Does a Kampo Medicine Containing Schisandra Fruit Affect Pharmacokinetics of Nifedipine Like Grapefruit Juice?

Toshiaki MAKINO,* Fumika MIZUNO, and Hajime MIZUKAMI

Department of Pharmacognosy, Graduate School of Nagoya City University; 3–1 Tanabe-Dori, Mizuho-ku, Nagoya 467–8603, Japan. Received June 18, 2006; accepted July 28, 2006; published online August 7, 2006

Herb–drug interaction has attracted attention as medicinal topics recently. However, the drug information is sometimes confusing. Previous in vitro studies revealed that schisandra fruit had strong inhibitory effect on CYP3A4 and claimed the possibilities of its herb–drug interaction. In the present study, we evaluated the inhibitory effects of schisandra fruit and shoseiryuto, an herbal formula in Japanese traditional kampo medicine containing eight herbal medicines including schisandra fruit, on rat CYP3A activity in vitro, and the effect of shoseiryuto on pharmacokinetics of nifedipine in rats, in comparison with those of grapefruit juice, a well-characterized natural CYP3A inhibitor. Shoseiryuto and its herbal constituents, schisandra fruit, ephedra herb and cinnamon bark exhibited in vitro inhibitory effect of CYP3A. Although shoseiryuto inhibited rat CYP3A activity in vitro with a degree comparable to grapefruit juice, shoseiryuto did not significantly affect a plasma concentration profile of nifedipine in rats as grapefruit juice did. These results indicate that in vivo experiments using the extract of herbal medicine prepared with the same dosage form as patients take are necessary to provide proper information about herb–drug interaction.

Key words pharmacokinetic interaction; cytochrome P450; Schisandra chinensis; shoseiryuto; nifedipine

Rapid developments in medicinal treatment have resulted in the declined mortality rate in industrialized countries, while the number of patients suffering from particular chronic diseases such as hypertension, diabetes, or allergic diseases has been increasing. Since most of the modern medicines are allopathic and cannot cure chronic diseases, and since they sometimes have the possibility of side effects, many patients choose to explore complementary/alternative medicines, including herbal medicines in particular.1 In Japan, kampo medicine (Japanese traditional herbal medicine) has been attracted as an alternative source of medicinal treatments for chronic diseases, having the potential to treat patients holistically by supporting the patient’s own healing power.2 Since kampo medicine and modern medicine have their own characteristics, it is desirable that these medicines should be combined, making up for defects of each other and expecting their synergistic action.

As the combined therapy using both modern and traditional herbal medicines is getting popular, the possibilities of the interaction between them have been described.3–5 Since Bailey et al. first found that grapefruit juice augments the blood concentration of nifedipine in clinical study,6 a number of reports claiming that herbal medicine may disturb usual medicinal treatments have been published.7,8 Among these reports, some insisted the possible dangerousness of herbal medicines based on their in vitro inhibitory effects with high concentration on drug metabolizing enzymes including cytochrome P450 (CYP). Furthermore, the assays were sometimes carried out by using methanol extracts of herbal medicines.9–11 However, these approaches have some limitations because (1) most of herbal medicines are used as the extract with boiled water and the solubilized compounds in the decoctions are much different from those extracted or dissolved in organic solvent, (2) most of herbal medicines are administered by oral route, and (3) some of the compounds in herbal extracts are absorbed from intestine to circulation after metabolized by intestinal bacteria, e.g. glycosides are hydrolyzed to give aglycones.12 In order to provide correct drug information about herb–drug interaction to physicians and pharmacists, it is necessary to use the same dosage form of herbal medicine as patients take, and to conduct in vivo experiments in which the decoctions of herbal medicines are orally administered.

