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The in-vivo effects of sho-saiko-to, a traditional Chinese herbal medicine, on two cytochrome P450 enzymes (1A2 and 3A) and xanthine oxidase in man

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Abstract

The Chinese herbal medicine sho-saiko-to is a mixture of seven herbal components (Bupleurum root, Pinellia tuber, Scutellaria root, Jujube fruit, Ginseng root, Glycyrrhiza root and Ginger rhizome) that is widely administered to patients with chronic hepatitis in Japan. We assessed the effects of sho-saiko-to on the activity of cytochrome P450 (CYP) 1A2, CYP3A and xanthine oxidase (XO) in man. Twenty-six healthy subjects were studied to evaluate their baseline activity of CYP1A2 and XO by the respective urinary metabolic ratios of an 8-h urine sample after an oral 150-mg dose of caffeine and of CYP3A by a urinary excretion ratio of 6β-hydroxycortisol (6β-HC) to free cortisol (FC). Thereafter, the subjects received a twice-daily 2.5-g dose of sho-saiko-to for five days, and underwent the caffeine test on day 1 and day 5. The mean activity of CYP1A2 decreased by 16% on both day 1 and day 5 compared with the baseline ($P = 0.001$). The mean activity of XO also significantly decreased by 25% on day 1 and 20% on day 5 ($P < 0.0001$) compared with the baseline value. The activity of CYP3A tended to be lower on day 5 than the baseline ($P = 0.146$). It is concluded that sho-saiko-to reduces CYP1A2 and XO activity in man.

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Introduction

The use of herbal medicines is increasing, and herb–drug interactions are recognised as a clinically important problem (O'Hara et al 1998; Fugh-Berman 2000; Izzo & Ernst 2001; Ioannides 2002; Zhou et al 2003). The synergistic interaction effects of herbal medicines may complicate the dosing schemes of long-term non-herbal medications, because users of herbal medicines tend to have chronic conditions treated with non-herbal or pharmaceutical drugs (O'Hara et al 1998; Fugh-Berman 2000; Izzo & Ernst 2001). Sho-saiko-to has been reported to decrease the area under the concentration–time curve for prednisolone (Homma et al 1995), while little is known about its influence on other drug dispositions. The cytochrome P450 (CYP) family comprises a group of enzymes with broad substrate specificity (Rendic & Di Carlo 1997), and this substrate specificity often leads to herb-induced drug interactions with selective CYP substrates, especially those catalysed by CYP3A or CYP1A2 (Izzo & Ernst 2001; Ioannides 2002; Zhou et al 2003).

In Japan, sho-saiko-to (Chinese name: Xiao-Chai-Hu-Tang) is the most commonly prescribed herbal remedy with clinical indication approved for the treatment of various infectious disorders (e.g. chronic hepatitis, bronchitis) as an adjunctive therapy (Yamamura 1988; Hirayama et al 1989). Sho-saiko-to is indicated for the improvement of liver dysfunction due to chronic hepatitis (Yamamura et al 1988; Hirayama et al 1989; Oka et al 1995; Shimizu et al 1999; Shiota et al 2002).

Xanthine oxidase (XO) plays an important role in the metabolism of endogeneous compounds (e.g., hypoxanthine, xanthine) and drugs (e.g. 6-mercaptopurine) (Pritsos 2000). XO has been implicated as the main generator of active oxygen species and as a potential target of drug therapy in various human disorders (e.g. cardiovascular diseases) (Simmonds et al 1995; Butler et al 2000; Wattanapitayakul & Bauer 2001; Clancy et al 2001; Doehner et al 2002). XO activity has also been implicated in several liver diseases

(Battelli et al 2001; Stirpe et al 2002). However, it remains totally unknown as to whether sho-saiko-to affects XO activity in man.

In this study, we assessed the in-vivo effects of sho-saiko-to on CYP1A2, CYP3A and XO in healthy subjects. To accomplish this aim, we employed a caffeine test for assessing CYP1A2 and XO, and a urinary assay of 6β-hydroxycortisol (6β-HC) and free cortisol (FC) for assessing CYP3A.

