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Sho-saiko-to and Saiko-keisi-to, the traditional Chinese and Japanese herbal medicines, altered hepatic drug-metabolizing enzymes in mice and rats when administered orally for a long time

Mitsuhiko Nose, Masayo Tamura, Nobukazu Ryu, Hajime Mizukami and Yukio Ogihara

Abstract

As the consumption of herbal remedies has increased, the opportunity that such herbal medicines are co-administered with other drugs has also risen gradually and we are, therefore, very much concerned about herb–drug interactions. We examined the effects of pre-administration of Kampo medicines (Sho-saiko-to, Saiko-keishi-to, Shigyaku-san and Dai-saiko-to) on the pentobarbital-induced sleeping time in mice and rats, to clarify the possibility that they could affect the drug-metabolizing enzymes. The administration of Sho-saiko-to and Saiko-keishi-to for 4 weeks significantly shortened the pentobarbital-induced sleeping time in mice and the administration of Sho-saiko-to for 2 weeks significantly reduced the sleeping time in rats. Furthermore, we tried to identify the molecular species of rat cytochrome P450s (CYPs) affected by Sho-saiko-to and Saiko-keishi-to by competitive RT-PCR. The oral administration of Sho-saiko-to for 2 weeks up-regulated the mRNA expression of CYP2B, CYP3A1, CYP2E1 and CYP4A1 in rats. The treatment with Saiko-keishi-to for 2 weeks also up-regulated the mRNA expression of CYP2B, CYP3A1 and CYP4A1. Sho-saiko-to and Saiko-keishi-to may potentially influence the drug-metabolizing enzymes in man, and would thus require much attention when used in the clinical situation.

Introduction

During the last decade, an explosion in the consumption of herbal remedies has been witnessed in North America and Europe. These regions now lead the world in the sales of such remedies and the intake of herbal remedies (including dietary supplements) may eventually increase the intake of phytochemicals much more than is consumed through the diet. Consequently, physicians and pharmacists are very much concerned about their toxicity and also the drug–drug interactions when using herbal medicines with other medicines (Fugh-Berman 2000; Ioannides 2002).

Recently, several herbal medicines such as St John's wort and *Ginkgo biloba* have been reported to demonstrate such drug–drug interaction with medicinal drugs (Fugh-Berman & Ernst 2001; Izzo & Ernst 2001; Shinozuka et al 2002). St John's wort, an extract of the plant *Hypericum perforatum*, is extensively used as an anti-depressant to treat mild-to-moderate depression in Europe, North Africa and North America (Josey & Tackett 1999; Barnes et al 2001). Intake of St John's wort enhances the expression of intestinal P-glycoprotein and the expression of CYP3A4 in the liver and intestine (Durr et al 2000; Markowitz et al 2000; Roby et al 2000). This combined up-regulation in intestinal P-glycoprotein and hepatic and intestinal CYP3A4 impairs the absorption and stimulates the metabolism of an immunosuppressant drug, ciclosporin, resulting in its subtherapeutic plasma concentrations. Thus, in transplant patients, self-medication with St John's wort has led to a drop in plasma level of ciclosporin, causing tissue rejection (Mai et al 2000; Ruschitzka et al 2000; Barone et al 2001).

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

Mitsuhiko Nose, Masayo Tamura, Nobukazu Ryu, Hajime Mizukami

Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468-8503, Japan

Yukio Ogihara

Correspondence: M. Nose, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan. E-mail: nose@phar.nagoya-cu.ac.jp

In Japan, Kampo medicines, traditional Chinese and Japanese herbal medicinal mixtures, have been used clinically for the treatment of many chronic diseases. Such herbal medicines have been used in China for thousands of years and are now being manufactured in Japan as drugs with standardized qualities and quantities of ingredients. Sho-saiko-to is one of the major prescriptions most frequently used for the treatment of infectious diseases, such as chronic viral hepatitis (Oka et al 1985; Hirayama et al 1989; Gibo et al 1994), and it has been extensively demonstrated that Sho-saiko-to has various pharmacological actions, including immunomodulating, anti-inflammatory and antihepatitis activity (Iwama et al 1987; Amagaya et al 1988, 1989; Amayaga & Ogihara 1990; Nose et al 1997, 2002; Shimizu et al 1999; Shiota et al 2002). However, the experimental data in the field of drug–drug interactions are limited.