Recently, Iwata et al. found that methanol fraction of the water extract of schisandra fruit (五味子) has strong inhibitory effect on human liver microsomal CYP3A4 and CYP2D6 among 78 herbal medicine in vitro,9,10 and identified the active ingredients in the alkaloid fraction of the decoction.13 Shoseiryuto (小青竜湯) contains schisandra fruit as one of eight herbal medicines, and is frequently used to treat common cold with nasal drip, allergic rhinitis and pollinosis in Japan.14,15 Kimura et al. evaluated in vitro inhibitory effect of shoseiryuto on rat CYP3A, and they found that the active herbal ingredients were not only schisandra fruit but also ephedra herb (麻黄) and cinnamon bark (桂皮).16 However, in their in vitro study, the concentrations of shoseiryuto and each herbal constituent were much higher than their clinical usage. Therefore, more accurate drug information is required to use shoseiryuto safely in clinics or pharmacies. In this study, we compared the inhibitory effects of shoseiryuto on rat CYP3A in vitro and in vivo with those of grapefruit juice, a well-characterized natural CYP3A inhibitor studied in patients and experimental animals.6,17–19

MATERIALS AND METHODS

Herbal Medicines All herbal medicines used in this study are registered in Japanese Pharmacopoeia VX20 and were purchased from Tsumura Co. Ltd. (Tokyo). The compositions of shoseiryuto are shown in Table 1. Each herbal medicine (20 g) or the mixture of herbal medicines constituting shoseiryuto (total 24 g) was boiled in 20-times weight of distilled water for 30—40 min by the volume of filtrate being approximately half of the original volume of water, as standardized by Health, Labor and Welfare Ministry, Japan.21 The decoctions were lyophilized, and kept in room tempera-
tature under desiccated condition until use. The yields of the extract from herbal medicines are shown in Table 1. These dried powder was used as an herbal medicine or shoseiryuto in the present study.

**Grapefruit Juice** A commercial grapefruit juice at an original concentration was used. Three different types of grapefruit juice (sample #1, Kirin Tropicana®, Koiiwai Nyugyo, Tokyo; sample #2, Dole®, Japan Milk Community, Tokyo; sample #3, Sunkist®, Morinaga Nyugyo, Tokyo) were purchased at local stores in Nagoya, Japan, and their inhibition effects on CYP3A were evaluated by the method mentioned below. Three samples of grapefruit juice significantly inhibited the 6β-hydroxylation of testosterone at one-tenth of their original concentration (relative activity; sample #1, 40.1 ± 3.7, p < 0.01; sample #2, 47.4 ± 3.5, p < 0.01; sample #3, 46.2 ± 2.2, p < 0.01; %). Since the sample #1 exhibited the strongest inhibitory effect on CYP3A, we used this juice as a positive control for CYP3A inhibitor. The dried weight of this juice (1 ml) was 100 μg, and the IC₅₀ for 6β-hydroxylation of testosterone was 7.65 μM/mL.

**In Vitro Study** An eight-week-old male rat (Japan SLC, Hamamatsu) was sacrificed under excessive anesthesia by ether. The experiments were handled in accordance with the Guiding Principles for the Care and Use of Experimental Animals of Graduate School of Nagoya City University. After perfusion with 90 ml of 1.15% KCl solution, the livers were excised and homogenized. The liver homogenate was centrifuged at 9000 × g for 20 min, and the supernatant was ultracentrifuged at 105000 × g for 60 min. The resulting microsomal pellets were suspended with phosphate buffered saline (PBS, 50 mM, pH 7.4) and stored at −80°C until use. The preparation mentioned above was carried out at 4°C. The protein concentration of this microsomal solution was measured according to the Bradford’s method.22 Fifty microliters of the sample solution, 160 μl of MgCl₂ (15 mM), 50 μl of glucose-6-phosphate (G6P) dehydrogenase (10 U/mL, Oriental Yeast, Tokyo), 25.6 μl of NADP⁺ (5 mM, Oriental Yeast), 25.6 μl of G6P disodium (50 mM, Nacalai Tesque, Tokyo), 164.8 μl of PBS, 4 μl of testosterone (25 mM, Nacalai) and 20 μl of rat microsome (protein 12.5 mg/mL) were mixed vigorously, and incubated at 37°C. After 20 min, the reaction was stopped by the addition of 4 ml of dichloromethane and 100 μl of n-butyl p-hydroxybenzoic acid (BHB, 1 mM, Nacalai) as an internal standard into the reactant, and mixed vigorously. After centrifugation (400 × g, 5 min), the lower layer was collected, dried up, and re-dissolved in 0.2 ml of 60% MeOH. The content of 6β-hydroxytestosterone was quantified by a HPLC system (LC-10A, Shimadzu, Kyoto, Japan) with UV detector (SPD-10A, Shimadzu) as following conditions: column, Inertilgods ODS-2 (4.6 × 250 mm), GL Science, Tokyo; mobile phase, H₂O/MeOH 40 : 60; flow rate, 1.0 ml/min; column-temperature, 40°C; detector, absorbance at 242 nm; injection volume of the sample, 20 μl; retention times, testosterone, 17.9 min; 6β-hydroxytestosterone, 6.2 min; BHB, 15.8 min. The standard of 6β-hydroxytestosterone was purchased from Sigma Aldrich (St. Louis, MO, U.S.A.) and linear regression of the concentration range of 0.39—6.25 mM of 6β-hydroxytestosterone by the peak-area ratio of 6β-hydroxytestosterone to BHB was calibrated with the least-squares method (r²=0.999). Data were expressed as relative activity (%), that was the ratio of the content of 6β-testosterone incubated with the sample to that incubated without the sample. The assay was conducted in triplicate, and the mean ± S.D. was exhibited. The 50% inhibitory concentration (IC₅₀) was determined by logistic regression analysis using Microsoft Excel.²³

