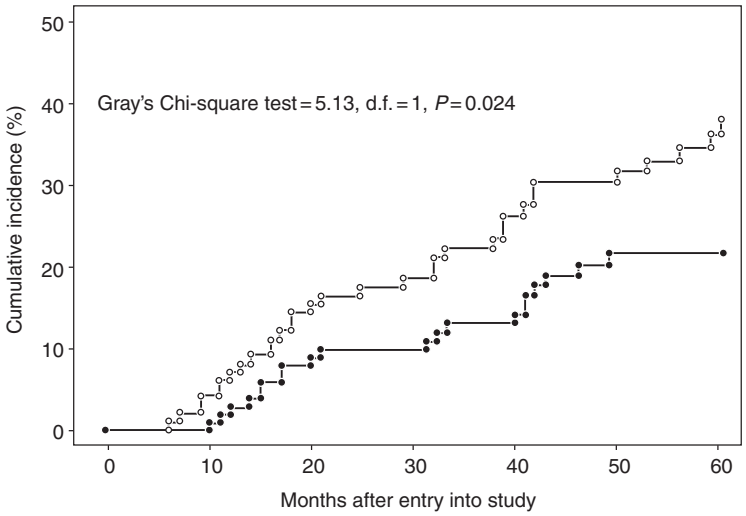

Figure 5.4 Cumulative incidence of HCC in patients without HBsAg. ●: trial group ($n = 111$); ○: control group ($n = 106$). Reproduced from Cancer (1995) with permission from Wiley Publishers.

The results for the other subgroups defined by the other classifying variables used in the randomization scheme were as follows. The difference in this incidence between the trial and control groups subgrouped by age and sex were not significant. The differences were not significant between the trial group and the control group in the subgroups of mild liver dysfunction (0 or 1) or severe dysfunction (4, 5 or 6). In the subgroup with dysfunction of moderate grade (2 or 3), 7 of the 59 patients in the trial group had HCC and 17 of the 52 patients in the control group had HCC. The cumulative incidence curve was significantly lower for the trial group than for the control group ($P < 0.05$).

Figure 5.5 shows the cumulative survival curves of the patients with cirrhosis (some with HCC) in the two groups. The survival curve was higher for the trial group (75%) than for the control group (61%) ($P = 0.053$). Figure 5.6 shows the cumulative survival curves of the patients without HBsAg. The survival curve for the trial group (76%) was significantly higher than that for the control group (60%).

Discussion

The principles underlying treatment of Chinese herbal medicine were established in China over thousands of years, and some are potentially useful, but only intervention trials in humans will provide definitive results.

This study was the first completed randomized controlled prospective trial of chemoprevention of HCC. The addition of Sho-saiko-to had a positive effect in both lowering the incidence of HCC and prolonging survival, but neither difference achieved statistical significance at $P < 0.05$. Regarding the patients without HBsAg, the difference between the two groups were significant. Experimental studies will be reviewed in the next part.

In our earlier prospective study (1994), the patients entering the study with AFP levels greater than 20 ng/ml had a significantly higher incidence of HCC evolution than those with normal AFP levels. Tsukuma *et al*. (1993) and Ikeda *et al*. (1993) reported similar results about the relationship

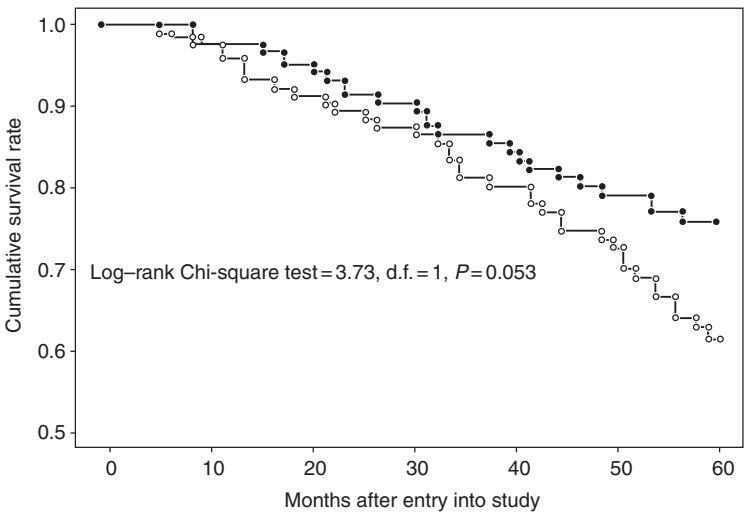

Figure 5.5 Cumulative survival rate of the patients with cirrhosis in the two groups. ●: trial group ($n = 127$); ○: control group ($n = 126$). Reproduced from Cancer (1995) with permission from Wiley Publishers.

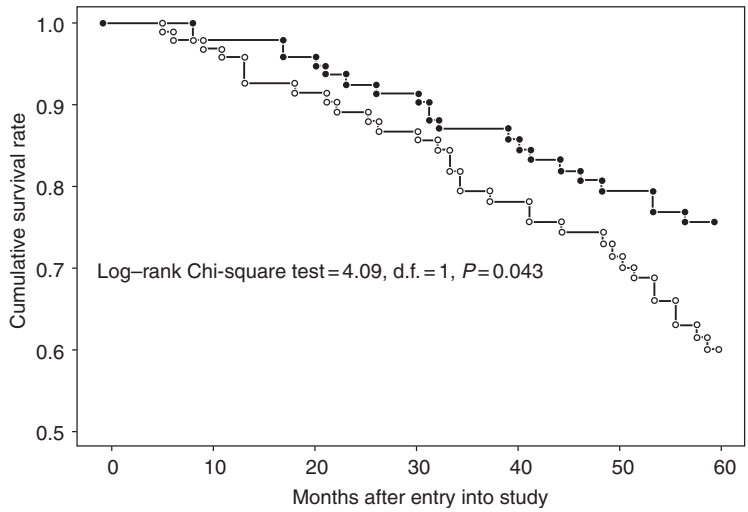

Figure 5.6 Cumulative survival rate of the patients negative for HBsAg. ●: trial group ($n = 111$); ○: control group ($n = 106$). Reproduced from Cancer (1995) with permission from Wiley Publishers.

between the serum level of AFP and the incidence of HCC. In this study, of the patients entering with AFP levels greater than 20 ng/ml, the incidence of HCC in those given Sho-saiko-to for up to 5 years was lower than in the control group. However, in the group of patients who had AFP levels below 20 ng/ml at entry, there was no difference between the two groups.

There is a long subclinical period between the appearance of one cancer cell after multiple genetic changes and the detection of a tumour mass by medical imaging. A tumour 1 cm in diameter contains several billion cancer cells. We speculated that Sho-saiko-to might have

inhibited the growth of cancer cells in the subclinical stage, when the number of cancer cells was much smaller than that in the clinical stage. The lower incidence of HCC in the trial group of patients with higher AFP levels supported this speculation.

It is unknown why the cumulative incidence of HCC was significantly lower in the trial group than in the control group in the patients without HBsAg. Most of the patients with cirrhosis without HBsAg seem to have anti-C-100-3 antibodies (Kiyosawa *et al*., 1990), so this result suggests that Sho-saiko-to is particularly effective for patients with hepatitis C virus in contrast to the result in the patients with chronic hepatitis. We speculated that in patients with HBV, HCC developed in earlier stage of cirrhosis or even in chronic hepatitis, but in patients with HCV, HCC mainly developed in cirrhosis. Sho-saiko-to prevents the fibrosis and development to cirrhosis of the liver, and it may result in the lower incidence of HCC in patients with HCV, though not in patients with HBV.

The higher survival rate of the cirrhotic patients in the trial group could mean that Sho-saiko-to had beneficial effects on the clinical course of patients with cirrhosis in addition to its inhibitory effect on HCC development.

Experimental studies

Some investigators (Odashima *et al*., 1979; Haranaka *et al*., 1985, etc.) have reported on the anti-tumour activities of Sho-saiko-to and its components over the last 20 years. For example, Kumazawa *et al*. (1988) found that intraperitoneal administration of Sho-saiko-to to mice resulted in marked activation of macrophages. Polysaccharide separated from these drugs exhibits anti-tumour activities at least partially through an increase in the immune response with macrophage involvement.

Recently, many papers have been published on the inhibitory effect of Sho-saiko-to on hepatocarcinogenesis in experimental animals. Tatsuta *et al*. (1991) reported about the effect of administration of Sho-saiko-to to rats on the development of enzyme-altered lesions of the liver induced by N-nitrosomorpholine (NNM). They used 60 (6 week old) male Sprague–Dawley rats, and randomly divided into three groups. Rats were given normal chow pellets containing 0.5% or 1.0% Sho-saiko-to until the end of the experiment and drinking water containing NNM for 8 weeks. Pre-neoplastic and neoplastic lesions staining for γ-glutamyl transpeptidase (GGT) or the placental type of glutathione-S-transferase (GST-P) were examined histochemically. In Week 15, quantitative histological analysis showed that prolonged treatment with 0.5% Sho-saiko-to significantly reduced the number and volume of GGT-positive and GST-P-positive hepatic lesions. Treatment with 1.0% Sho-saiko-to inhibited the development of GGT-positive and GST-P-positive lesions, but was less effective than 0.5% Sho-saiko-to. Administration of 0.5% Sho-saiko-to also caused a significant increase in the proportion of helper T lymphocytes and a significant decrease in the labelling index of pre-neoplastic lesions. They speculated these effect of Sho-saiko-to might be caused by free radical processes, because Sho-saiko-to has a high content of flavonoids, which are scavengers of superoxide anions (Roback *et al.*, 1988), or by an effect on the immune system.

Okita *et al*. (1993) investigated the effects and the mechanism of the components of Sho-saiko-to (baicalein, baicalin, saikosaponin-a, saikosaponin-c, ginsenoside Rb1, ginsenoside Rg1) on cultured human hepatoma cells (HuH-7). Cell cycle analysis was carried out with flow cytometry and the bromodeozyuridine (BrdU)-labelling method. The results showed that baicalein, baicalin and saikosaponin-a inhibited cell proliferation dose-dependently but independently of the cell cycle. The result from bivariate DNA/BrdU distribution suggested that baicalein elongated the total cell cycle time without changing the relative rates among G_1, S and

G_2M phases. The same phenomenon was observed in the study of baicalin, although its anti-proliferation activity was half that of baicalein. Furthermore, it was found that saikosaponin-a possesses a strong cell-killing effect. On the other hand, saikosaponin-c, ginsenoside Rb1 and ginsenoside Rg1 had no effect on cell proliferation. The result from bivariate DNA/BrdU distribution suggested that baicalein elongated the total cell cycle time without changing the relative rate among G_1, S and G_2M phases. The same phenomenon was observed in the study of baicalin, although its anti-proliferation activity was half that of baicalein. They speculated that apoptosis, which was defined by Kerr *et al*. (1972) as 'apoptosis, or programmed cell death, is a process in which cells die in a controlled manner, in response to specific stimuli, following an intrinsic program', has a major role in cell death in this experiment because a distinctly dense, small, pyknotic pattern was observed.