Materials and Methods

Chemicals

Sho-saiko-to (TJ-9) was obtained from Tsumura Co. (Tokyo, Japan). Seven and a half grams of sho-saiko-to contain 4.5 g of dried extract, which is prepared from boiled water extract of seven herb components as follows: 7.0 g of Bupleurum root (Bupleurum falcatum Linne), 5.0 g of Pinellia tuber (Pinellia ternata Breitenbach), 3.0 g of Scutellaria root (Scutellaria baicalensis Georgi), 3.0 g of Jujube fruit (Zizyphus jujuba Miller), 3.0 g of Ginseng root (Panax ginseng C. A. Meyer), 2.0 g of Glycyrrhiza root (Glycyrrhiza uralensis Fisher) and 1.0 g of Ginger rhizome (Zingiber officinale Roscoe) (TSUMURA Sho-saiko-to Extract Granules for Ethical Use). 1,7-Dimethyl-uric-acid (17U), 1-methyxanthine (1X) and 1-methyl-uric-acid (1U) were purchased from Sigma Chemical Co. (St Louis, MO). 5-Acetyl-amino-6-formyla mino- 3-methyluracil (AFMU), a precursor of 5-acetylamino-6-amino-3 -methyluracil (AAMU), was purchased from Welfide Co. (Osaka, Japan). Cortisol and 6β-HC were obtained from Wako Pure Chemical Industries (Osaka, Japan) and Stabiligen (Nancy, France), respectively.

Subjects

After approval by the Institutional Review Board of Kumamoto University School of Pharmacy, 26 healthy Japanese subjects, who were all university students (12 females and 14 males; age, 23.0 ± 1.2 years; weight, 60.2 ± 12.8 kg; mean $\pm$ s.d.), gave written informed consent to participate in the study. All subjects were judged to be healthy according to their medical history and physical examination; each was required to be a non-smoker. Participants were excluded for the following reasons: allergy to sho-saiko-to, any herb, any non-herbal medicine or caffeine; history of any infectious disease within 4 weeks before enrollment; use of sho-saiko-to or other herbal medicines within 4 weeks; use of prescription or over-the-counter medications or alcohol within 2 days; use of an investigational drug within 2 months; history of alcohol or drug abuse; and current pregnancy or its suspicion.

Study design

This was an open-label, fixed-order study and each subject served as his or her own control. Throughout the study period the fixed-order, but not randomised crossover, design (i.e. caffeine test without sho-saiko-to and then with sho-saiko-to on the postdose days 1 and 5) was used. This was because this herbal medicine has several components as mentioned above and also because the pharmacokinetic parameters (e.g. elimination half-life) of each of the components remain unknown and, therefore, the dissipation time of the interaction effect(s), if any, of sho-saiko-to or the length of a washout period is considered unpredictable, when a randomised, crossover design was employed. From 48 h before the first caffeine test through the end of the study, subjects were not allowed to eat any food or beverage containing xanthines (e.g. coffee, tea, Japanese tea, cola, chocolate). Subjects were instructed to take twice-daily 2.5 g-doses of sho-saiko-to before a meal for 5 days. The caffeine test was performed three times – 2 days before (baseline), and on the first day (day 1) and fifth day (day 5) after the start of sho-saiko-to. Compliance was assessed by self-reporting of missed doses at the end of the study. Grapefruit or grapefruit juice intake was prohibited throughout the study period, because they might affect the caffeine disposition and the 6β-HC/FC ratio (Fuhr et al 1993; Seidegard et al 1998).

Caffeine test

After emptying their bladders, the subjects received an oral dose of 150 mg caffeine before they went to sleep. An overnight urine sample was collected from each subject (approximately from 2300 h to 0700 h). The mean ($\pm$ s.d.) length of time for urine collection was 8.4 ± 0.9 h. Thereafter, 1 mL of urine sample acidulated by ascorbic acid (10 mg mL^{-1}) and 10 mL of urine sample without ascorbic acid were stored at $-20\,^{\circ}$C until HPLC analyses of caffeine metabolites and of 6β-HC and FC, respectively. For the data correction, we applied spot urine samples before taking caffeine on day 1 and day 5, and spot 2300 h and accumulated from 2300 h to 0700 h samples of 3 days after dosing with sho-saiko-to in randomly selected 5 out of the 26 subjects.

Analytical procedure

Urinary concentrations of three caffeine metabolites, 17U, 1U, and 1X, were quantified by HPLC with UV detection as described previously (Saruwatari et al 2002) with a minor modification of acetate buffer (0.01 M, pH 3.8) of the mobile phase, to separate these metabolites from constituents of sho-saiko-to. After the urine sample (40 μL) was extracted with 5 mL of ethyl acetate–2-propanol (93:7 v/v), 3 mL of the organic phase was evaporated to dryness at 55 °C under a steam of nitrogen. The residue was reconstituted in 300-μL mobile phase, and a 40-μL sample was injected onto Hitachi instruments (Hitachi, Tokyo, Japan) as follows: an L-7200 autosampler, an L-7100 intelligent pump, an L-7400 UV-VIS detector and a D-7000 HPLC interface module.