**In Vivo Study** Nine-week-old male rats (Japan SLC) were anesthetized with intraperitoneal injection of ketamine chloride (80 mg/kg, Sankyo, Tokyo) and xylazine (8 mg/kg, Wako Pure Chemicals, Osaka). The jugular vein was cannulated with a PE-50 tubing (Becton Dickinson Co., Sparks, MD, U.S.A.) to collect blood samples with an established heparin-lock using 100 U/ml heparin in saline. Eighteen hours after the surgery, nifedipine (Wako) was administered orally at doses of 2 mg/10 ml/kg. Then, blood samples (0.3 ml) were chronologically collected from the cannula. Shoseiryuto (50 mg/10 ml/kg), grapefruit juice (10 ml/kg) or the vehicle was orally administered 30 min before nifedipine treatment (n = 6). These dosages were selected based on the first report that 500 ml of grapefruit juice interacted with calcium antagonists in clinical study.²⁴ To compare the inhibitory effects of shoseiryuto with grapefruit juice, the general dosage of herbal medicine (about 50 mg/kg) were dissolved in water with the same volume of grapefruit juice.

One hundred and fifty microliters of plasma sample, 100 μl of BHB (50 mM in EtOH) as internal standard, 4 ml of ether were mixed vigorously, and centrifuged at 300 × g for 5 min. The supernatant was collected, dried up, and dissolved in 0.2 ml of 60% MeOH. HPLC-conditions were as follows: column, Inertilgods ODS-3, 4.6 × 150 mm (GL Science); mobile phase, H₂O/MeOH 40 : 60; flow rate, 1.0 ml/min; column-temperature, 40°C; detector, absorbance at 238 nm; injection volume of the sample, 25 μl; retention times, nifedipine, 7.8 min and BHB, 12.3 min. For standards, nifedipine was dissolved in normal rat plasma, and the sample was prepared as mentioned above. Linear regression at the concentration range of 0.0313—8.00 μM/g of nifedipine by the peak-area ratio of nifedipine to BHB was calibrated with the least-squares method (r²=0.999). The coefficients of intra-day and inter-day variation of the assay were less than 3.5 and 5%. The plasma concentration-time data of orally administered nifedipine of each rat was assessed by non-compartment analysis using MOMENT (Microsoft Excel) based on the moment analytic method,²⁵ and pharmacokinetic parameters including AUC, mean residence time (MRT) and

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Table 1. The Composition of Herbal Medicines* in Shoseiryuto

<table>
<thead>
<tr>
<th>Herbal Medicine</th>
<th>Weight (g)</th>
<th>Ratio of yield (%)</th>
</tr>
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<tbody>
<tr>
<td>Ephedra herb</td>
<td>3.0</td>
<td>12.9</td>
</tr>
<tr>
<td>Cinnamon bark</td>
<td>3.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Peony root</td>
<td>3.0</td>
<td>22.6</td>
</tr>
<tr>
<td>Processed ginger</td>
<td>3.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Asiasaurum root</td>
<td>3.0</td>
<td>12.8</td>
</tr>
<tr>
<td>Pinellia tuber</td>
<td>6.0</td>
<td>10.1</td>
</tr>
<tr>
<td>Glycyrrhiza</td>
<td>3.0</td>
<td>25.9</td>
</tr>
<tr>
<td>Schisandra fruit</td>
<td>3.0</td>
<td>25.2</td>
</tr>
<tr>
<td>Shoseiryuto</td>
<td>Mixt. of above</td>
<td>18.5</td>
</tr>
</tbody>
</table>