In the same way, Yano *et al*. (1994) reported that the complete water-soluble ingredients of Sho-saiko-to dose-dependently suppressed cell proliferation of a HCC cell line (KIM-1) and a cholangiocarcinoma cell line (KMC-1), and that the complete water-soluble ingredients of Sho-saiko-to suppressed cell proliferation much more strongly than did each of the ingredients glycyrrhizin, baicalin and baicalein. KIM -1 and KMC-1 were originally established in their laboratory (Murakami, 1984; Iemura *et al*., 1992). In this study, they used these two lines and two control cell groups, that is, normal hepatocytes isolated from male Wistar rats using the collagenase perfusion method (Sugihara *et al*., 1990) and normal human peripheral blood lymphocytes isolated from healthy adults and separated from whole blood by the density centrifugation method using Ficoll-Paque.

The numbers of KIM-1 and KMC-1 cells in 6-well plated were adjusted to reach confluence in about 11 days. The seeded cells were cultured for 1 day in basal medium. On the next day, the culture medium was replaced with medium containing one of six different concentrations of Sho-saiko-to or medium alone as a control. Exchange of culture medium and determination of living cells by the trypan blue dye exclusion test were repeated every 2 days thereafter until day 11 of culture. The ED_{50} value was determined on day 3 of culture.

PBL and normal rat hepatocytes were seeded on 6-well plates at a density of 5×10^5 cells/well and were cultured for 3 days in medium with or without Sho-saiko-to. The number of living cells was counted using the trypan blue dye exclusion test on day 3 of culture, and the numbers were compared with those of KIM-1 and KMC-1 cells. KIM-1 and KMC-1 cells in Sho-saiko-to cultures were examined on day 2. These cells were then collected using a scraper, stained with hematoxylin and eosin, and then observed for morphological changes.

Flow cytometric analyses of KIM-1 and KMC-1 cells were carried out using a FACScan (Becton Dickinson Immunocytometry Systems USA, San Jose, CA), from day 1 to day 6 of Sho-saiko-to. To monitor DNA synthesis, determination of BrDU incorporation was performed.

To determine the reversibility of growth inhibition by the drug, the kinetics of entry into DNA synthesis were analysed releasing the cells from the treatment. After treatment with Sho-saiko-to (400 and 1000 μg/ml) for 96 h, the cells in monolayer culture were washed 3 times with PBS and the culture was continued for an additional 48 h with basal medium containing 100 μM BrDU. BrDU incorporation into the nuclei of cultured cells was identified under a microscope by immunohistochemical detection. Because these cells complete one cell cycle within 48 h during the logarithmic growth phase, judging from their doubling time, cells which did not incorporate BrDU within 48 h after the release of the drug treatment were tentatively assessed as an irreversibly growth-arrested cell population.

As a result, Sho-saiko-to showed dose-dependent inhibitory effects on the proliferation of both KIM-1 and KMC-1 cells. ED_{50} values (day 3) were 353.5 ± 32.4 μg/ml for KIM-1 and 236.4 ± 26.5 μg/ml for KMC-1. In the cultures of lymphocytes, this drug showed no effects at

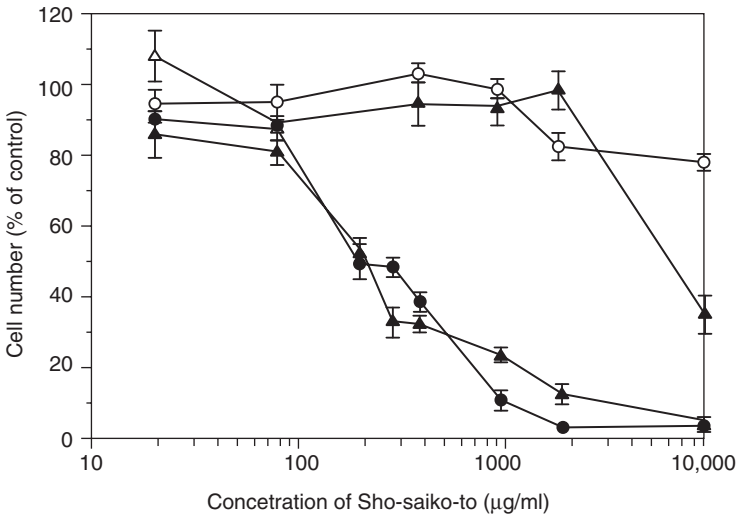

Figure 5.7 Relationship between the concentration of Sho-saiko-to and the number of viable cells, represented as percentage of control. (without addition of Sho-saiko-to), on day 3 of culture with Sho-saiko-to. Dose-dependent growth-inhibitory effects were observed only in cancer cell lines, that is, KIM-1 (●) and KMC-1(▲), whereas almost no effect was identified in normal peripheral lymphocytes (○) or normal rat hepatocytes (△). There was a significant difference in the number of viable cells between cancer cell lines (KIM-1 or KMC-1) and normal cells (human peripheral lymphocytes or rat hepatocytes) at 400, 1000, 2000 and 10 000 μg/ml ($P < 0.03$–0.002). In cancer cell lines, a significant difference was present between KIM-1 and KMC-1 at 1000 and 2000 μg/ml ($P < 0.01$). Reproduced from Yano *et al*. (1994), Cancer Research with permission from American Association for Cancer Research.

concentraions less than 2000 μg/ml, and the number of lymphocytes slightly decreased at 2000 μg/ml of higher concentrations. In cultures of normal rat hepatocytes, the proliferation of cells was slightly accelerated by a low dose (20 μg/ml) and slightly suppressed by a concentration of 10 000 μg/ml (Figure 5.7).

In comparisons between cell numbers in cultures with Sho-saiko-to (complete water-soluble ingredients) or its ingredients and in control cultures (without any added ingredients) on day 3, Sho-saiko-to showed significant inhibitory effects on cell proliferation; Sho-saiko-to decreased cell number to $36.6 \pm 0.9\%$ of the control, whereas baicalin, which is the most efficacious ingredient, decreased cell number to $55.3 \pm 2.5\%$ (Figure 5.8).

In both KIM-1 and KMC-1 cell cultures, they observed apoptotic cells, characterized by shrinkage of cell, chromatin condensation and nuclear fragmentation, on day 2. In the KMC -1 cell line, the BrDU incorporation rate decreased time and dose dependently. On the other hand, in the KMC-1 cell line, an equivalent decrease in corporation occurred in cultures with 2000 μg/ml, but no significant difference was observed in cultures with 400 μg/ml. In the DNA analysis, a ladder of fragmented DNA of 180–190 base pairs was detected in both cell lines on day 2 of culture with 400 μg/ml or higher concentrations of Sho-saiko-to. DNA fragmentation in a ladder pattern indicated internucleosomal chromatin cleavage, which is characteristic of apoptosis.

In this study, Yano *et al*. (1994) demonstrated that Sho-saiko-to directly inhibited the proliferation of HCC cells and cholangiocarcinoma cells, and that at concentrations as low as 20 μg/ml Sho-saiko-to slightly promoted cell proliferation of normal rat hepatocytes. Most interesting

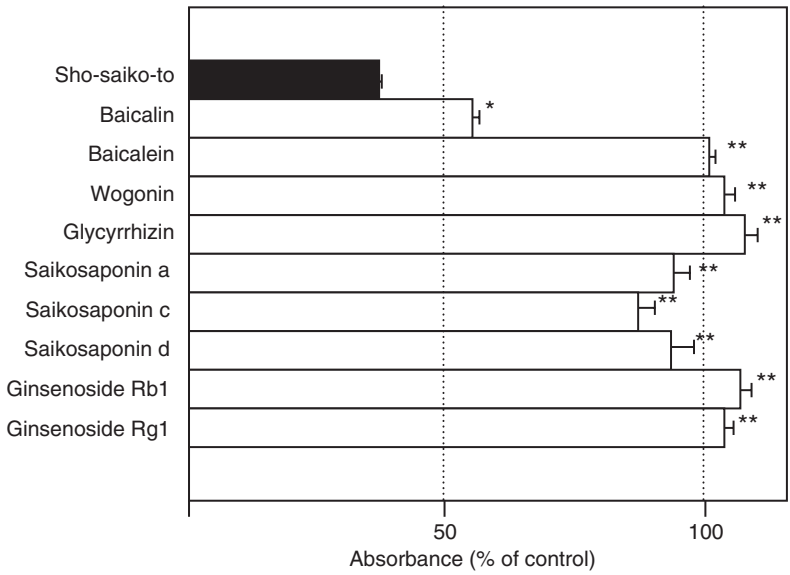

Figure 5.8 Growth-inhibitory effects of Sho-saiko-to and its ingredients. Sho-saiko-to (400 μg/ml) or the amount of each ingredient contained in 400 μg/ml Sho-saiko-to was added on day 1. Each value was determined as a percentage of the viable cell number, compared with that in control cultures (without any added ingredient), on day 3. The relative cell number was quantified by a colorimetric method. Values represent mean ± SD ($n = 8$–16/points). Significant difference between values for Sho-saiko-to and various ingredients are shown; * $P < 0.05$; ** $P < 0.005$. Reproduced from Yano *et al*. (1994), Cancer Research with permission from American Association for Cancer Research.

result is that Sho-saiko-to was confirmed to have much stronger anti-proliferative effects than the effects of each ingredient, such as the flavonoids. They used only water-soluble ingredients of Sho-saiko-to in this study; and flavonoids are barely soluble in water and also are unstable in water, so a water solution of Sho-saiko-to does not contain high level of flavonoids which can produce significant anti-tumour effects. The efficacy of Sho-saiko-to cannot be explained only by the presence of flavonoids. Therefore, they suggested that there could be synergistic or additive effects of various ingredients of Sho-saiko-to and that there could be unknown substances present in Sho-saiko-to that also inhibit tumour cell proliferation.

The ED_{50} values of this drug were 353.5 ± 32.4 μg/ml for KIM-1 and 236.3 ± 26.5 μg/ml for KMC-1. Though these values seem to be higher than those of other anti-neoplastic agents, they considered these levels to be clinically applicable. They used the level of glycyrrhizin as an index of the level of ingredients. The maximum level of glycyrrhizin after administration of 7.5 g of Sho-saiko-to is approximately 1.2 μg/ml, while the level of this ingredient in 300 μg/ml Sho-saiko-to (ED_{50} value in this *in vitro* study) is approximately 1.5 μg/ml. Therefore, the ED_{50} value of Sho-saiko-to in this study is considered to be useful in clinical situations.

They presumed that Sho-saiko-to have at least two different mechanisms of action to inhibit tumour cell proliferation; one is the effect of the flavonoids which induce apoptosis in actively proliferating tumour cells, and the other is the effect of glycyrrhizin, which induces arrest at the G_0/G_1 phase and a decrease in DNA synthesis.

From the point of the production of cytokines, Yamashiki *et al*. (1996) demonstrated by *in vitro* study that there were dose-dependent increases in production levels of tumour necrosis factor-α (TNF-α) and granulocyte colony-stimulating factor (G-CSF) in peripheral mononuclear cells of patients with HCC when they added Sho-saiko-to to cell cultures.