In general, Kampo medicine is prescribed for relatively long-term use (e.g., 2–4 weeks), and during that period some other medicines are occasionally co-administered. Moreover, there is a possibility that the patient will self-medicate and some Kampo medicines are prescribed with some other synthetic medicines. It is, therefore, necessary (highly desirable) to clarify what kinds of drug-metabolizing enzymes are influenced by intake of these herbal remedies and what kinds of drug-metabolizing enzymes are concerned in the metabolism of ingredients in these herbal medicines.

In this study, we demonstrated that so-called Saiko-agents (a general term of prescriptions containing Bupleuri Radix as a main crude drug in Japan), such as Sho-saiko-to, Saiko-keishi-to and Dai-saiko-to, reduced pentobarbital-induced sleeping time in mice or rats. Furthermore, we investigated the effects of these Kampo medicines on the expressions of rat cytochrome P450 (CYP) mRNAs by competitive RT-PCR and identified the molecular species influenced by long-term administration of these Kampo medicines.

Materials and Methods

Preparation of Kampo medicines

Most of the medicinal herbs were authenticated and provided by Tsumura Co. Ltd (Tokyo, Japan). The crude drug composition of four kinds of Kampo medicines, Sho-saiko-to, Shigyaku-san, Saiko-keishi-to and Dai-saiko-to, is given in Table 1. The mixture of crude drugs was extracted with 600 mL of water at 100 °C for 1 h. The decoction was filtered and then lyophilized to obtain a powder extract. The yield of each extract was also noted in Table 1. The dose of each Kampo medicine was roughly equivalent to five times the daily human dose as follows: 0.55 g kg^{-1} for Sho-saiko-to, 0.37 g kg^{-1} for Shigyaku-san, 0.5 g kg^{-1} for Saiko-keishi-to and 0.58 g kg^{-1} for Dai-saiko-to, respectively.

Animals

Female ICR mice, 6 weeks old, were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and female Sprague-Dawley rats, 6 weeks old, were purchased from Charles River Japan, Inc. (Tokyo, Japan). The animals were housed in standard plastic cages in a temperature- and humidity-controlled environment, with food (CE-2, Clea Co., Tokyo, Japan) and water freely available. A period of at least 7 days of acclimatization was allowed before experimentation. Experimental procedures were approved by the Animal Care Committee at Graduate School of Pharmaceutical Sciences, Nagoya City University, in accordance with the guidelines of the Japanese Council on Animal Care.

Table 1 Crude drug composition and yield of Kampo medicine.

Crude drug	Dai-saiko-to	Shigyaku-san	Sho-saiko-to	Saiko-keishi-to
Bupleuri Radix	6.0 g	5.0 g	7.0 g	5.0 g
Pinelliae Tuber	4.0 g		5.0 g	4.0 g
Scutellariae Radix	3.0 g		3.0 g	2.0 g
Paeoniae Radix	3.0 g	4.0 g		2.0 g
Zizyphi Fructus	3.0 g		3.0 g	2.0 g
Aurantii Fructus Immaturus	2.0 g	2.0 g		
Zigiberis Rhizoma	1.0 g		1.0 g	1.0 g
Rhei Rhizoma	1.0 g			
Ginseng Radix			3.0 g	2.0 g
Glycyrrhizae Radix		1.5 g	2.0 g	2.0 g
Cinnamomi Cortex				2.0 g
Yield (g/day)	6.92 ± 0.18	4.39 ± 0.07	6.65 ± 0.14	6.00 ± 0.22

Crude drug was extracted with 600 mL of water at 100 °C for 1 h and then lyophilized to obtain a powder extract, which is the daily human dose (g). The yield (g/day) of each Kampo medicine was expressed as the mean ± s.e.m. of 5 preparations.