Chromatography was performed at 30 °C on a Capcell Pack C18 column (25 cm $\times$ 4.6 mm i.d.; Shiseido Co., Tokyo, Japan) equilibrated with the 1% methanol and 99% acetate buffer (0.01 M, pH 3.8) at a flow rate of 1.0 mL min^{-1}, after which the chromatogram was developed

with a multistep gradient produced by applying the following changes in methanol and flow rate: 1% (1.0 mL min^{-1}, 0 min), 1% (1.5 mL min^{-1}, 10 min), 1–15% (1.7–1.55 mL min^{-1}, 10.1–40 min), 15% (1.55 mL min^{-1}, 40.1 min) and 1% (1.0 mL min^{-1}, 51 min). Ultraviolet absorbance at 273 nm was used to monitor the separation of analytes. Within- and between-day coefficients of variation for determining these 3 metabolites at 2.5 $\mu g\,mL^{-1}$ were < 5% (n = 5 each). Calibration curves for urinary 17U, 1U and 1X assays constructed over the respective concentration ranges 1–75 $\mu g\,mL^{-1}$ were linear ($r > 0.99$, $P < 0.01$).

After complete conversion of AFMU into AAMU at pH 10, the AAMU was measured by HPLC with ultraviolet detection as described previously (Saruwatari et al 2002). AAMU was eluted by a TSK-20 column (30 cm × 7.8 mm i.d.; Tosoh Inc., Tokyo, Japan) with 0.1% acetic acid containing 0.2 M $NaNO_3$ at a flow rate of 0.8 mL min^{-1} and monitored by ultraviolet absorbance at 263 nm. Within- and between-day coefficients of variation for determining AAMU at 20 $\mu g\,mL^{-1}$ were < 5% (n = 5). Calibration curves for urinary AAMU assay constructed over the concentration range 0.5–100 $\mu g\,mL^{-1}$ were linear ($r > 0.99$, $P < 0.01$).

Urinary concentrations of 6β-HC and FC were assayed by an HPLC with a ultraviolet detection according to the method of Ushiama et al (2002). For the urinary 6β-HC assay, a 1-mL urine sample was extracted twice with ethyl acetate (6 mL each). The combined organic layer was then mixed with 0.5 mL of 0.25 M sodium hydroxide, saturated with anhydrous sodium sulfate and vigorously vortexed for 5 min. After centrifuging, the organic layer was separated and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 200 μL of the mobile phase as described below, and 50 μL was injected onto the HPLC column. The mobile phase consisting of acetonitrile–water (8:92 v/v) was adjusted to pH 2.5 with 0.05% trichloracetic acid and was delivered at a flow rate of 1.0 mL min^{-1}. Urinary FC assay was performed using a method essentially similar to that employed for the 6β-HC assay, except for a greater sample volume (5 mL) and a different mobile phase (acetonitrile–water 19:81). 6β-HC and FC were eluted by a Prodigy 5μ ODS column (150 × 4.6 mm, i.d.; Phenomenex, Torrance, USA) at 244 nm. Within- and between-day coefficients of variance for determining urinary 6β-HC at 200 ng mL^{-1} were < 5% and those for FC at 20 ng mL^{-1} were < 10% (n = 6 each). Calibration curves for urinary 6β-HC and FC assay constructed over the respective concentration ranges 25–800 ng mL^{-1} and 10–100 ng mL^{-1} were linear ($r > 0.98$, $P < 0.01$).

Statistical methods

The CYP1A2, XO and CYP3A activity was assessed by use of the molar concentration ratios (AAMU + 1U + 1X)/17U, 1U/(1U + 1X) and 6β-HC/FC, respectively. Differences of the ratios between the study phases were considered statistically significant at the 5% level of probability with repeated measures analysis of variance. Bonferroni adjustment was made for multiple comparisons. These statistical analyses were performed with an SPSS software (version 11.0, SPSS Inc., Chicago, IL).

Results

All enrolled subjects completed the study protocol and reported that they had taken all dispensed study medications as directed. Sho-saiko-to was well tolerated without any adverse reactions.

The mean (±s.d.) and individual values of three drug-metabolising enzyme activities at baseline, and on day 1 and day 5 after dosing with sho-saiko-to, are shown in Figure 1. The mean activity of CYP1A2 and XO significantly differed among the three phases ($P = 0.001$ and $P < 0.0001$, respectively). The mean activity of CYP1A2 decreased significantly on day 1 and day 5 after dosing with sho-saiko-to, with the mean reduction value of 16% on both the postdose days compared with the baseline value (Figure 1A). The mean activity of XO decreased significantly, with mean reduction values of 25% on day 1 and 20% on day 5 (Figure 1B). The 5-day dosing with sho-saiko-to inhibited the mean CYP3A activity by 19% from the baseline, though not significantly ($P = 0.146$, Figure 1C).