TNF-α is a 17 kDa molecular weight glucose protein that is generated by all monocyte or macrophage cells throughout the body. It was originally discovered as a substance that induces tumour necrosis. Recent studies have demonstrated that TNF-α is an important mediator in the body defence mechanism. In addition to anti-tumour effects, it performs functions such as immune-response regulation, activation of inflammatory cells, the promotion of proliferation of various cells, anti-viral activities and suppression of the growth of pathogenic micro-organisms. G-CSF is a haematopoietic-stimulating factor, which was discovered in 1965 by researchers investigating differentiation and proliferation mechanism of haematopoietic stem cells. More recently, as gene recombinant techniques have been developed, recombinant G-CSF came to be mass-produced and became available as a therapy for neurtropaenia.

In this study, blood samples were collected from 11 patients with HCC, from 8 patients with cirrhosis of the liver positive for HCV antibody, and from 23 healthy volunteers in order to monitor the effects of Sho-saiko-to on the induction of these cytokines in cell culture.

The mononuclear cell fraction was obtained by densimetric centrifugation and prepared to 1×10^6 cells/mL using RPMI solution. The prepared mononuclear cell fraction (1 mL) and 0.2 mL of heat-inactivated foetal bovine serum were added to each well of a culture plate. Sho-saiko-to powder was dissolved in the RPMI solution to make a final concentration of 0, 3.1, 12.5, 50 and 200 μg/mL, and 50 μL of each preparation was used to observe dose production levels.

In order to analyse TNF-α and G-CSF production by Sho-saiko-to, the following drugs or reagent were prepared in an identical manner and used in the experiment: lipopolysaccaride as a control stimulant, Dai-saiko-to (TJ-8) and Saiko-keishi-to (TJ-10) which consist of similar herbal ingredients and have a similar pharmaceutical effects as the reference herbal medicines and Sho-seiryu-to (TJ-19) which consists of very different herbal ingredients from above three herbal medicines as the control herbal medicine. All four herbal medicines were supplies by Tsumura Co., Ltd and were made up in a final concentration of 200 μg/mL. Each preparation was then added to the wells and incubated for 24 h in a CO_2 incubator at 37°C. The supernatant was collected for analysis.

TNF-α production levels increased significantly with increasing concentrations of Sho-saiko-to as shown in Figure 5.9.

Stimulation index of TNF-α production for each preparation is shown in Figure 5.10.

The stimulation index for TJ-19 was significantly lower than Sho-saiko-to, TJ-8 and TJ-10. Furthermore, the stimulation index for Sho-saiko-to was significantly higher than that for the similar herbal medicines, TJ-8 and TJ-10. There were significant differences observed in the levels of TNF-α between the healthy controls and patients with HCC when Sho-saiko-to was added. TNF-α production levels in patients with HCC also tended to be higher than in the healthy controls when the control stimulant, LPS, was added.

G-CSF production levels were measured in the same manner as for TNF-α (Figure 5.11). G-CSF production levels significantly increased with increasing concentration of Sho-saiko-to. The stimulation index of G-CSF production for TJ-19 was significantly lower than for TJ-8, TJ-10 and Sho-saiko-to (Figure 5.12).

Japanese physicians who have studied Chinese medicine often administer not only Sho-saiko-to, but also TJ-8 and TJ-10 according to the patient's *Sho*. TJ-8 is mixture of 8 herbs (bubleurum root, pinellia tuber, scutellaria root, jujube fruit, peony root, immature orange, ginger rhizome and rhubarb rhizome), and TJ-10 is mixture of 9 herbs, in which 7 herbs are common with Sho-saiko-to

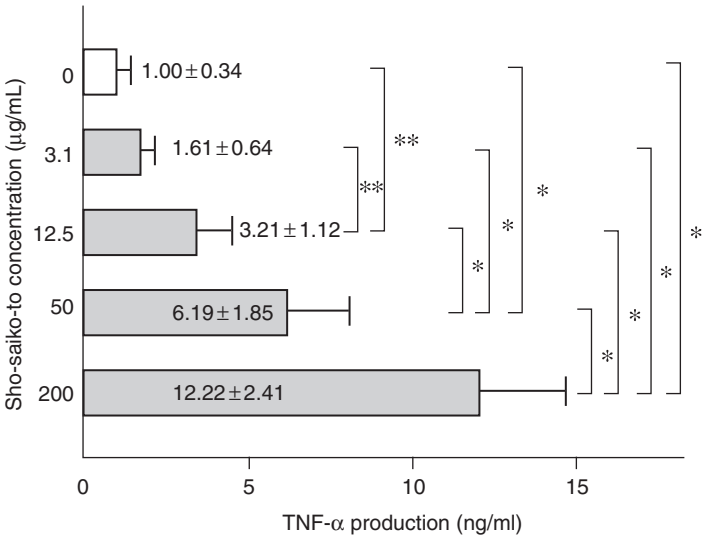

Figure 5.9 *In vitro* TNF-α production levels in (PBMC: peripheral blood mononuclear cell). Figures represent mean ± s.e.m. The level of production increased significantly in a dose-dependent manner. *$P < 0.01$; **$P < 0.05$. From M Yamashiki *et al*. (1996) *Journal of Gastroenterology and Hepatology*, reproduced with permission.

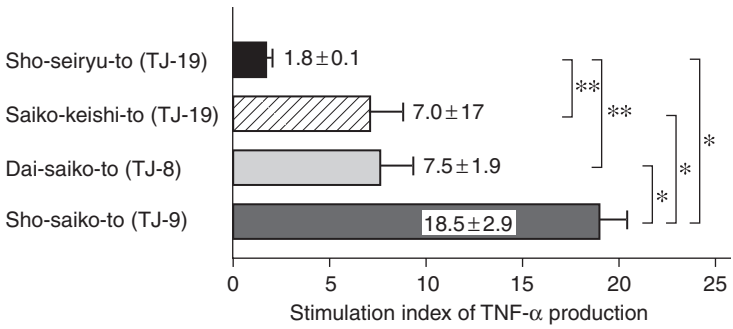

Figure 5.10 Stimulation index of TNF-α production for each preparation. The stimulation index for TJ-19 was significantly lower than TJ-9 (* $P < 0.01$), TJ-8 and TJ-10 (** $P < 0.05$) From M Yamashiki *et al*. (1996) *Journal of Gastroenterology and Hepatology*, reproduced with permission.

and the other 2 herbs are cinnamon bark and peony root. TJ-19 is mixture of 8 herbs: pinellia tuber, glycyrrhiza root, cinnamon bark, schisandra fruit, gardenia fruit, peony root, ephedra herb and ginger rhizome. Of these, only two (pinella tuber and glycyrrhiza root) are common with Sho-saiko-to. The reason why TJ-19, which contains the glycyrrhiza root, did not influence cytokine production levels is thought to be that another component of TJ-19 that is capable of immunosuppression (schisandra fruit) may conceal the effects of gylcyrrhiza root on cytokine production.

Yano *et al*. (1994) reported that the level of Sho-saiko-to reached approximately 300 μg/mL after 1 week of administering 7.5 g/day. As the levels used in this *in vitro* study were lower than 200 μg/mL, it can be assumed that similar effects of Sho-saiko-to on cytokine production may be observed *in vivo*.

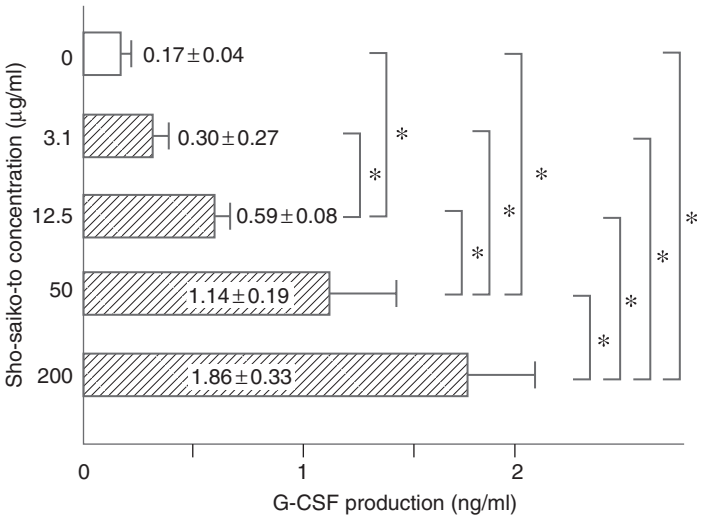

Figure 5.11 *In vitro* G-CSF production levels in PBMC. The levels of production increased significantly in a dose-dependent manner. * $P < 0.01$. From M Yamashiki *et al*. (1996) *Journal of Gastroenterology and Hepatology*, reproduced with permission.

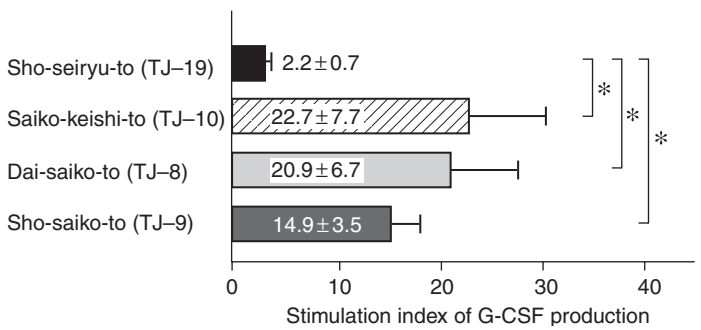

Figure 5.12 Stimulation index of G-CSF production for each preparation. The stimulation index for TJ-19 was significantly lower than TJ-8, TJ-9 and TJ-10 (*$P < 0.05$). From M Yamashiki *et al*. (1996) *Journal of Gastroenterology and Hepatology*, reproduced with permission.

Summary

In our prospective controlled study, Sho-saiko-to had a positive effect in both lowering the incidence of HCC and prolonging survival. Many experimental studies that supported this result were shown in this chapter. We speculate that baicalin and baicalein, flavonoids contained in Scutellaria root may cause apoptosis, that glycyrrhizin, saponin contained in Glycyrrhiza root may induce arrest at G_0/G_1 phase and to decrease DNA synthesis, and saikosaponin-a contained in Bupleurum root may inhibit proliferation of cancer cells.

Geerts (1999) described that complex herbal medicine can probably be simplified without loss of activity. However, Yano *et al*. (1994) showed Sho-saiko-to had much stronger anti-proliferative effects than that effects of each ingredients. The method of Western medicine is to purify the active components and synthesize them, but the method of Oriental medicine is quite different. Synergistic effects of various unknown ingredients of herbal medicine are supposed to be important.

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6 Recent topics on Sho-saiko-to and other Kampo medicines

Sakae Amagaya

Reduction of the adverse effect induced by Western medicines

Sho-saiko-to has multiple actions as described in the previous sections. In particular, the pharmacological and clinical data suggest that the anti-inflammatory and/or immunoregulatory actions are important to explain the effect of Sho-saiko-to. On the other hand, the immunosuppressive action of glucocorticoids or anti-tumour drugs induces severe adverse effect clinically, and the usage of these drugs is restricted in many clinical cases. To decrease the doses of these drugs with keeping the therapeutic effect, or to decrease their adverse effects are important to elevate both the therapeutic index and the compliance of patients. By these reasons the combination therapy with glucocorticoid or anti-tumour drugs and Sho-saiko-to is studied.

