Short Communication

Effects of Kampo medicines on Cyp3a and P-glycoprotein activity in vivo

Kiyomi ITO,a) Ayaka TAKASAKI,a) Nobutomo IKARASHI,a) Junko WATANABE,b) Masanao KANITANI,b) and Kiyoshi SUGYAMAa)∗

a)Department of Clinical Pharmacokinetics, Hoshi University; 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan. b)TSUMURA Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan. *(Received May 13, 2009. Accepted August 20, 2009.)

Abstract

In order to evaluate the possibility of Kampo medicines causing pharmacokinetic drug-drug interactions, the effects of 3 Kampo medicines (rikunshito, yokukansan and boiogito) on the activities of cytochrome P450 (CYP) and P-glycoprotein (P-gp) were investigated in a mouse in vivo study using triazolam and digoxin, respectively, as probe substrates.

A significant increase in the plasma triazolam concentration was observed by co-administration of ketoconazole, a Cyp3a inhibitor, whereas none of the Kampo medicines tested showed any significant effects on triazolam pharmacokinetics in mice. Similarly, plasma concentration of digoxin was significantly elevated by quinidine co-administration, while unaffected by any of the Kampo medicines investigated.

These findings indicate that rikunshito, yokukansan and boiogito are unlikely to affect the pharmacokinetics of co-administered drugs which are substrates of CYP3A and/or P-gp.

Key words Kampo medicine, cytochrome P450, P-glycoprotein, triazolam, digoxin, drug interaction.

Introduction

In order to investigate the possibility of pharmacokinetic interaction between Kampo medicines and Western drugs, we have evaluated the effects of Kampo medicines on the in vitro activities of cytochrome P450 (CYP), a superfamily of drug-metabolizing enzymes involved in biotransformation of xenobiotics, and P-glycoprotein (P-gp), one of the major drug transporters involved in drug absorption, distribution and excretion. Three commonly-used Kampo medicines (rikunshito, yokukansan and boiogito) have been found to cause little inhibitory effects on the major CYP isoymes and P-gp in in vitro studies using human CYP recombinants and P-gp membranes, respectively.1) However, the possibility of their metabolites, formed in the liver or by intestinal flora, causing drug interactions cannot be evaluated in such in vitro studies. In the present study, therefore, the effects of these Kampo medicines on the activities of CYP and P-gp were investigated in a mouse in vivo study.

CYP3A4 is known to be most abundant in human livers and involved in the metabolism of the largest number of drugs among the CYP isozymes. Therefore, the pharmacokinetics of triazolam, a substrate of mouse Cyp3a corresponding to human CYP3A4, were investigated in mice following oral administration of the 3 Kampo medicines (rikunshito, yokukansan and boiogito). Their effects on the P-gp activity were also evaluated in
a similar in vivo experiment using digoxin as a probe substrate.

Materials and Methods

Materials: Male ddY mice, 5-7 weeks of age, were purchased from Sankyo Labo Service Co., Ltd. (Tokyo, Japan), maintained on a 12-hr light/dark cycle and given food and water ad libitum. Dried extract of rikkunshito, yokukansan and boiogito were provided by Tsumura & Co. (Tokyo, Japan). Ketoconazole, polyethylene glycol (PEG) 400, quinidine sulphate dihydrate, and triazolam were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Digoxin (Digosin® injection) was purchased from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). All other reagents were of analytical grade.

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Committee on Animal Research of Hoshi University.

Preparation of Kampo test solutions: 10 mL of distilled water was added to 200 mg dried extract of rikkunshito, yokukansan or boiogito and the suspensions were sonicated and vortexed followed by centrifugation at 1,600 x g, at room temperature for 15 min. The supernatants were used in the following experiments after dilution with distilled water.

Effects on Cyp3a activity: The effects of Kampo medicines on the Cyp3a activity were determined using triazolam as a substrate and ketoconazole as a positive control inhibitor. Triazolam and ketoconazole were dissolved in PEG 400/saline (1:1) and PEG 400, respectively.