Discussion

Sho-saiko-to contains several pharmacologically active ingredients derived from seven herbal components (Table 1). These ingredients are emerging as inhibitors of CYP1A2, 3A and XO in-vitro (Table 1). Our results showed that the in-vivo activity of CYP1A2 and XO is inhibited by sho-saiko-to, which may cause pharmacokinetic herb–drug interactions. In this study, the subjects received 2/3 of the usual dose (7.5 g daily) for only 5 days, because over 80 cases of interstitial pneumonia have been reported to come from sho-saiko-to therapy (Kawasaki et al 1994; Ishizaki et al 1996).

As a safe and non-invasive test, urinary caffeine metabolic ratios are widely used for assessing CYP1A2 and XO activity in drug–drug or food–drug interaction screening surveys (Kashuba et al 1998; Fuchs et al 1999; Lampe et al 2000). Fluvoxamine and apiaceous vegetables are reported to decrease the caffeine metabolic ratio for CYP1A2, without affecting the ratio for XO (Kashuba et al 1998; Lampe et al 2000). On the other hand, allopurinol decreases the ratio for XO and increases the ratio for CYP1A2 (Fuchs et al 1999). These results support the clinical significance of our observation that sho-saiko-to affects the metabolic ratios for both CYP1A2 and XO, leading to a possible interaction with drugs metabolised by CYP1A2 (e.g. theophylline, clozapine) or XO (e.g. 6-mercaptopurine). The 1U/(1U + 1X) ratio has been widely used as a measure of XO activity. Although several equations have been proposed for assessing CYP1A2 activity by a caffeine test, we employed an equation (AAMU + 1U + 1X)/17U, which has been suggested to be the best CYP1A2 index among urinary caffeine metabolic ratios (Streetman et al 2000).

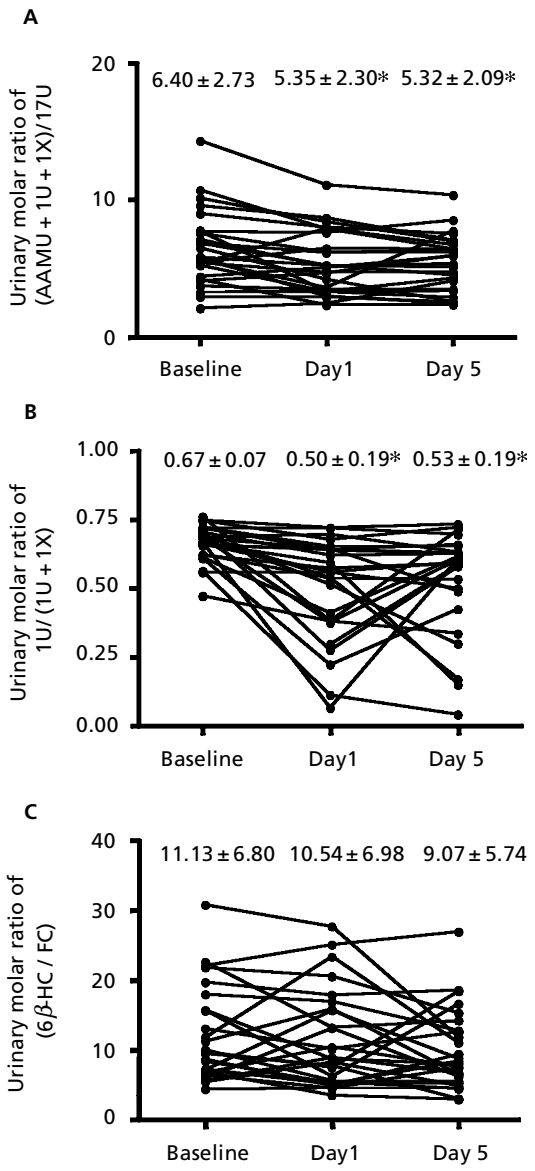

Figure 1 Individual values of the urinary molar concentration ratios of (AAMU + 1U + 1X)/17U for CYP1A2 (A), 1U/(1U + 1X) for XO (B) and 6β-HC/FC for CYP3A (C) activity in 26 healthy subjects before (baseline), on the first day (day 1) and on the fifth day (day 5) after dosing with sho-saiko-to. Results are expressed as mean ± s.d. *P < 0.05 compared with the respective baseline values after correction for multiple comparisons (Bonferroni adjustment). AAMU, 5-Acetylamino-6-amino-3-methyluracil; 1U, 1-methyl-uric-acid; 1X, 1-methyxanthine; 17U, 1,7-dimethyl-uric-acid; 6β-HC, 6β-hydroxycortisol; FC, free cortisol; CYP, cytochrome P450; and XO, xanthine oxidase.