Combination with glucocorticoid and Sho-saiko-to

Autoimmune diseases, hepatitis, nephritis, rheumatoid, collagen disease etc., occur on the basis of constitution of patients, and many of them are intractable. Glucocorticoid is very effective for such autoimmune diseases, and its value is evaluated and used widely all over the world. There is no known therapy to inhibit glucocorticoid-induced adverse effect, and many patients are apt to avoid the usage of glucocorticoid due to its severe adverse effect. Arichi and Abe (1979) and Toda *et al*. (1979a,b) reported that the combination of glucocorticoid and Kampo medicine (Sho-saiko-to, Gorei-san or Keishi-bukuryo-gan) can decrease the adverse effect of glucocorticoid, or decrease the doses of glucocorticoid with keeping the therapeutic effect of glucocorticoid. Furthermore, the treatment of these Kampo medicines enabled the withdrawal of glucocorticoid in patients with nephritis, chronic hepatitis and rheumatoid.

Ishida *et al*. (1983) also reported the combined therapy of glucocorticoid and Sho-saiko-to in dermatitis. In 30 patients treated with glucocorticoid, Sho-saiko-to was orally administered in combination, and the doses of glucocorticoid could be decreased for 7–103 days therapeutic periods. The combination of glucocorticoid and Sho-saiko-to in the aim of the withdrawal of glucocorticoid was valuable in 90% patients. These actions of Sho-saiko-to were also proved in an animal model by Shimizu *et al*. (1984). Effect of combined use of Sho-saiko-to and prednisolone was examined in anti-inflammatory effects using the carrageenan oedema and the cotton pellet method. In this experiment, Sho-saiko-to showed mild anti-inflammatory action and significantly increased the anti-inflammatory effect of prednisolone in the combination. In a cotton pellet model, Sho-saiko-to inhibited the prednisolone-induced decrease of adrenal weight. Sho-saiko-to is reported to stimulate the adrenal cortex and increase the endogenous glucocorticoid (corticosterone) release. Iwama *et al*. (1986) reported the effect of Sho-saiko-to on the adrenal atrophy induced by the treatment of prednisolone for 45 days. In this case, Sho-saiko-to inhibited the prednisolone-induced decrease of blood corticosterone level. Further, Sho-saiko-to

inhibited the prednisolone-induced suppression of haemolytic plaque forming cells (HPFC), rosette forming cells (RFC) and 7S HA titre in serum. Sho-saiko-to inhibited the adrenal atrophy and immunosuppression induced by prednisolone with increasing the anti-inflammatory effect of prednisolone, and Sho-saiko-to was suggested to increase the chance of glucocorticoid treatment in chronic inflammatory diseases. These results indicate that Sho-saiko-to is useful for the alleviation of adverse effect of glucocorticoid in the combination treatment, which is different from the traditional usage of Sho-saiko-to. These results suggest the new development of medical treatment on the intractable autoimmune diseases.

Combination with anti-cancer agents and Sho-saiko-to

Ito and Shimura (1986) reported that Sho-saiko-to showed the anti-tumour effect in an experimental model of lung metastasis induced by Lews lung carcinoma in C57BL mice, and Sho-saiko-to suppressed the development of lung metastases of 5-fluorourasil or cyclophosphamide. Further, Sho-saiko-to was reported to improve the survival time of mice treated with mytomicine C. The immunoregulatory action of Sho-saiko-to will play an important role in the combination with anti-tumour drugs. These findings raise the possibility that Sho-saiko-to may have clinical value in the prevention of cancer metastasis by chemotherapy.

Combination with irinotecan hydrochloride and Hange-shashin-to

In recent years, several studies using other Kampo medicine have been reported. Murakoshi *et al*. (1999), Tanaka *et al*. (1999) and Mori *et al*. (1998) reported that Hange-shashin-to was effective for the diarrhoea induced by irinotecan hydrochloride (CPT-11). A randomized comparative trial to study the clinical usefulness of Hange-shashin-to was performed. A combination of irinotecan hydrochloride (160 mg/m^2, intravenous infusion, day 1) and cisplatin (20 mg/m^2/day, 5-day continuous intravenous infusion) was administered to patients with non-small cell lung cancer. They were given Hange-shashin-to to prevent the occurrence of irinotecan-induced diarrhoea. Hange-shashin-to was reported to show a significant improvement in the grade of diarrhoea and a lower incidence of diarrhoea. However, no differences were seen among the studied groups in terms of the frequency of diarrhoea and a duration of diarrhoea. As side-effects, two patients developed a low degree of constipation. These findings showed that Hange-shashin-to was effective in preventing and alleviating the incidence of diarrhoea induced by CPT-11. Kamataki *et al*. (1997) reported the mechanism of preventing diarrhoea induced by irinotecan hydrochloride. The concept is based on the following metabolic pathway of irinotecan hydrochloride.

1 The drug is metabolized by esterase to give SN-38, an active metabolite, which is subsequently conjugated by glucuronosyl transferase to give SN-38-glucuronide.
2 The glucronide is excreted in urine to reach the intestine where the glucuronide is cleaved by β-glucuronidase present in gut flora to yield SN-38 and glucuronic acid.
3 The SN-38 thus formed was assumed to be the cause of cytotoxic diarrhoea.

In the study, baicalin, one of the natural glucuronide components of Hange-shashin-to or Sho-saiko-to, was found to be a potent competitive inhibitor of β-glucuronidase. Hange-shashin-to- and Sho-saiko-to, which contain baicalin, potently inhibited the late diarrhoea by irinotecan hydrochloride in rats.

In an animal chronic diarrhoea model, Kase *et al*. (1997a) reported the anti-diarrhoea effect of Hange-shashin-to. Repeated oral administration of Hange-shashin-to at 125 and 500 mg/kg significantly prevented the reduction in body weight and the onset of chronic diarrhoeal symptoms

due to irinotecan hydrochloride in a dose-dependent manner, even though it failed to show a definite effect on acute diarrhoeal symptoms. In addition, administration of Hange-shashin-to increased the healing of the intestinal tract injury induced by the repeated dosing of irinotecan hydrochloride and inhibited significantly the increase of colonic prostaglandin E2 (PGE2) synthesis which is closely related to the onset of diarrhoea. Hange-shashin-to also improved colonic water absorption impaired by repeated dosing of irinotecan hydrochloride in rats. These results demonstrate that Hange-shashin-to is an effective medicine for the prevention and/or treatment of irinotecan hydrochloride-induced colonic diarrhoeal symptoms.

Kase *et al*. (1997b) reported the effect of Hange-shashin-to on the water-absorbing capacity in the large intestine. Repeated oral administration of Hange-shashin-to revealed a significant decrease in PGE2 content in the colonic mucosa and also the promotion of colonic water absorption. However, there was no significant influence on the concentration of aldosterone and electrolytes in the serum. It is suggested that Hange-shashin-to prevents the loss of water content caused by diarrhoea. A study to determine the mechanisms by which Hange-shashin-to reduces PGE2 was also performed by Kase *et al*. (1998a). Hange-shashin-to orally administered to rats caused a dose-dependent increase in blood corticosterone levels. Moreover, Hange-shashin-to inhibited the cyclo-oxygenase-2 (COX-2) activity, while COX-1 activity was not inhibited. These results suggest that the effect of Hange-shashin-to in decreasing PGE2 is partially mediated by corticosterone and inhibition of COX-2.

The effect of Hange-shashin-to on diarrhoea was also studied using castor oil by comparing its action with that of loparamide by Kase *et al*. (1996). The oral administration of Hange-shashin-to caused the dose-dependent suppression of castor oil-induced diarrhoea. No significant suppression was noted by Hange-shashin-to for diarrhoea induced by pilocarpine, serotonin or barium chloride. Oral treatment with loperamide markedly suppressed diarrhoea induced by castor oil and barium chloride. Contraction of isolated guinea-pig ileum in response to acetylcholine, histamine or barium chloride was little affected by Hange-shashin-to. The responses elicited by the three contractive drugs were dose-dependently suppressed by loperamide. Hange-shashin-to did not affect the small intestinal transit, but loperamide significantly suppressed it. These results suggest that Hange-shashin-to can effectively control castor oil-induced diarrhoea and that its anti-diarrhoea action is not based on the suppression of intestinal motility.

The effect of Hange-shashin-to on cholera toxin-induced intestinal fluid secretion has been studied by Kase *et al*. (1998b). Hange-shashin-to suppressed the intestinal fluid secretion induced by cholera toxin in a dose-dependent manner. It also inhibited the luminal PGE2 level as well as the treatment with indomethacin. On the other hand, serotonin release was not affected by Hange-shashin-to. Hange-shashin-to had little effect on the phasic contraction of isolated guinea-pig ileum induced by serotonin, while ondasetron suppressed the phasic contraction caused by serotonin. These results indicate that Hange-shashin-to is useful in suppressing cholera toxin-stimulated intestinal fluid secretion, and that this effect is partially due to its suppressive action on the PGE2 synthesis. Furthermore, the active crude drugs on diarrhoea were studied, and Scutellariae Radix, Glycyrrhizae Radix, Ginseng Radix and Coptidis Rhizoma were found to show anti-diarrhoea action. Among the active crude drugs mentioned above, Scutellariae Radix, Glycyrrhizae Radix and Ginseng Radix are common components in Sho-saiko-to.

Combination with anti-cancer agents and Juzen-taiho-to

Clinical studies were performed to confirm the effect of Juzen-taiho-to against the side-effect of anti-cancer agents in post-operative patients with malignant tumour. Moreover, immunological examination was conducted on administration of Juzen-taiho-to for post-operative gastric cancer

by Kurokawa *et al*. (1989). Eighty-eight post-operative cancer patients were examined for the clinical effect of Juzen-taiho-to against the gastrointestinal side-effects due to various anti-cancer agents. In this study, anorexia was improved in 83% of early, advanced and terminal stage of cancers, and other gastrointestinal symptoms were also improved. Blastformation of mouse lymphocyte to which patient serum was added was examined. TNF activity, LAK activity and 2-5AS activity before and after administration of Juzen-taiho-to were measured in 23 gastric cancer patients. Blastformation of mouse lymphocytes after the administration of Juzen-taiho-to showed a great tendency to increase. TNF activities and 2-5AS activities were shown to decrease.