Mice were fed chow containing rikkunshito (1.0 g/kg/day), yokukansan (0.81 g/kg/day) or boiogito (0.94 g/kg/day) for 15 days before triazolam (0.3 mg/kg) was injected intraperitoneally. In the positive control study, mice were injected intraperitoneally with ketoconazole (100 mg/kg) or vehicle (PEG 400) 60 min before triazolam (0.3 mg/kg). At 15, 30, 45 and 60 min after triazolam injection, mice were anesthetized with diethylether and the blood was obtained from abdominal veins.

Plasma concentration of triazolam was determined as reported previously2) with slight modification. 300 μL isopropanol was added to 300 μL of plasma and centrifuged at 1,000 x g for 6 min after vortex mixing. To the supernatant (360 μL), 450 μL borate buffer (50 mM, pH 11.0) and 1,620 μL chloroform were added and the mixture was centrifuged at 1,000 x g for 10 min. 1,360 μL of the organic layer was evaporated to dryness under a gentle stream of nitrogen. The residues were reconstituted with 60 μL of HPLC mobile phase and 20 μL was injected into the HPLC system.

The HPLC system consisted of a CCPM pump (Tosoh, Tokyo, Japan), a PX-8010 controller (Tosoh), an UV-8010 UV detector (Tosoh) set at 222 nm and Inertsil ODS-3 column (5 μm, 4.6 × 250 mm; GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of a 60/38/2 (v/v/v) mixture of water, acetonitrile and methanol. The column temperature was kept at 40°C using a column oven (CO-8010; Tosoh) and the flow rate was 1.0 mL/min. All chromatograms were recorded using an integrator (Chromatocorder 21; System Instruments Co., Ltd., Tokyo, Japan) and triazolam was quantified based on its peak areas.

Effects on P-gp activity: The effects of Kampo medicines on the P-gp activity were determined using digoxin as a substrate and quinidine as a positive control inhibitor. Mice were fed chow containing rikkunshito (1.0 g/kg/day), yokukansan (0.81 g/kg/day) or boiogito (0.94 g/kg/day) for 15 days before digoxin (0.05 mg/kg) was injected intraperitoneally. In the positive control study, mice were injected intraperitoneally with quinidine (100 mg/kg) or saline 30 min before digoxin (0.05 mg/kg). At 45, 120, 240 and 480 min after digoxin injection, mice were anesthetized with diethylether and the blood was obtained from abdominal veins. Plasma concentration of digoxin was determined by fluorescence polarization immunoassay using the Abbot TDx analyzer (Abbot Laboratories, Chicago, IL) after 20-fold dilution and protein precipitation.

Data analyses: The plasma concentrations of triazolam and digoxin are shown as means ± S.D. of 4 mice each for each time point. Student’s t-test was used to evaluate statistical significance.
The area under the plasma concentration-time curve (AUC) from time zero to the final sampling time was calculated by the trapezoidal rule using the mean concentration at each time point.

Results

Effects of Kampo medicines on Cyp3a activity: The plasma concentration profiles of triazolam are shown in Fig 1. The plasma triazolam concentrations for the ketoconazole-treated mice were higher than those for the control mice at 30 min post-injection and thereafter, with a statistically significant difference at 45 min post-injection (p<0.01; Fig. 1A). The AUC of triazolam from 0 to 60 min (AUC_{0-60}) was 2.4-fold higher for the ketoconazole-treated mice (5.18 µg • min/mL) compared with the control mice (2.06 µg • min/mL).

On the other hand, none of the Kampo medicines showed a significant effect on plasma triazolam concentration (Fig. 1B). The AUC_{0-60} values were also similar for the mice treated with Kampo medicines (3.07, 3.06, and 3.47 µg • min/mL for rikkunshito, yokukansan and boiogito-treated mice, respectively) and the control mice (3.36 µg • min/mL).

![Fig. 1](image1.png)

**Fig. 1** Effects of ketoconazole (Panel A) and Kampo medicines (Panel B) on the plasma concentration profiles of triazolam in mice.