Sho-saiko-to contains Ginseng root (Table 1), one of the most popular herbs in the world (O'Hara et al 1998). A recent study with healthy subjects has reported that the oral intake of Panax ginseng extract (500 mg daily) for 28 days did not decrease the CYP1A2 activity in-vivo (Gurley et al 2002), despite the dose of Ginseng extract being higher than that (i.e. 375 mg) contained in sho-saiko-to of the present study (TSUMURA Sho-saiko-to Extract Granules for Ethical Use). Because three flavonoids of Scutellaria root or unknown ingredient(s) of Ginseng root have been reported to inhibit the CYP1A2 activity in human liver microsomes (Table 1), the inhibitory effect of sho-saiko-to on the CYP1A2 activity shown in our study may come from those active ingredients (Kim et al 2002) or the unknown ingredient(s) (Chang et al 2002). However, there is, to our knowledge, no report concerning the in-vivo interaction between sho-saiko-to and CYP1A2 substrates in man. Further studies are definitely required to elucidate the clinical significance of sho-saiko-to as a candidate CYP1A2 inhibitor.

Some ingredients contained in sho-saiko-to were reported to inhibit CYP3A4 activity in-vitro (Table 1) (Henderson et al 1999; Kent et al 2002; Kim et al 2002). In addition, extract of Glycyrrhiza root (Glycyrrhiza glabra) is known to inhibit CYP3A4 activity in-vitro (Budzinski et al 2000). The urinary ratio of 6β-HC/FC has been used as a non-invasive measure of CYP3A (Bienvenu et al 1991; Ohno et al 2000; Streetman et al 2000; Ushiama et al 2002), and the ratio obtained from the 24-h or the first morning spot urine collection is preferred for assessing the CYP3A activity (Ohno et al 2000; Streetman et al 2000). In this study, therefore, all subjects were asked to collect overnight urine samples. The mean 6β-HC/FC ratio tended to be lower on the postdose day 5 with sho-saiko-to than the baseline value, although the difference was not statistically significant. Sho-saiko-to has been reported to increase the conversion of cortisol to cortisone and prednisolone to prednisone, which are mediated by 11β-hydroxysteroid dehydrogenase (Homma et al 1995). However, whether this mechanism might have influenced the non-significant reduction (i.e. 19%) of the 6β-HC/FC ratio observed on day 5 remains obscure from our data.

We performed a power analysis to estimate the ability to detect significant distances between the smallest and largest of the mean values (Cohen 1969). Our analysis for the CYP3A activity obtained with at least 80% β-power at 5% α-level of significance showed that we had to detect a 53% difference among the 3 phases. For the difference in the CYP3A activity to be statistically significant ($\alpha = 0.05$ and $\beta = 0.2$), 211 subjects would be needed (Cohen 1969). Therefore, larger numbers of the sample size would be required to elucidate the lack of significant difference in the CYP3A activity obtained from our study to avoid the type II error.

We observed statistically significant reduction in the XO activity on day 1 and day 5. Baicalein, liquiritigenin and isoliquiritigenin contained in sho-saiko-to have been reported to inhibit XO in-vitro (Table 1) (Kong et al 2000; Shieh et al 2000). However, our data on the change in the XO activity showed an inter-individual variability: in the 7 subjects the mean XO activity decreased to 45.7% of the baseline on day 1, that was considerably lower than that (85.6% of the baseline) in the remaining 19 subjects. Nevertheless, in the former 7 subjects the mean XO activity on day 1 was recovered up to 93.1% of the baseline on day 5. It has been reported that sho-saiko-to increased the levels of tumour necrosis factor-α, interferon-γ or interleukin-6 (Ohtake et al 2000; Huang et al 2001), and those cytokines increase the XO activity (Pritsos 2000). Further studies are

Table 1 Herbal components and major active ingredients of sho-saiko-to, and the in-vitro effects on CYP1A2, CYP3A and XO activity reported in the literature.