Adachi and Watanabe (1989) reported that Juzen-taiho-to suppressed the adverse effect induced by the treatment of cisplatin, cyclophosphamide, adriamycin, methotrexate, tegafur etc. The patients were randomized with the envelope method, dividing into groups A or B. Group A was treated with Juzen-taiho-to combined with chemo-endocrine therapy. Group B was treated with only chemo-endocrine therapy. About 118 patients were evaluable and 58 patients belonged to group A and 61 to group B. In Juzen-taiho-to treated breast cancer patients, survival rate is significantly higher. Quality of life was expressed as self-assessment scores for physical condition, appetite and the coldness of extremities. Quality of life was significantly improved, in particular the inhibition of bone marrow suppression induced by chemotherapy was the important factor in the improvement of quality of life. Further, Kuboki *et al*. (1991) reported the effect of Juzen-taiho-to on the alleviating effect on bone marrow suppression during EAP (etoposide, adriamicin and cisplatin) therapy and its clinical effect. This study was conducted on 32 patients with advanced, inoperable gastric cancer or advanced pancreatic cancer in whom EAP therapy was performed. Duration of the period with white blood cells (WBC) less than 2000 m/m^3 was significantly shorter at 5.5 days in the treated group, against 10.7 days in the control group. Mean WBC after 4 weeks of EAP therapy was 5640 m/m^3 in the treated group and 2880 m/m^3 in the non-treated group, reflecting a significant recovery of WBC in response to Juzen-taiho-to treatment. Alleviation of nausea and vomiting as well as pain due to cancer before and after treatment was also noted in the Juzen-taiho-to treated group. Juzen-taiho-to thus appeared to be effective as an adjuvant therapeutic agent in the chemotherapy. Juzen-taiho-to is also reported to alleviate the radiation-induced injury by Takegawa (1991) and Konno *et al*. (1997). Juzen-taiho-to inhibited the decrease of WBC, platelet and haemoglobin induced by the radiation.

Combination with anti-cancer agents and Hochu-ekki-to

Kuroda *et al*. (1985) reported the clinical trial of Hochu-ekki-to. Hochu-ekki-to was administered to 162 patients who complained of anorexia or lassitude because of genito-urinary cancer. The efficacy rate was 63%. The rate of effectiveness on anorexia was 48.8% and that on lassitude was 36.6%. Side-effects were observed in 12 patients (7.4%), but most of them were mild gastrointestinal disorders. No severe adverse effects were noted.

Malaise frequently occurs as a side-effect of cancer chemotherapy, sometimes constituting a dose-limiting factor. A study on the utility of Hochu-ekki-to for malaise accompanying lung cancer chemotherapy was performed by Mori *et al*. (1992). The subjects were 43 patients with primary lung cancer who were treated with multi-drug therapy, including 5-day continuous infusion of cisplatin. The study was conducted by the random comparative method. Hochu-ekki-to was administered from 1 week before chemotherapy to at least 4 weeks after completion of therapy. Evaluations were carried out once a week, with a survey being conducted using a questionnaire on the presence/absence and severity of malaise, mood and digestive symptoms. Evaluation was possible in a total of 41 patients with 21 in the Hochu-ekki-to administration group and 20 in the non-administration group. The Hochu-ekki-to administration group showed a significantly lower frequency of malaise than the non-administration group, and their

malaise was mild. Improvement in mood and the regaining of appetite were observed. There were no side-effects. It appears that Hochu-ekki-to is useful in preventing malaise accompanying cancer chemotherapy.

Topics of related Kampo medicine to Sho-saiko-to (Saiko-Zai)

Some Kampo medicine, which have 'Saiko: Bupleuri Radix' as a composed crude drug, is called 'Saiko-Zai', and have similar efficacy to Sho-saiko-to. Small changes of the combination of the composed crude drug differentiate the clinical indications.

Dai-saiko-to

Saku *et al*. (1992) reported the effects of Dai-saiko-to on blood pressure (BP), pulse rate, serum lipids, lipoproteins and apolipoproteins in 30 patients with mild to moderate hypertension in an open and randomized trial. After the Dai-saiko-to treatment, BP and pulse rate remained unchanged. Serum total cholesterol and triglyceride values did not change, but high-density lipoprotein-cholesterol increased significantly. Apo-AII tended to increase 3 months after treatment. These data indicate that Dai-saiko-to has a preferential effect on lipid metabolism.

Iizuka *et al*. (1998a) reported the inhibitory effects of Dai-saiko-to on the progression of the atherosclerotic lesions by using the spontaneous familial hypercholesterolaemia (FH) model, Kurosawa and Kusanagi-hypercholesterolaemic (KHC) rabbits. Changes in blood chemistry, pathology and low-density lipoprotein (LDL) oxidation were measured in a control group and a Dai-saiko-to group. In the control group, the area of atheromatous plaques of the aorta progressed between week 12 (29.1%) and 26 (51.5%). This progression of atherosclerotic lesions did not happen between week 12 (26%) and 26 (27.4%) in the Dai-saiko-to treated group. Anti-oxidative effects on LDL were seen in the Dai-saiko-to treated group in weeks 16 and 18. Dai-saiko-to did not improve the hypercholesterolaemia in the KHC rabbits. These results suggest that Dai-saiko-to has inhibitory effects on the development of atheromatous plaque formation in spontaneous FH model rabbits. It is possible that the anti-oxidative effects of Dai-saiko-to on LDL led to the beneficial effects.

Saiko-ka-ryukotsu-borei-to

Saiko-ka-ryukotsu-borei-to is used clinically for the treatment of hypertension, atherosclerosis and sleeping disorder. The effects of Saiko-ka-ryukotsu-borei-to on BP, pulse rate, serum lipid, lipoproteins and apolipoproteins were studied in 30 patients with mild to moderate hypertension in an open and randomized trial by Saku *et al*. (1992). After the Saiko-ka-ryukotsu-borei-to treatment, BP remained unchanged, but pulse rate declined significantly after 3 months. Serum total cholesterol and triglyceride values did not change, but high-density lipoprotein-cholesterol increased significantly. Apo-AI and Apo-AII tended to increase 3 months after treatment. These data indicate that Saiko-ka-ryukotsu-borei-to has a preferential effect on lipid metabolism with little anti-hypertensive action.

Wei *et al*. (1997) reported the effects of Saiko-ka-ryukotsu-borei-to on the contraction of rat thoracic aorta induced by norepinephrine, 5-hydroxytriptamine and high potassium. In this experiment, Saiko-ka-ryukotsu-borei-to was found to inhibit the norepinephrine-induced vasoconstriction to show anti-hypertensive effect. In addition, Saiko-ka-ryukotsu-borei-to showed an inhibitory effect on nitric oxide-induced relaxation.

Koshikawa *et al*. (1998) reported the effect of Saiko-ka-ryukotsu-borei-to on behavioural despair and acetic acid-induced writhing in mice. In this experiment, Saiko-ka-ryukotsu-borei-to was found to have anti-depressive and anti-nociceptive properties.

Effect of Saiko-ka-ryukotsu-borei-to on irritable characteristics in El mice was studied by Iizuka *et al*. (1998b). The circadian rhythm of locomotor activity of El mice and the duration of sodium pentobarbital-induced sleep during the light and dark period were examined. The spontaneous locomotor activity of El mice during the dark period was not so different from that of ddY mice, whereas the activity during the light period was significantly higher. The duration of sodium pentobarbital-induced sleep of El mice was very short during the light period, nearly equal to that during the dark period. The administration of Saiko-ka-ryukotsu-borei-to caused marked reduction in the locomotor activity during the light period and dose-dependent prolongation in the duration of sodium pentobarbital-induced sleep time during the light period. These findings suggest that the El mouse is a model with a tendency to be easily excitable during the light period. Saiko-ka-ryukotsu-borei-to reduced the excitation in El mice and might be useful against sleep disorder due to excitation.

The effect of Saiko-ka-ryukotsu-borei-to on the stress-induced increase of monoamines in brain regions was investigated in a mouse emotional stress model by Sasaki *et al*. (1998b). Dopamine (DA) and 3,4-dihydroxy-phenyl acetic acid (DOPAC) contents were elevated by electric shock stress, psychological stress and conditioned fear stress in thalamus, hypothalamus and amygdala. The DA and DOPAC levels were decreased by pre-administration of Saiko-ka-ryukotsu-borei-to in the last two models, but were not altered in electric shock stress. Therefore, Saiko-ka-ryukotsu-borei-to seems to be effective in stress involving emotional factor. Saiko-ka-ryukotsu-borei-to was suggested to affect the brain monoamine neurons leading to psychological change in mice.

Sanae *et al*. (1999) reported the effects of Saiko-ka-ryukotsu-borei-to on theophylline-induced tachycardia in anaesthetized rats and theophilline-induced locomotion and convulsions in mice. An intraduodenal administration of Saiko-ka-ryukotsu-borei-to prevented theophylline-induced tachycardia in rats. Saiko-ka-ryukotsu-borei-to also attenuated an increase in arterial BP with a low reduction in heart rate of rats treated with theophylline. Saiko-ka-ryukotsu-borei-to showed no influence on the plasma theophylline concentration. However, Saiko-ka-ryukotsu-borei-to did not change the beating rate of the right atrium isolated from rats. The locomotor activity of theophylline in mice was reduced by the treatment with Saiko-ka-ryukotsu-borei-to. Furthermore, the latency of convulsions in mice induced by theophylline was prolonged by treatment with Saiko-ka-ryukotsu-borei-to and 7 out of 15 mice were saved from death due to convulsions. These results suggest that theophylline-induced tachycardia and central nervous stimulation are suppressed by Saiko-ka-ryukotsu-borei-to and that Saiko-ka-ryukotsu-borei-to may reduce the undesirable actions of theophylline on the cardiovascular and central nervous systems.

Hochu-ekki-to

Hochu-ekki-to is used for the prevention of methicillin-resistant *Staphylococcus aureus* (MRSA) candidiasis or influenza infection in patients with glucocorticoid or surgical operation-induced immunosuppression. It is, further, used for male patients with infertility due to oligozoospermia.

Since the management of MRSA carriers is not established, these patients are isolated individually, limited in activity and delayed in rehabilitation. In this study, the effects of Hochu-ekki-to or Juzen-taiho-to on MRSA carriers was examined in neurosurgery by Karibe *et al*. (1997). In the control group, 52 MRSA carriers were isolated individually. In the treatment group, Hochu-ekki-to or Juzen-taiho-to was given in addition to isolating the 44 patients. Hochu-ekki-to or Juzen-taiho-to was selected by the individual's constituents which were called 'SHO' in traditional literature. It was shown that Hochu-ekki-to or Juzen-taiho-to had effectively changed

MRSA carriers to negative. Effective rate of control group was 29%, and that of treated group was 66%. They also shortened the duration required to bring about the change of MRSA carriers to negative. The duration of control group was 88.4 ± 12.8 (days), and that of treated group was 47.0 ± 5.5 (days). As a result, the total number of MRSA carriers was reduced. The number in control group was $28.1 \pm 2.4\%$, and that in treated group was $13.4 \pm 2.2\%$. These results suggested that Hochu-ekki-to or Juzen-taiho-to may be useful for the management of MRSA carriers in neurosurgery.

The prospective effect of Hochu-ekki-to on experimental candidiasis in immunosuppressed mice was investigated by Abe *et al*. (1999). ICR mice were immunosuppressed by the injection of prednisolone or cyclophosphamide, given Hochu-ekki-to orally and challenged intravenously with *Candida albicans.* Treatment with Hochu-ekki-to for 8 days from day -4 or for 4 days from day 0 significantly prolonged the life span of the Candida-infected mice pre-treated with prednisolone. Hochu-ekki-to inhibited the colonization of Candida in the kidneys of the infected mice. These results suggest that Hochu-ekki-to can be used as a therapeutic agent against candidiasis in patients with glucocorticoid-induced immunosuppression.