* Registered in Japanese Pharmacopoeia VX.²⁰  \(^{b)} \) Weight of each herbal medicine in a dairy dosage of shoseiryuto. c) The decoctions were prepared according to Materials and Methods, and ratio of yield means % of dried weight of the decoction to the original herbal medicine.
half life \((t_{1/2})\) were obtained. The peak plasma concentration \((C_{\text{max}})\) and the time to reach \(C_{\text{max}} (T_{\text{max}})\) of orally administered nifedipine were obtained from the actual data observed after oral administration.

**Statistical Analysis** The statistical analysis was conducted using Student’s or Welch’s \(t\)-test for the differences between 2 groups, and using one-way ANOVA followed by Bonferroni’s multiple analysis for the differences among multiple groups by the computer software “Statcel2”.24) A difference of \(p<0.05\) was considered statistically significant.

**RESULTS**

We compared the inhibitory effect of shoseiryuto, a kampo medicine containing schisandra fruit, with that of grapefruit juice on the activity of CYP3A in vitro. As shown in Fig. 1, shoseiryuto significantly \((p<0.05)\) inhibited 6β-hydroxylation of testosterone in a dose dependent manner, and its \(IC_{50}\) was 52.6 \(\mu\)g/ml, that were higher than that of grapefruit juice \((7.65 \mu\text{g/ml})\). Next, we prepared the decoctions of each herbal medicine prescribed in shoseiryuto, and evaluated the inhibitory effect of an herbal medicine on CYP3A. Among eight herbal medicines, peony root, processed ginger, asiasarum root, pinellia tuber or glycyrrhiza did not show any inhibitory effect on CYP3A at the dosage corresponded to 1mg/ml of shoseiryuto (data not shown). The water extract of schisandra fruit, ephedra herb and cinnamon bark at the concentration corresponded to 1mg/ml of shoseiryuto (data not shown). The water extract of schisandra fruit, ephedra herb and cinnamon bark at the concentration corresponded to 1mg/ml of shoseiryuto significantly inhibited CYP3A with the relative activity of 51.8, 15.1, and 11.1%, respectively. As shown in Fig. 2, the extract of schisandra fruit significantly \((p<0.05)\) inhibited 6β-hydroxylation of testosterone in a dose dependent manner, and the \(IC_{50}\) of the extract was 123 \(\mu\)g/ml, that was higher than that of shoseiryuto. The extract of ephedra herb and cinnamon bark dose-dependently inhibited 6β-hydroxylation of testosterone with statistically significance \((p<0.01)\) and with \(IC_{50}\) of 64.1 and 52.2 \(\mu\)g/ml, respectively.

Then, we conducted in vivo experiments using rat in order to examine the compatibility between in vitro and in vivo studies. Figure 3 exhibits the profiles of plasma nifedipine concentration in shoseiryuto- or grapefruit juice-treated rats, and their pharmacokinetic parameters are shown in Table 2. The grapefruit juice significantly \((p<0.01)\) augmented the

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**Fig. 1. Effect of Shoseiryuto on Rat CYP3A in Vitro**

CYP3A activity was evaluated as the activity of 6β-hydroxylation on testosterone of rat liver microsomes. Data were represented as means±S.D. \((n=3)\). GFJ, grapefruit juice \((1 \text{mg/ml})\) used as a positive control. *\(p<0.05\), **\(p<0.01\) compared with control.

**Fig. 2. Effect of Schisandra Fruit (A), Ephedra Herb (B) and Cinnamon Bark (C) Extract on Rat CYP3A in Vitro**

CYP3A activity was evaluated as the activity of 6β-hydroxylation on testosterone of rat liver microsomes. Data were represented as means±S.D. \((n=3)\). Extracts of herbal medicines were prepared as described in Materials and Methods. GFJ, grapefruit juice \((1 \text{mg/ml})\) used as a positive control. *\(p<0.05\), **\(p<0.01\) compared with control.