**Effects of Kampo medicines on P-gp activity:** The plasma concentration profiles of digoxin are shown in Fig. 2. The plasma digoxin concentrations for the quinidine-treated mice were higher than those for the control mice, with a statistically significant difference at 240 min post-injection (p<0.05; Fig. 2A). The AUC of digoxin from 0 to 480 min (AUC_{0-480}) was 1.5-fold higher for the quinidine-treated mice (21.0 µg • min/mL) compared with the control mice (14.4 µg • min/mL).

On the other hand, none of the Kampo medicines showed a significant effect on plasma digoxin concentration (Fig. 2B). The AUC_{0-480} values were also similar for the mice treated with Kampo medicines (16.0, 16.2, and 14.8 µg • min/mL for rikkunshito, yokukansan and boiogito-treated mice, respectively) and the control mice (15.5 µg • min/mL).

![Fig. 2](image2.png)

**Fig. 2** Effects of quinidine (Panel A) and Kampo medicines (Panel B) on the plasma concentration profiles of digoxin in mice.


Data are expressed as means ± S.D. (n=4). * p<0.05 vs control

Discussion

The effects of continuous administration of 3 Kampo medicines (rikkunshito, yokukansan and boiogito) on the activities of Cyp3a and P-gp, a major drug metabolizing enzyme and drug transporter, respectively, have been investigated in the present in vivo study using
mice. The doses used for the Kampo medicines were 15-times higher than their respective clinical doses. Rikkunshito improves the function of the alimentary system and is used for a number of conditions including gastritis and stomach atony. Yokukansan is used to treat psychoneurosis when patients complain of overexcitability, proneness to anger, irritability, or insomnia. Boiogito is a hydrostatic modulating drug used for conditions that include osteoarthritis of the knee and obesity. All these Kampo medicines are being used more and more widely in clinical practice, thus increasing the chances that they will be administered together with other drugs.

Triazolam has been shown to be metabolized mainly by Cyp3a in an in vitro study using mouse liver microsomes. Furthermore, the plasma concentration of triazolam after intraperitoneal injection in mice is reported to increase following co-administration of ketoconazole, an inhibitor of Cyp3a. The combination of triazolam and ketoconazole in humans is also reported to result in the elevation of plasma triazolam levels via inhibition of CYP3A4. In the present study, therefore, triazolam and ketoconazole were used as a probe substrate and a positive control inhibitor, respectively, of mouse Cyp3a. In the mice given an intraperitoneal injection of ketoconazole (100 mg/kg) followed by triazolam (0.3 mg/kg), the plasma triazolam concentration was significantly higher than in the control mice which received triazolam alone (Fig. 1A), which is consistent with the inhibitory effect of ketoconazole on Cyp3a. On the other hand, the plasma triazolam concentration profile in the mice treated with rikkunshito, yokukansan or boiogito for 15 days was similar to that in the control mice (Fig. 1B), suggesting that Cyp3a activity is unaffected by continuous administration of these Kampo medicines.

It has been reported that digoxin is transported by P-gp in mice and that plasma digoxin concentration after intravenous administration in mice is elevated by co-administration of quinidine, an inhibitor of P-gp. Quinidine is also reported to inhibit the biliary and renal elimination of digoxin in humans possibly by inhibiting P-gp. In the present study, therefore, digoxin and quinidine were used as a probe substrate and a positive control inhibitor, respectively, of mouse P-gp. In the mice given an intraperitoneal injection of quinidine (100 mg/kg) followed by digoxin (0.05 mg/kg), the plasma digoxin concentration was significantly higher than in the control mice which received digoxin alone (Fig. 2A). This finding is consistent with the inhibitory effect of quinidine on P-gp, resulting in the inhibition of renal and biliary excretion of digoxin. On the other hand, the plasma digoxin concentration profile in the mice treated with rikkunshito, yokukansan or boiogito for 15 days was similar to that in the control mice (Fig. 2B), suggesting that the P-gp activity is unaffected by continuous administration of these Kampo medicines.

In conclusion, it was demonstrated that rikkunshito, yokukansan and boiogito have minimal effect on the activities of Cyp3a and P-gp. The findings in the present study suggest that these Kampo medicines are unlikely to affect the pharmacokinetics of co-administered drugs which are substrates of CYP3A and/or P-gp.

References