Herbal components	Species	Main active ingredients	Effects of active ingredients on enzyme activity (species)	References
Bupleurum root	Bupleurum falcatum Linne	Saikosaponin-d		
Pinellia tuber	Pinellia ternata Breitenbach	Adenine		
Scutellaria root	Scutellaria baicalensis Georgi	Baicalin		
		Baicalein	CYP1A2 ↓ (man)	Kim et al 2002
			CYP3A ↓ (man)	Kim et al 2002
			XO ↓ (unknown)	Shieh et al 2000
		Wogonin	CYP1A2 ↓ (man)	Kim et al 2002
		Oroxylin A	CYP1A2 ↓ (man)	Kim et al 2002
Jujube fruit	Zizyphus jujuba Miller	Cyclic AMP		
Ginseng root	Panax ginseng C. A. Meyer	Ginsenoside Rd	CYP3A4 ↓ (man)	Henderson et al 1999
		Ginsenoside Rb1		
		Ginsenoside Rg1		
Glycyrrhiza root	Glycyrrhiza uralensis Fisher	Glycyrrhizin		
		Glabridin	CYP3A ↓ (man)	Kent et al 2002
		Liquiritigenin	XO ↓ (bovine)	Kong et al 2000
		Isoliquiritigenin	XO ↓ (bovine)	Kong et al 2000
		Liquiritin		
		Glycyrrhetic acid		
Ginger rhizome	Zingiber officinale Roscoe	6-Gingerol		
		6-Shogaol		
		Zingerone		

↓, Inhibition; CYP, cytochrome P450; XO, xanthine oxidase.

obviously required to assess whether the change in those cytokines by this herbal medicine might occur to an inter-individually different extent.

Sho-saiko-to is known to improve liver function in chronic hepatitis (Hirayama et al 1989; Shimizu et al 1999; Shiota et al 2002). The serum XO concentrations in patients with hepatitis and cirrhosis were significantly higher than in healthy subjects (Battelli et al 2001), and the XO activity in the livers from patients with virus-related cirrhosis was higher than that in the normal livers (Stirpe et al 2002). These findings, taken together, suggest that the enhanced XO activity status would promote liver injury by an increased production of reactive oxygen species by XO. Therefore, the inhibitory effect of sho-saiko-to on the XO activity observed in this study may offer a likely explanation for the protective action against liver injury by sho-saiko-to. In addition, XO has been implicated as a potential target of drug therapy in non-hepatic human disorders (e.g. post-ischaemic reperfusion tissue injury, cardiovascular diseases) (Simmonds et al 1995; Butler et al 2000; Clancy et al 2001; Wattanapitayakul & Bauer 2001; Doehner et al 2002). However, to our knowledge, whether sho-saiko-to offers protection in these non-hepatic disorders remains totally unknown.

Conclusion

We demonstrated that sho-saiko-to appeared to affect CYP1A2 and XO activity with certain inter-individual variabilities in the changes in the enzymatic activity. Further in-vivo studies are obviously required in man to assess whether sho-saiko-to would interact with drugs metabolised by CYP1A2 or XO to a clinically significant extent. In addition, based upon our findings, we postulated that sho-saiko-to may protect against hepatocellular damage mediated, at least in part, via an inhibition of XO activity.

References

Battelli, M. G., Musiani, S., Valgimigli, M., Gramantieri, L., Tomassoni, F., Bolondi, L., Stirpe, F. (2001) Serum xanthine oxidase in human liver disease. Am. J. Gastroenterol. **96**: 1194–1199

Bienvenu, T., Rey, E., Pons, G., d'Athis, P., Olive, G. (1991) A simple non-invasive procedure for the investigation of cytochrome P-450 IIIA dependent enzymes in humans. Int. J. Clin. Pharmacol. Ther. Toxicol. **29**: 441–445

Budzinski J. W., Foster B. C., Vandenhoek S., Arnason J. T. (2000) An in-vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. Phytomedicine **7**: 273–282

Butler, R., Morris, A. D., Belch, J. J., Hill, A., Struthers, A. D. (2000) Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension **35**: 746–751

Chang, T. K., Chen, J., Benetton, S. A. (2002) In vitro effect of standardized ginseng extracts and individual ginsenosides on

the catalytic activity of human CYP1A1, CYP1A2, and CYP1B1. Drug Metab. Dispos. **30**: 378–384

Clancy, R. R., McGaurn, S. A., Goin, J. E., Hirtz, D. G., Norwood, W. I., Gaynor, J. W., Jacobs, M. L., Wernovsky, G., Mahle, W. T., Murphy, J. D., Nicolson, S. C., Steven, J. M., Spray, T. L. (2001) Allopurinol neurocardiac protection trial in infants undergoing heart surgery using deep hypothermic circulatory arrest. Pediatrics **108**: 61–70

Cohen, J. (1969) F tests on means in the analysis of variance and covariance. In: Cohen, J. (ed.) Statistical power analysis for the behavioral sciences. 1st edn, Academic Press, New York, pp 266–397