Pentobarbital-induced sleeping time test

The animals were all given sodium pentobarbital (Nembutal Injection (50 mg mL^{-1}); Abbot Laboratories, IL; 60 mg kg^{-1} for mice and 30 mg kg^{-1} for rats) intraperitoneally, 48 h after the final administration of each Kampo medicine. The time from the loss of righting reflex to recovery was recorded as the sleeping time.

Total RNA extraction and competitive RT-PCR

Sho-saiko-to and Saiko-keishi-to were orally administered for the indicated periods. Whole livers from four control rats, four Sho-saiko-to-treated rats and four Saiko-keishi-to-treated rats were collected after perfusion carefully with ice-cold phosphate-buffered saline (pH 7.4) to remove as much blood as possible, 24 h after the final administration. The collected organs were pulverized in liquid nitrogen and stored at −80 °C until use. Each sample was pooled and used for extracting total RNA using the RNA Extraction Kit (Amersham Pharmacia Biotech Inc., NJ,) according to the manufacturer's instruction. Each total RNA preparation was treated with deoxyribonuclease I (DNase I, Amplification Grade; Invitrogen Corp., Carlsbad, CA) to avoid the contamination of genomic DNA. First-strand cDNA synthesis and following competitive PCR were performed using a rat Cytochrome P450 Competitive RT-PCR Set (TaKaRa Bio Inc., Shiga, Japan) according to the manufacturer's instruction. The PCR products were viewed on a 2% agarose gel (Agarose S; Nippon Gene Co. Ltd, Tokyo, Japan) with ethidium bromide and analysed with an ImageQuant analysis software (Molecular Dynamics, Inc., Sunnyvale, CA) using a FluoroImager (Molecular Probes, Eugene, OR).

Statistical analysis

With respect to the effects of Kampo medicines on pentobarbital-induced sleeping time, results were indicated as the mean ± s.e.m.; mean values were compared by analysis of variance and Bonferroni's multiple *t*-test.

Results

Effects of Sho-saiko-to, Shigyaku-san, Saiko-keishi-to and Dai-saiko-to on pentobarbital-induced sleeping time

To clarify whether drug-metabolizing enzymes are induced by oral administration of Kampo medicines such as Sho-saiko-to, Shigyaku-san, Saiko-keishi-to and Dai-saiko-to, we studied the effects of these Kampo medicines on pentobarbital-induced sleeping time, a classical behaviour pharmacological technique to detect the induction of drug-metabolizing enzymes. To avoid the direct suppressive actions of these Kampo medicines on the central nervous system, the sleeping-time test was performed 48 h after the final administration.

In female ICR mice, the consecutive administration of Sho-saiko-to and Saiko-keishi-to for 4 weeks significantly shortened pentobarbital-induced sleeping time, whereas Dai-saiko-to and Shigyaku-san slightly reduced the sleeping time (Table 2). Sho-saiko-to and Saiko-keishi-to slightly shortened the sleeping time even after a 1-week administration.

It is well known that the activity of drug-metabolizing enzymes differs by species, sex and age. Thus, we investigated the effects of Sho-saiko-to and Saiko-keishi-to on the pentobarbital-induced sleeping time using female rats (Table 3). Sho-saiko-to significantly shortened the sleeping time when administered for 2 weeks and slightly reduced the sleeping time when administered for 4 weeks. Saiko-keishi-to also slightly reduced the sleeping time when administered for 2 weeks and for 4 weeks.

These results suggested that Sho-saiko-to and Saiko-keishi-to might induce drug-metabolizing enzymes, although the extent of their effects differed depending on the animal species.

Table 2 Effects of orally administered Dai-saiko-to, Shigyaku-san, Sho-saiko-to and Saiko-keishi-to on pentobarbital-induced sleeping time in mice.