**Fig. 3. Plasma Concentration Profile of Nifedipine in the Rats Orally Treated with Shoseiryuto**

Each point represents the mean±S.E. of 6 rats. Shoseiryuto \((50 \text{mg/kg})\), grapefruit juice \((10 \text{ml/kg})\), or the vehicle was orally administered 30 min before nifedipine treatment \((2 \text{mg/kg})\). Open circle, control; closed square, shoseiryuto-treated rat; closed triangle, grapefruit juice-treated rat. *\(p<0.05\), **\(p<0.01\) compared with control.
plasma concentration of nifedipine at a phase of absorption (5—30 min after the administration). The concentration of nifedipine in the shoseiryuto-treated rats were slightly higher than that of control, however, it was lower than expected from *in vitro* studies and the increase was not statistically significant.

**DISCUSSION**

In the present study, we revealed the inhibitory effect of shoseiryuto and its herbal components on rat CYP3A *in vitro*. In Japanese clinics or pharmacies, shoseiryuto is usually used as an extracted powder (ca. 5 g/d) of the water extract prepared from the original herbal medicines shown in Table 1, and was used in two to three portions in a day. Therefore, single dosage of shoseiryuto in patients is 1.7—2.5 g as extracted powder. If a patient takes powdered drug using a cup of water, the concentration of shoseiryuto is about 8.5—12.5 mg/ml. The IC$_{50}$ of shoseiryuto on *in vitro* CYP3A activity was 52.6 μg/ml, that was higher than that of grapefruit juice (7.65 μg/ml). However, the original concentration of shoseiryuto was 10 times higher than that of grapefruit juice used in this study (1.0 mg/ml). Considered with the difference of their original concentration, the inhibitory effect of shoseiryuto could be highly comparable with that of grapefruit juice, a natural CYP3A inhibitor well-characterized by clinical studies.5,17—19) Thus, the result suggests that shoseiryuto may clinically interact with calcium antagonists as grapefruit juice does.

Next, we evaluated the inhibitory effect of each herbal component in shoseiryuto on rat CYP3A *in vitro*. Water extract of schisandra fruit significantly inhibited rat CYP3A *in vitro* in a dose-dependent manner with IC$_{50}$ of 123 μg/ml, which was similar to the value for the inhibition on erythromycin N-demethylation in the microsome of human liver and small intestine.13) However, among eight herbal components in shoseiryuto, ephedra herb and cinnamon bark had higher inhibitory activity with IC$_{50}$ of 64.1 and 52.2 μg/ml, respectively, than schisandra fruit. Kimura *et al.* found the higher inhibitory effect of three herbal medicines on CYP3A *in vitro* among 8 herbal medicines.16) However, the concentrations of these herbal medicines in their study were 250 and 500 μg/ml, which were too high for the comparison of the activity among these herbal medicines. The contribution of schisandra fruit to the inhibitory effect of shoseiryuto on CYP3A *in vitro* would be much less than those of ephedra herb and cinnamon bark. Iwata *et al.* investigated the inhibitory effects of 78 herbal medicines on human liver microsomal CYP3A4, and revealed that the inhibitory effects of ephedra herb and cinnamon bark were much lower than that of schisandra fruit.20) The differences between our or Kimura’s study16) and theirs could be the extracting procedure for herbal medicines. Though Kimura *et al.* did not mention the extracting procedure of herbal medicines, we used the water extract, which is the same dosage form as patients take, while Iwata *et al.* used the methanolic fraction of the water extract. It is suggested that the active ingredients in ephedra herb and cinnamon bark on CYP3A might be too hydrophilic to dissolve in methanol. Actually, the patients take shoseiryuto as water extract of herbal medicines, and the organs where the drug interactions occur are not only liver but small intestine. In fact, grapefruit juice affects the blood concentration of orally administered cyclosporine but did not affect when cyclosporine was intravenously injected, suggesting that grapefruit juice could inhibit drug-metabolising enzymes in only intestine.25) Since the whole ingredients containing herbal extract can reach intestinal lumen when patients take, the evaluation of its fraction is not sufficient to confirm herb–drug interaction.