Doehner, W., Schoene, N., Rauchhaus, M., Leyva-Leon, F., Pavitt, D. V., Reaveley, D. A., Schuler, G., Coats, A. J., Anker, S. D., Hambrecht, R. (2002) Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies. Circulation **105**: 2619–2624

Fuchs, P., Haefeli, W. E., Ledermann, H. R., Wenk, M. (1999) Xanthine oxidase inhibition by allopurinol affects the reliability of urinary caffeine metabolic ratios as markers for N-acetyltransferase 2 and CYP1A2 activities. Eur. J. Clin. Pharmacol. **54**: 869–876

Fugh-Berman, A. (2000) Herb–drug interactions. Lancet **355**: 134–138

Fuhr, U., Klittich, K., Staib, A. H. (1993) Inhibitory effect of grapefruit juice and its bitter principal, naringenin, on CYP1A2 dependent metabolism of caffeine in man. Br. J. Clin. Pharmacol. **35**: 431–436

Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B., Cui, Y., Ang, C. Y. (2002) Cytochrome P450 phenotypic ratios for predicting herb–drug interactions in humans. Clin. Pharmacol. Ther. **72**: 276–287

Henderson, G. L., Harkey, M. R., Gershwin, M. E., Hackman, R. M., Stern, J. S., Stresser, D. M. (1999) Effects of ginseng components on c-DNA-expressed cytochrome P450 enzyme catalytic activity. Life Sci. **65**: PL209–PL214

Hirayama, C., Okumura, M., Tanikawa, K., Yano, M., Mizuta, M., Ogawa, N. (1989) A multicenter randomized controlled clinical trial of Shosaiko-to in chronic active hepatitis. Gastroenterol. Jpn. **24**: 715–719

Homma, M., Oka, K., Ikeshima, K., Takahashi, N., Niitsuma, T., Fukuda, T., Itoh, H. (1995) Different effects of traditional Chinese medicines containing similar herbal constituents on prednisolone pharmacokinetics. J. Pharm. Pharmacol. **47**: 687–692

Huang, X. X., Yamashiki, M., Nakatani, K., Nobori, T., Mase, A. (2001) Semi-quantitative analysis of cytokine mRNA expression induced by the herbal medicine Sho-saiko-to (TJ-9) using a Gel Doc system. J. Clin. Lab. Anal. **15**: 199–209

Ioannides, C. (2002) Pharmacokinetic interactions between herbal remedies and medicinal drugs. Xenobiotica **32**: 451–478

Ishizaki, T., Sasaki, F., Ameshima, S., Shiozaki, K., Takahashi, H., Abe, Y., Ito, S., Kuriyama, M., Nakai, T., Kitagawa, M. (1996) Pneumonitis during interferon and/or herbal drug therapy in patients with chronic active hepatitis. Eur. Respir. J. **9**: 2691–2696

Izzo, A. A., Ernst, E. (2001) Interactions between herbal medicines and prescribed drugs: a systematic review. Drugs **61**: 2163–2175

Kashuba, A. D., Nafziger, A. N., Kearns, G. L., Leeder, J. S., Gotschall, R., Rocci, M. L., Kulawy, R. W., Beck, D. J., Bertino, J. S. (1998) Effect of fluvoxamine therapy on the activities of CYP1A2, CYP2D6, and CYP3A as determined by phenotyping. Clin. Pharmacol. Ther. **64**: 257–268

Kawasaki, A., Mizushima, Y., Kunitani, H., Kitagawa, M., Kobayashi, M. (1994) A useful diagnostic method for drug-induced pneumonitis: a case report. Am. J. Chin. Med. **22**: 329–336

Kent, U. M., Aviram, M., Rosenblat, M., Hollenberg, P. F. (2002) The licorice root derived isoflavan glabridin inhibits the activities of human cytochrome P450S 3A4, 2B6, and 2C9. Drug Metab. Dispos. **30**: 709–715

Kim, J. Y., Lee, S., Kim, D. H., Kim, B. R., Park, R., Lee, B. M. (2002) Effects of flavonoids isolated from Scutellariae radix on cytochrome P-450 activities in human liver microsomes. J. Toxicol. Environ. Health A **65**: 373–381

Kong, L. D., Zhang, Y., Pan, X., Tan, R. X., Cheng, C. H. (2000) Inhibition of xanthine oxidase by liquiritigenin and isoliquiritigenin isolated from Sinofranchetia chinensis. Cell. Mol. Life Sci. **57**: 500–505