A decline in the immunopotential of the host plays an essential role in the occurrence of infections with MRSA or other multi-drug resistant micro-organisms. In the present study, Matsui *et al*. (1997) examined the bacteriostatic action as well as the immunopotentiating action of the promising Hochu-ekki-to in mytomicin C (MMC)-treated mice with or without the infection of MRSA. Basic experimental data showed the drug to be effective in the treatment of MRSA infection. About 8–10-week-old male C57BL mice were injected with MMC at a dose of 5 mg/kg/day to inhibit the bone marrow, thus creating a mouse model with reduced immunopotential. Hochu-ekki-to was administered orally at a dosage of 500 mg/kg/day for seven consecutive days. For the infection of MRSA, 1×10^9 cells were injected intraperitoneally. Peritoneal macrophages were prepared by the adherence technique. Macrophage migration, phagocytic activity and the bactericidal activity were examined by the boyden chamber method, by the phagocytosis for fluorescent-activated latex beads, and by the nitroblue tetrazolium (NBT) reduction test, respectively. After the administration of Hochu-ekki-to, the number of WBCs in the MMC-treated mice recovered to 80% of the normal value. In addition, the phagocytic activity of macrophages increased to 50%, although that of the non-treated group was only 20%. The bactericidal activity also recovered to a level close to the normal value. The ratio of neutrophils in the Hochu-ekki-to administered MMC-treated group increased to 2.2% (normal mice, 2.6%) whereas that MMC-treated control group was 0.5%. In this study, IL-1β and IFNγ levels were recovered by the treatment with Hochu-ekki-to, as observed by IL-1β and IFNγ monitoring. The bacterial count in the liver of the MRSA challenged mice, with or without Hochu-ekki-to administration peaked 6 h after the challenge. The bacteria count in the blood showed an increase 12 and 24 h after the challenge. Even 24 h after the challenge, a significant number of bacterial cells existed in the blood of the group without Hochu-ekki-to administration, whereas only a small number of cells were detected 6 h after the challenge. All of the control mice died 8 days after the MRSA challenge, whereas the survival rates were 60% for Hochu-ekki-to treatment, 40% for the vancomycin treatment and 80% for the Hochu-ekki-to plus vancomycin treatment, respectively. The authors believe Hochu-ekki-to, which activates the immunopotential, will be very helpful in the treatment of opportunistic infections that are common among elderly patients.

Yoshida *et al*. (1986) orally administered Hochu-ekki-to to 45 patients with primary male infertility due to oligozoospermia (sperm density was below 40 million/ml) for over 12 weeks. After the treatment, significant increases in sperm concentration, rate of motility and total counts of normal spermatozoa, which were calculated as seminal volume by sperm density and

by rate of normal form of spermatozoa, were observed, especially in the group of moderate oligozoospermia (sperm density ranged from 20 to 40 million/ml). The rate of pregnancy was 20% (9/45) and the rate of clinical efficacy was 51.1% (23/45). No changes of laboratory examinations were observed. These data indicate that Hochu-ekki-to is more effective for the treatment of oligozoospermia, especially moderate oligozoospermia, without any serious side-effects.

Sairei-to

The most appropriate initial treatment for children with steroid-responsive nephritic syndrome is controversial. Initial treatment was with 18-week prednisolone and Sairei-to. Sairei-to may prevent subsequent relapse. To determine whether a similar result can be obtained with a combination of just initial 8-week prednisolone and Sairei-to, the effects of treatment with 18-week prednisolone and Sairei-to in 197 children with steroid responsive nephritic syndrome were studied by Yoshikawa *et al*. (1998).

Thirty-seven children with steroid-dependent nephritic syndrome (SDNS) were administered with Sairei-to under corticosteroid by Liu (1995). After treatment with Sairei-to relapse was markedly improved, time for negative conversion of proteinuria shortened, prednisolone dosage significantly reduced, and side-effects eased. Thirty-two children with SDNS treated with prednisolone and cyclophosphamide served as a control. Results showed that short-term and long-term relapse and average prednisolone dosage were similar between these two groups. It is considered that Sairei-to may be useful for patients with SDNS who fail to respond to or manifest severe toxic effects from cytotoxic agents.

Sairei-to increases the synthesis and secretion of ACTH by stimulating hypothalamic CRH release. In the present study, Tozawa *et al*. (1998) reported the effect of Sairei-to on the recovery of the hypothalamic-pituitary-adrenal axis function after treating male rats with prednisolone for 14 days, Sairei-to was administered during and after prednisolone administration. Tail-pinch stress was applied to the rats. The plasma ACTH response to tail-pinch stress in the prednisolone and Sairei-to group recovered to the control level on day 1, but that in the group given prednisolone alone recovered on day 3. The ACTH level in the anterior pituitary and the CRH level in the median eminence of the prednisolone and Sairei-to group returned to the control level on day 3, and that in the group given prednisolone alone returned to it on day 5. These results indicate that the administration of Sairei-to reduces the negative feedback effect of prednisolone on the hypothalamus and pituitary and accelerates the recovery of the hypothalamic CRH and pituitary ACTH level after glucocorticoid treatment.

Yoshikawa *et al*. (1997) undertook a prospective control study to determine the effect of Sairei-to in children with newly diagnosed IgA nephropathy showing focal/minimal mesangial proliferation. One hundred and one patients were randomly assigned to receive Sairei-to for 2 years (group 1) or no drug for 2 years (group 2). Forty-six of the 50 patients in group 1 and 48 of the 51 patients in group 2 completed their trial. At entry, the two groups of patients did not differ in the clinical, laboratory and pathologic findings. At the end of the trials, urinary protein excretion and haematuria were significantly reduced in group 1, but were unchanged in group 2. Twenty-one group-1 patients (46%) had normal urine, only 5 group-2 patients (10%) had normal urine at the end of the trial. BP and creatinine clearance were normal at the end of the trial in all but one group-2 patient, who developed chronic renal failure. The study demonstrates that 2-year Sairei-to treatment early in the course of disease is effective in children with IgA nephropathy showing focal/minimal mesangial proliferation.

In order to clarify the anti-nephritic mechanisms of Sairei-to, several studies using animals were performed. Hattori and Shindo (1995) reported the effect of Sairei-to on the expression of

adhesion molecules in rats with anti-glomerular basement membrane (GBM) nephritis. This study showed that Sairei-to inhibited cresent formation in glomeruli at the 10th day compared to control rats with anti-GBM nephritis. Sairei-to prevented the increase in the ICAM-1- or VCAM-1-positive area in the glomeruli of nephritic rats, and inhibited the increase in the number of LFA-1- or LFA-4-positive cells in the glomeruli. These results indicate that anti-nephritic activation of Sairei-to may be partially due to inhibition of adhesion molecule expression in the glomeruli. Further, Hattori *et al*. (1997) reported the effects of Sairei-to on the synthesis and expression of endothelin-1 in rats with anti-GBM nephritis. Sairei-to prevented the proteinuria and histopathological changes in the glomeruli of nephritic rats. Sairei-to inhibited the elevation of the endothelin-1 concentration in the supernatant from cultured glomeruli of nephritic rats and endothelin-1-positive area in the glomeruli. Sairei-to, further, inhibited the elevation of systolic BP and the number of proliferating cell nuclear antigen (PCNA)-positive cells per glomeruli.

Horikoshi *et al*. (1995) reported that Evans's syndrome patient was treated with prednisolone, but the anaemia and thrombocytopaenia progressed. When Sairei-to was administered combined with prednisolone, the platelet count increased from $6.1 \times 10\,849$/ml to 12.3×10^4/ml after 1 week, while haemoglobin level rose from 9.5 to 12.0 g/dl after 3 weeks. Patients maintained a good physical condition after the reduction of the prednisolone dosage. Sairei-to seems to be a promising therapeutic agent for steroid-resistant ITP and AIHA, and seems to have no side-effects.

Takakuwa *et al*. (1996) administered Sairei-to to 12 patients with recurrent abortion who had shown positive anti-phospholipid antibodies. The patients had experienced a total of 27 spontaneous abortions in their previous pregnancies and had no other pregnancy history except for one patient. The patients were treated with Sairei-to before their next pregnancy. The positive value of anti-phospholipid antibodies returned to negative in 9 out of 12 patients through the treatment. Out of 12 patients, in 10 patients, the new pregnancy continued uneventfully and delivered an offspring (success rate 83.3%). Thus, the current treatment was considered to be an effective therapy for patients with recurrent abortion who were found to be positive for anti-phospholipid antibodies. Takakuwa *et al*. (1997) sequentially analysed the alterations of peripheral blood lymphocyte subsets in patients with recurrent foetal wastage who were treated with Sairei-to. The titre of anti-phospholipid antibody was significantly decreased by administration of Sairei-to at 1 and 2 months after commencement of treatment and in the newly pregnant state compared. The percentage of CD19-positive cells significantly decreased at 2 months after commencement of Sairei-to treatment and at the newly pregnant state. The percentage of CD4-positive cells significantly increased 2 months after commencement of Sairei-to treatment. The CD4/CD8 ratio was increased significantly after 2 months administration of Sairei-to in both successful pregnancy and total cases. Thus, it is suggested that the administration of Sairei-to might induce the predominance of CD4-positive cells in parallel with the suppression of anti-phospholipid antibodies. Moreover, the suppression of CD19-positive cells (B cell) was induced by administration of Sairei-to which might be involved in successful continuation of pregnancy in the patients.

In anovulatory patients, ovulation is usually induced by clomiphene citrate (CC) or gonadotropin therapy, but in the case of polycystic ovary syndrome (PCOS), diagnosed by the presence of several micropolycysts in the ovaries and a high LH/FSH ratio in the serum, CC is only minimally effective, and side-effects are often a problem with gonadotropin therapy. Sakai *et al*. (1999) administered Sairei-to which appears to have a steroidal effect to anovulatory PCOS patients. As a result of the treatment, serum LH and the LH/FSH ratio significantly decreased ($P < 0.01$) and the ovulatory rate was 70.6%. Serum testosterone levels were within normal limits before the treatment and did not significantly change during the treatment. Sairei-to may therefore be useful for the treatment of anovulation in PCOS patients.

Shida *et al*. (1994) conducted a joint multi-institution study on the efficacy of Sairei-to, centering on urinary tract fibrosis. The subjects consisted of 18 patients with retroperitoneal fibrosis (including 3 women), 77 patients with plastic induration of the penis, 5 patients with sclerotic lipogranuloma (all men), and 67 patients with haemorrhagic cystitis (including 6 men). As a rule, Sairei-to was administered in monotherapy for periods of 4 weeks or longer. Efficacy was most pronounced in the patients with sclerotic lipogranuloma and plastic induration of the penis, with overall improvement rates (percentage of patients with ratings of effective or better) of 80% in the former and 77.9% in the latter group. The overall improvement rate in the patients with retroperitoneal fibrosis was 61.1%. In the above diseases, there were numerous patients concurrently administered drugs such as anti-inflammatory enzyme preparations and corticoid preparations, and the improvement rates were somewhat higher in these patients treated concurrently with other drugs. Outstanding efficacy was also seen in haemorrhagic cystitis. Dividing the patients into irradiation and non-irradiation groups, respective overall improvement rates of 77.8% and 82.8% were obtained, with the non-irradiation group showing a slightly higher rate. The non-irradiation group showed slightly higher improvement rates in the subjects treated concurrently with drugs such as anti-bacterial drug. Conversely, the irradiation group showed significantly superior rates for monotherapy. Side-effects such as mild gastrointestinal disturbances were seen in only 13 of 167 patients (7.8%), and the utility of this drug in treatment of the above diseases should be held in high regard.