In addition, we carried out *in vivo* experiments using an orally administered CYP3A substrate, nifedipine. Grapefruit juice, used as a positive control, significantly augmented the plasma concentration of nifedipine without affecting the half life of it, which is consistent with the previous clinical study;23) suggesting that grapefruit juice could inhibit metabolic enzymes in the small intestine but not in the liver. In contrast, shoseiryuto slightly augmented the plasma concentration of nifedipine at the same condition with grapefruit juice. However, it was not statistically significant and this effect was much lower than that expected from the result that shoseiryuto inhibited CYP3A *in vitro* comparably to grapefruit juice. These results suggested that *in vitro* inhibitory effects of herbal medicines on CYPs would not refer to the activity *in vivo*.

In rats, the expression of the CYP3A subfamily is different in liver and small intestine. Normal male Wistar rats predominantly have CYP3A9 and CYP3A18 in small intestine, while they have CYP3A1, CYP3A2 and CYP3A18 in liver.26) Since *in vitro* experiments in this study were carried out using rat liver microsome, differences between *in vitro* and *in vivo* inhibitory effects of herbal medicines on metabolic enzymes might be depended on the specificity of CYP3A subfamilies, although grapefruit juice inhibited CYP3A subfamilies both in rat liver and small intestine. Generally, the differences between *in vitro* and *in vivo* pharmacological effects of herbal medicines are usually arisen from the characteristics for the absorption of active ingredients in herbal medicines. Herbal medicines are a complex mixture of organic compounds, some of which can be absorbed after metabolized by intestinal bacteria. Such metabolites exert their pharmacological activities in the target organ and/or tissues.12) Another reason for the difference between *in vivo* and *in vitro* inhibitory effect of shoseiryuto might be the ability of active ingredients to be absorbed from intestinal lumen to the inside of epithelial cells in intestine. As mentioned above, the inhibitory ingredients of shoseiryuto on CYP3A *in vitro* are expected to be much hydrophilic, which could hardly penetrate through cell membrane. Since the transportation of active ingredients through cell membrane is omitted in *in vitro* assay, the sensitivity would be much higher than *in vivo* experiments. Therefore, *in vitro* experiments to evaluate the in-

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<th>Control</th>
<th>Grapefruit juice</th>
<th>Shoseiryuto</th>
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<tbody>
<tr>
<td>$C_{\max}$ (μg/ml)</td>
<td>1.25±0.17</td>
<td>3.75±0.78*</td>
<td>2.57±1.05</td>
</tr>
<tr>
<td>$T_{\max}$ (min)</td>
<td>9.17±2.37</td>
<td>10.0±0.60</td>
<td>11.7±3.8</td>
</tr>
<tr>
<td>$AUC$ (μg·ml⁻¹·min)</td>
<td>180±39</td>
<td>256±52</td>
<td>230±57</td>
</tr>
<tr>
<td>$MRT$ (min)</td>
<td>114±18</td>
<td>93.8±17.4</td>
<td>100±12</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>143±50</td>
<td>77.7±28.2</td>
<td>83.7±26.0</td>
</tr>
</tbody>
</table>

Data were represented as means±S.E. ($n=6$). *p<0.05 vs. control.

Table 2. Effects of Grapefruit Juice and Shoseiryuto on Pharmacokinetics of Nifedipine
hibitory effects of herbal medicine on drug metabolizing enzymes by directly adding the sample into the enzyme systems could not prove their pharmacological effects accurately.

Of course, it is still limited that this in vivo result of animal experiment accommodates to the case of human beings. Although the bioavailability of orally administered nifedipine is reported to range from about 45 to 58% in the rat, which compares favorably to human beings, the contribution of metabolic enzymes in small intestine and liver to the first-pass effect was expected to be different between rats and human beings. In this study, we used grapefruit juice, a well-characterized natural CYP3A inhibitor studied in patients, as a positive control, that would be helpful to approximate the data of animal experiment to the case of human beings.

In conclusion, although shoseiryuto inhibits the activity of CYP3A in vitro with a degree comparable to grapefruit juice, the inhibition would not be significant in vivo. In order to offer the drug information about herbal medicine to physicians or pharmacists, a careful experimental design is required using at least in vivo experiments with herbal extracts prepared as patients actually take.

REFERENCES