Lampe, J. W., King, I. B., Li, S., Grate, M. T., Barale, K. V., Chen, C., Feng, Z., Potter, J. D. (2000) Brassica vegetables increase and apiaceous vegetables decrease cytochrome P450 1A2 activity in humans: changes in caffeine metabolite ratios in response to controlled vegetable diets. Carcinogenesis **21**: 1157–1162

O'Hara, M., Kiefer, D., Farrell, K., Kemper, K. (1998) A review of 12 commonly used medicinal herbs. Arch. Fam. Med. **7**: 523–536

Ohno, M., Yamaguchi, I., Ito, T., Saiki, K., Yamamoto, I., Azuma, J. (2000) Circadian variation of the urinary 6β-hydroxycortisol to cortisol ratio that would reflect hepatic CYP3A activity. Eur. J. Clin. Pharmacol. **55**: 861–865

Ohtake, N., Suzuki, R., Daikuhara, H., Nakai, Y., Yamamoto, M., Amagaya, S., Ishige, A., Sasaki, H., Komatsu, Y., Fukuda, K., Hayashi, S. (2000) Modulation of lung local immune responses by oral administration of a herbal medicine Sho-saiko-to. Int. J. Immunopharmacol. **22**: 419–430

Oka, H., Yamamoto, S., Kuroki, T., Harihara, S., Marumo, T., Kim, S. R., Monna, T., Kobayashi, K., Tango, T. (1995) Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). Cancer **76**: 743–749

Pritsos, C. A. (2000) Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. Chem. Biol. Interact. **129**: 195–208

Rendic, S., Di. Carlo, F. J. (1997) Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab. Rev. **29**: 413–580

Saruwatari, J., Nakagawa, K., Shindo, J., Tajiri, T., Fujieda, M., Yamazaki, H., Kamataki, T., Ishizaki, T. (2002) A population phenotyping study of three drug-metabolizing enzymes in Kyushu, Japan, with use of the caffeine test. Clin. Pharmacol. Ther. **72**: 200–208

Seidegard, J., Dahlstrom, K., Kullberg, A. (1998) Effect of grapefruit juice on urinary 6β-hydroxycortisol/cortisol excretion. Clin. Exp. Pharmacol. Physiol. **25**: 379–381

Shieh, D. E., Liu, L. T., Lin, C. C. (2000) Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. Anticancer. Res. **20**: 2861–2865

Shimizu, I., Ma, Y. R., Mizobuchi, Y., Liu, F., Miura, T., Nakai, Y., Yasuda, M., Shiba, M., Horie, T., Amagaya, S., Kawada, N., Hori, H., Ito, S. (1999) Effects of Sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. Hepatology **29**: 149–160

Shiota, G., Maeta, Y., Mukoyama, T., Yanagidani, A., Udagawa, A., Oyama, K., Yashima, K., Kishimoto, Y., Nakai, Y., Miura, T., Ito, H., Murawaki, Y., Kawasaki, H. (2002) Effects of Sho-Saiko-to on hepatocarcinogenesis and 8-hydroxy-2′-deoxyguanosine formation. Hepatology **35**: 1125–1133

Simmonds, H. A., Sebastian, R., Nishino, T. (1995) Hereditary xanthinuria. In: Sciver, C. R., Eaudet, A. L., Sly, W. S., Valle, D. (eds) The metabolic and molecular basis of inherited disease. 7th edn, McGraw-Hill, New York, pp 1781–1797

Stirpe, F., Ravaioli, M., Battelli, M. G., Musiani, S., Grazi, G. L. (2002) Xanthine oxidoreductase activity in human liver disease. Am. J. Gastroenterol. **97**: 2079–2085

Streetman, D. S., Bertino, J. S., Nafziger, A. N. (2000) Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. Pharmacogenetics **10**: 187–216

Ushiama, H., Echizen, H., Nachi, S., Ohnishi, A. (2002) Dose-dependent inhibition of CYP3A activity by clarithromycin during Helicobacter pylori eradication therapy assessed by changes in plasma lansoprazole levels and partial cortisol clearance to 6β-hydroxycortisol. Clin. Pharmacol. Ther. **72**: 33–43

Wattanapitayakul, S. K., Bauer, J. A. (2001) Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. Pharmacol. Ther. **89**: 187–206

Yamamura, Y. (1988) The history of Kampo medicine and its development in modern Japan. In: Hosoya, E., Yamamura, Y. (eds) Recent advances in the pharmacology of Kampo (Japanese herbal) medicines. Excerpta Medica, Tokyo, pp 3–13

Zhou, S., Gao, Y., Jiang, W., Huang, M., Xu, A., Paxton, J. W. (2003) Interactions of herbs with cytochrome P450. Drug Metab. Rev. **35**: 35–98