Treatment	Sleeping time (min)	
	1 week	4 weeks
Control	79.4 ± 3.7	100.3 ± 9.0
Sho-saiko-to	72.3 ± 5.3	65.6 ± 7.8*
Saiko-keishi-to	68.8 ± 4.3	63.6 ± 3.0**
Dai-saiko-to	80.0 ± 9.5	92.8 ± 10.9
Shigyaku-san	77.2 ± 4.1	83.4 ± 6.07

Each Kampo medicine was administered orally for the indicated period. Forty-eight hours after the final administration, mice were given pentobarbital (60 mg kg^{-1}) intraperitoneally. The time from the loss of righting reflex to recovery was recorded as the sleeping time. The data were expressed as the mean ± s.e.m. of 5 or 6 mice. $^{**}P < 0.01$, $^{*}P < 0.05$ vs the control group (analysis of variance and Bonferoni's multiple *t*-test).

Table 3 Effects of orally administered Sho-saiko-to and Saiko-keishi-to on pentobarbital-induced sleeping time in rats.

Treatment	Sleeping time (min)	
	2 week	4 week
Control	155.7 ± 4.3	155.0 ± 12.8
Sho-saiko-to	134.0 ± 8.7*	134.0 ± 10.3
Saiko-keishi-to	140.8 ± 7.1	143.6 ± 10.7

Each Kampo medicine was administered orally for the indicated period. Forty-eight hours after the final administration, rats were given pentobarbital (30 mg kg^{-1}) intraperitoneally. The time from the loss of righting reflex to recovery was recorded as the sleeping time. The data were expressed as the mean ± s.e.m. of 5–7 rats. *$P < 0.05$ vs the control group (analysis of variance and Bonferoni's multiple *t*-test).

Effects of Sho-saiko-to and Saiko-keishi-to on the expression of cytochrome P450 mRNA in rat liver by competitive RT-PCR

We attempted to determine the molecular species of cytochrome P450 induced by Sho-saiko-to and Saiko-keishi-to, using competitive RT-PCR methods.

As shown in Table 4, oral administration of Sho-saiko-to for 1 week up-regulated the expression of CYP2B1/2, CYP2E1, CYP3A1 and CYP4A1 mRNAs, whereas Saiko-keishi-to up-regulated the mRNA expression of CYP2B1/2 alone. Furthermore, the extent of up-regulated mRNA expression was about 2 times higher when Sho-saiko-to was administered for 2 weeks, compared with the 1-week administration (Table 5).

Saiko-keishi-to also up-regulated transcription of CYP2B1/2, CYP3A1 and CYP4A1, although CYP2B1/2 expression was decreased to the control level and CYP3A1 and CYP4A1 expression was down-regulated after 4-week administration. In contrast, the CYP2E1 transcription was increased only when Saiko-keishi-to was administered for 4 weeks (Table 6).

Discussion

In classifying drug–drug interactions, it has been considered that the interaction in the process of drug metabolism is about 40% and most of them are based upon the modification of cytochrome P450 enzymes. Cytochrome P450 exists in the liver, kidney, lung and intestine of mammalian species as a superfamily of isoenzymes and plays an important role in the metabolism of xenobiotics, including drugs and other chemicals. Metabolic processes catalysed by cytochrome P450 are the primary oxidative processes involved in the detoxication and bioactivation of a number of drugs and environmental pollutants, and this process is the rate-limiting process in the elimination of drugs and other chemicals from our body.

The safety of traditional herbal remedies such as Kampo medicines has been believed to be secured through the experience of their use over a long time. However, the case of co-administration with other synthetic medicines is excepted from this rule. Especially, drug–drug interactions

Table 4 Analysis of cytochrome P450 mRNA expression by competitive RT-PCR in rats administered Kampo medicine for 1 week.