Saiboku-to

Nakajima *et al*. (1993) noted a remarkable 'steroid sparing' effect of Saiboku-to within 6–12 months of treatment in steroid-dependent asthmatic patients. Saiboku-to inhibited the down-regulation of glucocorticoid receptor of human lymphocytes, and increased the plasma ACTH, and cortisol levels. It also inhibited the down-regulation of beta 2 receptor by beta 2 agonists and suppressed mACh receptor at the same time. Saiboku-to increased tyrosine aminotransferase (TAT) production, which was inhibited by actinomycin-D, and showed steroid-like activity. In mite-allergic asthma, Saiboku-to inhibited the induction of expression of IgE-Fc epsilon R/CD23 in the lymphocytes by mite allergen. It also inhibited IgE production by mite allergens. In experimental asthma in guinea-pigs, the use of Saiboku-to resulted in a decrease in the number of eosinophils in the bronchoalveolar lavage fluid during late asthmatic response. These findings suggest that Saiboku-to may be effective in inhibiting both the expression of IgE-Fc epsilon R2 and the induction of expression of IgE-Fc epsilon R1. Saiboku-to also has a steroid-like action and polyhedral anti-asthmatic activities.

In recent years, bronchial asthma has come to be regarded as a chronic inflammatory disease of the respiratory tract, with mast cells, lymphocytes and eosinophils playing important roles in its pathogenesis. Proteins contained in eosinophil granules, especially major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO), can cause tissue injury. When stimulated, eosinophils release mediators such as leukotriene C4 (LTC4) and platelet activating factors (PAF). Thus, they are recognized as effector cells that are actively involved in the development of allergic inflammation. Tohda *et al*. (1999a) used eosinophils from healthy volunteers to investigate the effects of Saiboku-to on eosinophils whose survival had been prolonged through stimulation with eosinophil-activating cytokines such as interleukin (IL)-3, IL-5 and granulocyte macrophage colony stimulating factors (GM-CSF). As a result, the cytokine-enhanced survival of eosinophils was significantly shortened by the addition of Saiboku-to. These findings suggest that Saiboku-to has the potential to inhibit allergic responses by directly affecting eosinophils which are related to allergic inflammation.

In recent years, bronchial asthma has come to be regarded pathologically as a chronic inflammatory disease of the respiratory tract. Inhalational steroids and anti-inflammatory drugs are recognized as being effective against bronchial asthma. Tohda *et al*. (1999b) reported the effects of Saiboku-to on asthmatic guinea-pigs sensitized to ovalbumin (OA). Following 7-day administration of Saiboku-to (500 μg/kg), the late asthmatic response (LAR) to an antigen challenge was found to be inhibited. The number of eosinophils in fluid obtained by bronchoalveolar lavage (BAL) 4 h after antigen challenge was decreased while the infiltration of eosinophils and T-lymphocytes into the lung parenchyma was inhibited. These findings suggest that Saiboku-to has the potential to become a useful drug in the treatment of bronchial asthma.

Yuzurihara *et al*. (1999) reported the effect of Saiboku-to on gastric lesions induced by restraint water-immersion stress and ethanol in rats. Thirty minutes after oral administration of Saiboku-to, the rats were placed in restraint cages and immersed in water at 23 °C for 7 h, or orally administered 99.5% ethanol (1 ml) and placed in normal cages for 1 h. The stress for 7 h or the ethanol treatment for 1 h induced erosion in the glandular area of the stomach. Histology showed that the surface epithelial cells were desquamated and part of the lamina propria mucosae was injured. The evaluation of the lesion index, the cumulative length of the gastric lesion, on the gross appearance of the stomach, revealed that Saiboku-to dose-dependently inhibited both the water-immersion stress-induced gastric erosion and ethanol-induced gastric erosion. To determine whether the anti-erosion effect of Saiboku-to was because of a mild irritant effect, Saiboku-to or 20% ethanol, which is known as a typical mild irritant, was given orally. After 30 min a strong irritant, 99.5% ethanol, was given orally. Histological examination was performed 30 min after administration of Saiboku-to or the mild irritant, and 1 h after administration of the strong irritant. The mild irritant induced a reduction in surface epithelial cells 30 min after administration. Furthermore, the mild irritant protected the stomach against mucosal erosion produced by the strong irritant. Saiboku-to protected the strong irritant-induced erosion without producing mild irritation as observed in stomach treated with 20% ethanol. Pre-treatment with Saiboku-to also inhibited the decrease in the levels of hexosamine, gastric mucus glycoprotein, induced by the strong irritant. In pylorus-ligated rats, Saiboku-to dose-dependently inhibited gastric acid secretion, a gastric aggressive factor. These results suggest that the anti-erosion effect of Saiboku-to, which is not a mild irritant, involves both inhibition of aggressive factors, such as gastric acid secretion, and augmentation of defensive factors, such as gastric mucus cells.

Tamaoki *et al*. (1995) reported the effect of Saiboku-to on the generation of nitric oxide (NO) from cultured canine tracheal epithelium using a highly specific amperometric sensor for this molecule *in vitro*. Immersion of the NO-selective electrode in the medium containing tracheal epithelial cells detected the baseline current of 16.8–57.0 pA, which corresponded to an NO concentration ([NO]) of 39.7 ± 8.1 nM. Addition of Saiboku-to increased [NO] in a concentration-dependent manner, the maximal increase from the baseline level and the concentration of Saiboku-to required to produce a half-maximal effect (EC50) being 127.5 ± 20.1 nM ($P < 0.001$) and 86 ± 9 μg/ml, respectively. Pre-treatment of cells with NG-nitro-L-arginine methylester (L-NAME) greatly inhibited the Saiboku-to-induced increase in [NO], whereas NG-nitro-D-arginine methylester (D-NAME) had no effect, and this inhibition was reversed by L-arginine but not by D-arginine. Cytochemical staining of the epithelial cells showed marked reactivity of NADPH diaphorase activity. These results suggest that NO is spontaneously released by the airway epithelium and that Saiboku-to stimulates the epithelial NO generation.

Saiboku-to has been used for the treatment of bronchial asthma. Kobayashi *et al*. (1996) reported the inhibitory action of this drug on arachidonate 5-lipoxygenase (5-LO) metabolism in rat basophilic leukaemia cells (RBL-1 cells). Saiboku-to significantly inhibited calcium ionophore-stimulated synthesis of cysteinyl leukotrienes (cLTs) and leukotriene B4 (LTB4).

Inhibition appeared 10 min after addition of the substance and reached a maximal value after 3 h. Saiboku-to did not inhibit the release of [3H]arachidonic acid (AA) from cell membrane by calcium ionophore stimulation, or the production of cLTs and LTB4, when LTA4-free acid was used as the substrate. However, it significantly inhibited the production of cLTs and LTB4 when free AA was used as the substrate. The production of thromboxane A2 (TXA2), a cyclooxygenase metabolite, was not inhibited when AA was used as the substrate in cell free study. These results indicate that Saiboku-to selectively inhibits 5-LO activity in the metabolic pathway of AA.

Saiko-keishi-to

Pancreatitis-associated protein (PAP) is almost absent in the normal pancreas but is overexpressed in acute pancreatitis. However, its expression in chronic pancreatitis (CP) is unknown. Saiko-keishi-to, has long been used clinically for CP, but there is no experimental evidence of the effect of Saiko-keishi-to on CP. Su *et al*. (1999) analysed the expression of PAP and the effect of Saiko-keishi-to in a spontaneous chronic pancreatitis model. Four-week-old male WBN/Kob rats were fed with a special pellet diet (MB-3), and Saiko-keishi-to (80 mg/100 g body weight/day) was orally administered for 16 weeks. Some of the rats were killed every 4 weeks, and their pancreas were histopathologically examined. PAP messenger RNA (mRNA) in the pancreas was detected with a reverse transcription–polymerase chain reaction (RT–PCR) method. The cellular localization of PAP mRNA and protein was analysed with *in situ* hybridization (ISH) and immunohistochemistry (IHC). PAP mRNA was expressed from 8 weeks, when the pancreas was still pathologically normal, and reached its peak at 12 weeks, when the pancreatitis first appeared. Then the expression of PAP mRNA was decreased gradually. Saiko-keishi-to suppressed the expression of PAP mRNA completely at 8 and 12 weeks. PAP mRNA was slightly expressed at 16 and 20 weeks. ISH and IHC confirmed the PAP mRNA and protein expression in the cytoplasm of acinar cells. These results suggest that PAP mRNA appears before CP, and its peak coincides with the onset of CP. Saiko-keishi-to suppressed the PAP expression and delayed the development of CP in the WBN/Kob rat.

Sugiyama *et al*. (1996) examined the mechanism underlying the anti-convulsant action of Saiko-keishi-to using whole-cell patch-clamp recording from cultured rat-dorsal-root ganglion cells. Neurones were held at −60 mV and perfused with an internal solution containing a high concentration of Cl^-. Under these circumstances, Saiko-keishi-to produced an inward current which reversed at +8 mV, and was identical to the gamma-aminobutyric acid (GABA)-induced chloride (I_{cl}) current. The Saiko-keishi-to-induced current was completely blocked by bicuculline. Saiko-keishi-to depressed a high voltage-activated calcium current (HVA-ICa), but this depression could not be antagonized by either phaclofen or saclofen. Of nine crude herbal drugs constituting Saiko-keishi-to, only Bupleuri radix, Ginseng radix, Zingiberis rhizome and Paeoniae radix produced the inward currents blocked by bicuculline. These results suggest that Saiko-keishi-to, and four of its crude herbal drugs, activate the I_{cl} mediated by the GABAA receptor. In addition, Saiko-keishi-to depresses HVA-ICa through some mechanism other than activation of GABAB receptors.

It was reported that Saiko-keishi-to which is often used for treating epileptic patients activates the GABAA receptor-mediated chloride current (I_{cl}). Sugiyama *et al*. (1997) examined whether the Saiko-keishi-to-induced Icl could be potentiated by several intravenous anesthetics known to interact with the GABAA receptor. It was also examined whether Saiko-keishi-to could potentiate the GABA-induced I_{cl}. Whole-cell patch-clamp recordings were made from cultured rat-dorsal-root ganglion cells. The peak amplitude of I_{cl} evoked by Saiko-keishi-to (2 mg/ml) increased after pentobarbital (50 μM) to 184 ± 26% ($n = 5$), diazepam (1 μM) to

166 ± 29% ($n = 5$), and propofol (5 μM) to 294 ± 93% ($n = 5$) from their respective controls, while the anaesthetics did not activate I_{cl} by themselves. The peak amplitude of I_{cl} evoked by GABA (10 μM) increased after propofol (5 μM) to 617 ± 189% of the control ($n = 4$), but decreased to 84 ± 7% of the control by Saiko-keishi-to (0.2 mg/ml, $n = 4$). These results indicate that the Saiko-keishi-to-induced I_{cl} can be potentiated by the intravenous anaesthetics, positive allosteric modulators of the GABAA receptor-Cl-channel complex and that Saiko-keishi-to is not a positive allosteric modulator, but a partial agonist for the GABAA receptor. Our study thus suggests that the combined use of Saiko-keishi-to and anti-convulsants such as barbiturates and benzodiazepines may be more effective in treating epileptic patients than Saiko-keishi-to alone.