CYP isoenzyme	Estimated expression level of mRNA (copies/ng total RNA)			Induction rate by Kampo medicine	
	Control	Sho-saiko-to	Saiko-keishi-to	Sho-saiko-to	Saiko-keishi-to
CYP1A1	ND	ND	ND	±	±
CYP1A2	ND	ND	ND	±	±
CYP2B1/2	8.0×10^4	1.6×10^5	1.6×10^5	×2	×2
CYP2E1	2.6×10^6	7.4×10^6	2.6×10^6	×2.8	±
CYP3A1	6.4×10^5	1.3×10^6	6.4×10^5	×2	±
CYP3A2	ND	ND	ND	±	±
CYP4A1	1.6×10^5	6.4×10^5	1.6×10^5	×4	±

ND, not detected. Sho-saiko-to or Saiko-keishi-to was administered orally for 1 week. Livers from control (n = 4), Sho-saiko-to-treated (n = 4) and Saiko-keishi-to-treated (n = 4) rats were excised and then pulverized in liquid nitrogen, respectively. Each sample was pooled as each group and then used for extracting total RNA and consequent competitive RT-PCR.

Table 5 Analysis of cytochrome P450 mRNA expression by competitive RT-PCR in rats administered Kampo medicine for 2 weeks.

CYP isoenzyme	Estimated expression level of mRNA (copies/ng total RNA)			Induction rate by Kampo medicine	
	Control	Sho-saiko-to	Saiko-keishi-to	Sho-saiko-to	Saiko-keishi-to
CYP1A1	ND	ND	ND	±	±
CYP1A2	ND	ND	ND	±	±
CYP2B1/2	1.6×10^5	6.4×10^5	3.2×10^5	×4	×2
CYP2E1	5.2×10^6	1.1×10^7	5.2×10^6	×2	±
CYP3A1	6.4×10^5	2.6×10^6	2.6×10^6	×4	×4
CYP3A2	ND	ND	ND	±	±
CYP4A1	1.6×10^5	1.3×10^6	6.4×10^5	×8	×4

ND, not detected. Sho-saiko-to or Saiko-keishi-to was administered orally for 2 weeks. Livers from control (n = 4), Sho-saiko-to-treated (n = 4) and Saiko-keishi-to-treated (n = 4) rats were excised and then pulverized in liquid nitrogen, respectively. Each sample was pooled as each group and then used for extracting total RNA and consequent competitive RT-PCR.

Table 6 Analysis of cytochrome P450 mRNA expression by competitive RT-PCR in rats administered Kampo medicine for 4 weeks.

CYP isoenzyme	Estimated expression level of mRNA (copies/ng total RNA)			Induction rate by Kampo medicine	
	Control	Sho-saiko-to	Saiko-keishi-to	Sho-saiko-to	Saiko-keishi-to
CYP1A1	ND	ND	ND	±	±
CYP1A2	ND	ND	ND	±	±
CYP2B1/2	1.6×10^5	1.6×10^5	1.6×10^5	±	±
CYP2E1	2.6×10^6	1.1×10^7	5.2×10^6	×4	×2
CYP3A1	1.3×10^6	2.6×10^6	3.2×10^5	×2	×0.25
CYP3A2	ND	ND	ND	±	±
CYP4A1	6.4×10^5	1.3×10^6	3.2×10^5	×2	×0.5

ND, not detected. Sho-saiko-to or Saiko-keishi-to was administered orally for 4 weeks. Livers from control (n = 4), Sho-saiko-to-treated (n = 4) and Saiko-keishi-to-treated (n = 4) rats were excised and then pulverized in liquid nitrogen, respectively. Each sample was pooled as each group and then used for extracting total RNA and consequent competitive RT-PCR.

may become more likely if the consumption of such traditional medicines is increased.

The interaction between dietary chemicals and drugs was first reported in the 1970s, showing that consumption of cruciferous vegetables resulted in lower plasma concentrations of an analgesic medicine phenacetin, and it was suggested that indole derivatives contained in the vegetables could be responsible for this interaction (Pantuck et al 1976, 1979). Since the drug–drug interaction between grapefruit juice and synthetic medicines has been reported (Ameer & Weintraub 1997; Bailey et al 1998a; Fuhr 1998), physicians and pharmacists pay much attention to the possibility of drug interaction with foods and the traditional herbal remedies. Furanocoumarins, such as bergamottin and its derivatives, are identified as promoting such drug–drug interactions with grapefruit constituents (Edwards et al 1996; Fukuda et al 1997; Schmiedlin-Ren et al 1997; Bailey et al 1998b; He et al 1998). Furanocoumarins occur in medicinal plants belonging to the Umbelliferae, Rutaceae, Leguminosae and Compositae families and, therefore, we are concerned about the effects of Kampo medicines on the drug–metabolizing enzymes.

In this study, we demonstrated that the oral administration of Sho-saiko-to and Saiko-keishi-to for 4 weeks significantly reduced pentobarbital-induced sleeping time in female mice and we could see the same tendency 1 week after administration. These results suggested that Sho-saiko-to and Saiko-keishi-to could induce drug-metabolizing enzymes in the liver. We confirmed this inducing activity of Sho-saiko-to in rats and therefore we next tried to identify the molecular species of cytochrome (CYP) induced by Sho-saiko-to, compared with the effect of Saiko-keishi-to. It is well documented that the barbitals, such as sodium phenobarbital and sodium pentobarbital, induce drug-metabolizing enzymes, especially CYP2B1/2 and CYP3A1 in rats. We confirmed that the mRNA

expressions of CYP2B1/2 and CYP3A1 were increased 16-fold and 4-fold, respectively, when sodium phenobarbital (80 mg kg^{-1}) was administered orally for 1 week, using the same experimental conditions (data were not shown). Oral administration of Sho-saiko-to resulted in up-regulation of CYP2B1/2 and CYP3A1 mRNA expression and these results gave us good agreement with the shortened effects of pentobarbital-induced sleeping time. Unexpectedly, Saiko-keishi-to did not significantly reduce the sleeping time in rats, which is consistent with the results that this Kampo medicine up-regulated neither CYP2B1/2 nor CYP3A1 expression. Furthermore, we found up-regulation of CYP2E1 and CYP4A1 mRNA expression by Sho-saiko-to and Saiko-keishi-to.

We demonstrated the effects of Sho-saiko-to and Saiko-keishi-to on the hepatic cytochrome P450 as described above, although we did not investigate their effects on intestinal cytochrome P450s. As it has been reported that grapefruit juice acts by inhibiting intestinal CYP3A4 activity and consequently elevates the serum concentration of co-administered drugs (Lown et al 1997), we should consider the possibility that Sho-saiko-to and Saiko-keishi-to could affect intestinal CYP species.

Although there have been a few reports of Sho-saiko-to and other Kampo medicines describing their effects on the hepatic drug-metabolizing enzymes or the pharmacokinetics of co-administered drugs in-vivo, this is the first report concerning the effect of Kampo medicine on the mRNA expression of major hepatic CYP species, especially when administered orally long term.

Homma et al (1995) performed cross-over open trials in healthy subjects to clarify prednisolone pharmacokinetics with pre-administration of Sho-saiko-to, Saiboku-to and Sairei-to. These major Kampo medicines consist of similar herbal prescriptions containing almost equal amounts of glycyrrhizin, which is a strong inhibitor of 11β-hydroxysteroid dehydrogenase. Unexpectedly, they found that Sho-saiko-to significantly decreased the AUC for prednisolone, while Saiboku-to increased the prednisolone AUC and Sairei-to had no influence. They suggested that some unknown metabolic enzyme modifiers might be in these prescriptions. It is well known that glucocorticoids are metabolized by CYP3A and also that CYP3A1 mRNA can be induced by glucocorticoids in rats. In this study, we found that Sho-saiko-to up-regulated CYP3A1 mRNA expression throughout the experiment, even in 1 week. Thus, we assumed that Sho-saiko-to might decrease prednisolone AUC by means of up-regulation of CYP3A in man.