Topics of placebo-controlled double-blind parallel study on Sho-saiko-to and other Kampo medicine

Sho-saiko-to

The efficacy of Sho-saiko-to on 222 patients with chronic active hepatitis was studied in a double-blind multicentre clinical study by Hirayama *et al*. (1989). One hundred and sixteen patients received Sho-saiko-to in a daily oral dose of 5.4 g for 12 weeks, followed by the same dose for a further 12 weeks. One hundred and six patients received a placebo containing 0.5 g of Sho-saiko-to for 12 weeks, followed by a cross-over to Sho-saiko-to for a further 12 weeks. Among the liver tests, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values decreased significantly with the administration of Sho-saiko-to. The difference of the mean value between the Sho-saiko-to group and the placebo group was significant after 12 weeks. In patients with chronic active type-B hepatitis, a tendency towards a decrease of HBeAg and an increase of Anti-HBe antibodies was also observed. No significant side-effects were noticed.

Keishi-ka-shakuyaku-to

The efficacy of Keishi-ka-shakuyaku-to on 286 patients with irritable bowel syndrome was studied in a double-blind clinical study by Sasaki *et al*. (1998a). One hundred and forty-eight patients received Keishi-ka-shakuyaku-to in a daily oral dose of 6.0 g for 4 weeks, followed by the same dose for a further 4 weeks. One hundred and thirty-eight patients received a placebo containing 0.16 g of Keishi-ka-shakuyaku-to for control. A tendency towards a decrease of abdominal pain was observed, particularly the improvement was observed in patients with diarrhoea. No significant side-effects were noticed.

Daio-kanzo-to

In the placebo-controlled, double-blind study of Daio-kanzo-to reported by Miyoshi *et al*. (1996) in the treatment of constipation that the authors previously conducted, there was a significant difference in efficacy between Daio-kanzo-to and placebo, indicating that Daio-kanzo-to is effective in the treatment of constipation, whereas no significant differences was noted in utility. It was concluded that Daio-kanzo-to was useful in the treatment of constipation. In this present study, new criteria for evaluation of overall improvement, efficacy and utility in patients who responded well to the drug was set up and the efficacy of the Daio-kanzo-to in the treatment of constipation was evaluated. This multi-institutional study was performed at 26 medical institutions. The subjects of the study were patients with a complaint of constipation who defecated only 3 days/week and required treatment. The following three different types of drugs

were used: Daio-kanzo-to usual-dosage-preparation (containing 1.5 g of the extracts at a daily dose); Daio-kanzo-to low-dose-preparation (containing 0.5 g of the extract at a daily dose); and placebo (containing 0 g of the extract at a daily dose). Each preparation was administered at 7.5 g daily, divided into three doses, for 2 weeks. In addition, magnesium oxide for 2-week administration at a daily dose of 1.0 g was prescribed for medication as a single dose whenever necessary. The number of patients enrolled was 53 for the usual dosage group, 49 for the low dose group and 54 for the placebo group; that is, 156 total. The number of patients accepted for the evaluation of safety and utility was 146 and 134, respectively. The number of patients accepted for the evaluation of efficacy was 44 for the usual dosage group, 41 for the low dose group, and 47 for the placebo group; that is, 132 in total. The evaluation results revealed significant differences in overall improvement rating, efficacy and utility between the usual dosage group and the placebo group, confirming that the study drug is effective and useful in the treatment of constipation.

Sho-seiryu-to

Baba *et al*. (1995) studied the efficacy and safety of Sho-seiryu-to in a joint double-blind trial in comparison with a placebo. The study was carried out on 220 patients with perennial nasal allergy at 61 hospitals. Granules in a dose of 3 g were administered three times daily for 2 weeks. Moderate to high improvement was recovered in 44.6% of the treated patients and in 28.2% of those receiving placebo. The difference is significant. Side-effects were noted in 6.5% of the treated patients and in 6.4% of the controls (not a significant difference). The side-effects were mild and had no influence on the daily life of the patients.

Shakuyaku-kanzo-to

In order to confirm the efficacy and safety of Shakuyaku-kanzo-to Extract Granules on muscle cramps in patients with liver cirrhosis, Kumada *et al*. (1999) conducted a randomized double-blind placebo controlled parallel study.

1 Hundred and twenty-six patients who experienced four times or more attacks of muscle cramps during 2 weeks were enrolled in this study. The patients were treated with Shakuyaku-kanzo-to or placebo in a daily dose of 7.5 g in 3 divided for 2 weeks. They were instructed to record frequency of muscle cramps, duration of the event and degree of pain in a diary every day. Twelve ineligible patients and 13 incomplete patients were excluded from the analysis. Efficacy of drug administration (improvement rate in frequency of muscle cramps and final general improvement rate) was assessed for 101 patients including 52 in the Shakuyaku-kanzo-to treated group and 49 in the placebo-administered group. Safety and usefulness were assessed in 90 cases, 49 in the Shakuyaku-kanzo-to group and 41 in the placebo group.
2 Improvement rate in frequency of muscle cramps during 14 days were studied: evaluation was divided into 'markedly improved' (IR = 0%), 'improved' ($0 < IR \leqq 50\%$), 'poor' ($50\% < IR \leqq 100\%$) and 'worsened' ($IR > 100\%$). The improvement rate in frequencies of muscle cramps was significantly superior to placebo group (Wilcoxon rank–sum test: $P = 0.022$). 'Markedly improved' or 'improved' were 67.3% in the Shakuyaku-kanzo-to group and 36.7% in the placebo group.
3 In final global improvement rate, changes in frequency of muscle cramps and degree of pain were added for comprehensive evaluation: Shakuyaku-kanzo-to was also found to be significantly better than placebo. 'Improved' or better response was 69.2% in the Shakuyaku-kanzo-to group and 28.6% in the placebo group.
4 There were no significant difference between the two groups in global safety rating.

5 The incidences of adverse reactions were 14.3% in the Shakuyaku-kanzo-to group and 4.95 in the placebo group. There was no significant difference between the two groups. Major adverse reactions included were pseudo-aldosteronism (BP raise, oedema and decrease in serum potassium level) in the Shakuyaku-kanzo-to group, while digestive symptoms were indicated in the placebo group. No severe adverse reaction was demonstrated.
6 The usefulness rate in the Shakuyaku-kanzo-to was significantly superior to placebo group. 'Useful' or better evaluation were 63.3% in the Shakuyaku-kanzo-to-treated group.
7 Despite the randomization, there was lack of balance between treatment groups, especially in two prognostic factors: the frequencies of muscle cramp during the 14-days baseline period; and the length of the patient's history of cirrhosis. Each imbalance was adjusted by the Mantel extension test. These stratified analyses did not alter the conclusions above. These results demonstrate that Shakuyaku-kanzo-to is a clinically useful medicine in treating muscle cramps.

Adverse effects

In type-C chronic hepatitis patients, IFNs are very effective to decrease the serum GPT and to kill the type-C hepatitis virus, and used first in selection of medicines. But the disappearance rate of virus is about 30% by the IFNs treatment in Japan. To increase its efficacy rate, the combination therapy of both IFNs and Sho-saiko-to was found to be very effective, and it was carried out in Japan for several years. In that situation the occurrence of interstitial lung pneumonia, the major adverse effect of IFNs, increased. The enhancement of the IFN-induced interstitial lung pneumonia by Sho-saiko-to is due to the similar action mechanisms of IFNs and Sho-saiko-to in immunological responses. Murakami *et al*. (1995) reported that Sho-saiko-to increased inflammatory cytokines, TNF α etc, and synergistically enhanced the IFNs action. The occurrence rate of interstitial lung pneumonia by the single use of Sho-saiko-to was less than 0.1%. And this adverse effect was reported to occur mainly in aged patients or weak constitution under cirrhosis. The infection with type-C hepatitis virus should make host some immunological changes those are relatively easy to occur interstitial pneumonia.

The syndrome of pseudoaldosteronism due to chronic ingestion of liquorice is well known. One of the composed crude drugs in Sho-saiko-to is liquorice which oral dose in Sho-saiko-to/day is the extract of 2 g of dried root of Glycyrrhizae Radix. Several cases of pseudoaldosteronism are reported in Sho-saiko-to treated patients. Kojima and Hoshino (1996) reported the case with pseudoaldosteronism induced in a patient taking Sho-saiko-to for treatment with chronic hepatitis. Hypokalaemia, hypertension and adrenal mass were discovered, and BP and serum potassium returned to normal 4 weeks after the discontinuation of Sho-saiko-to. They concluded that the patient had liquorice-induced pseudoaldosteronism as well as non-functioning adrenal adenoma.

A liver injury induced by Sho-saiko-to are also reported. Takeshima *et al*. (1997) reported the case of liver injury. Serum AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase) increased to 631, 1150 and 1329 IU/l, respectively, in one patient after taking Sho-saiko-to, and he rapidly recovered from liver dysfunction after discontinuance of Sho-saiko-to. Allergic liver injury should be made by Sho-saiko-to.

Conclusion

Kampo medicines have been used in Japan for more than 1000 years. Kampo medicine is a mixture of crude medicinal drugs, and Sho-saiko-to is composed of seven crude medicinal drugs. Most of the composed crude drugs in Sho-saiko-to are reported to show anti-inflammatory effects, and some of them possess immunopotentiative and/or immunosuppressive effects. The multiple crude

drugs with the similar pharmacological actions are composed in one formula and the crude drugs with both agonistic and antagonistic actions are also combined in one formula. These characteristics resulted from the fact that one formula has both opposite actions depending on the patient's constitutions. Namely Sho-saiko-to shows immunostimulatory action in the immunosuppressive state and immunosuppressive action in immunostimulative state. Although the efficacy of the most ingredients is mild, the action as a formula, a mixture of crude drugs, is worth noting. Synergistic actions of the plural active ingredients or plural crude drugs should be the essence of Kampo medicine. Although it may take a long time to clarify the pharmacological action mechanisms of Kampo medicine due to the existence of the numerous active ingredients, the accumulation of the clinical experiences for 1000 years is an important signpost for medical doctors. This is the main reason that the Japanese government admitted the Kampo medicine as medicines covered by health insurance. The clinical indications of Kampo medicine is nowadays determined by the proof of the clinical experience for more than 1000 years. The duty of the medical scientists is to prove their clinical effect by scientific methods, and add scientific signpost in the traditional clinical system. The fusion of Western and Eastern medicine system should contribute to the health of patients with serious diseases that are intractable with known medical methods.

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