Hosoya et al (1993) suggested that the decrease in serum phenytoin concentration induced by treatment with Sho-saiko-to for 1 week in rabbits might be mainly a result of the stimulation of hepatic phenytoin-oxidizing metabolic enzyme activity by Sho-saiko-to. In contrast, Nishimura et al (1998) reported that single co-administration of Sho-saiko-to slightly hastened the gastrointestinal absorption of tolbutamide but did not affect its hepatic drug-metabolizing enzyme. Both phenytoin and tolbutamide are metabolized by CYP2C9 in man. This discrepancy may be due to difference in experimental conditions such as animals used and administration periods. Further studies are needed.

Ohnishi et al (1996) revealed that the administration of Sho-saiko-to for 2 weeks resulted in a 25% increase in the content of cytochrome P450 and the metabolic rates for substrates of CYP2E1 were significantly enhanced in female rat liver, while the content of cytochrome P450 and the metabolic activity towards various xenobiotics in male rats were unchanged. In general, the content of cytochrome P450 and the metabolic activity towards various xenobiotics in the male are much higher than those in the female and, therefore, we used females for this study. We confirmed that Sho-saiko-to increased CYP2E1 at the transcriptional level in female rats as well.

With respect to the crude drugs that compose Sho-saiko-to and other Kampo medicines we investigated here, there are several reports concerning the CYP-inducing activity of Glycyrrhizae Radix. Ethanolic or water extract of Glycyrrhizae Radix and glycyrrhizin, one of the important ingredients of this herb, were shown to affect the activity of CYP3A using in-vitro and in-vivo systems (Paolini et al 1998; Budzinski et al 2000). Furthermore, Paolini et al (1999) revealed that repeated administration of Glycyrrhizae Radix extract for 4 days doubled the CYP1A-mediated *O*-deethylation of ethoxyresorufin and also stimulated the *O*-demethylation of methoxyresorfin in rats, but in females only. They also reported that liquorice intake stimulated CYP3A-mediated 6β-hydroxylation of testosterone and CYP2B-mediated *O*-dependentylation of pentoxyresorufin in the female rats. These observations may suggest the potential involvement of Glycyrrhizae Radix in the up-regulation of the CYP mRNA expression by Sho-saiko-to and Saiko-keishi-to we demonstrated here. There are a few reports concerning Ginseng Radix (Kim et al 1997; Henderson et al 1999; Coon & Ernst 2002). However, we do not have enough information about the effects of these crude drugs and their ingredients on drug-metabolizing enzymes. Furthermore, we do not fully understand even the metabolism of the major constituents of these crude drugs and Kampo medicines. Thus, we also should pay attention to the pharmacokinetics of their ingredients to clarify the active principles in these modifications of CYP mRNA expression.

On the other hand, recent studies revealed the important roles of orphan nuclear receptor superfamily members, such as constitutive androstane receptor (CAR), pregnane X receptor (PXR) and peroxisome proliferator-activated receptor (PPAR), in mediating the induction of hepatic CYPs belonging to the families of CYP2, CYP3 and CYP4, respectively (Waxman 1999). These nuclear receptors are thought to act as the sensors to xenochemicals and their ligands are thought to be low-molecular-weight and lipophilic compounds, and, therefore, we may isolate the active principles by screening whether ingredients interact with their nuclear receptors in the near future.

In conclusion, we found that Sho-saiko-to and Saiko-keishi-to could induce the drug-metabolizing enzymes in mice and rats, and, especially, Sho-saiko-to

up-regulated the mRNA expressions of CYP2B, CYP3A1, CYP2E1 and CYP4A1 in rats. Although we should further confirm the up-regulation of these CYPs at the protein level and at the point of their activity, these Kampo medicines may potentially influence drug-metabolizing enzymes in man, and indicate a special requirement to pay much attention to their use in clinical practice.

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