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Inhibitory Effects of Kampo Medicine on Human UGT2B7 Activity

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Summary: Kampo medicine is traditional Japanese medicine modified from the Chinese original. Kampo medicine is a mixture of several medicinal herbs and includes many ingredients such as glycosides. Glycosides are hydrolyzed to aglycons by intestinal bacterial flora and absorbed into the body. Aglycons such as baicalein and glycyrrhetinic acid can be conjugated by UDP-glucuronosyltransferase (UGT) in human liver or small intestine. UGT2B7 is one of the major isoforms responsible for drug conjugation including morphine 3- and 3'-azido-3'-deoxythymidine (AZT) glucuronidation. The present study investigates the effects of 51 Kampo medicines, 14 medicinal herbs and 11 ingredients on UGT2B7 activity in human liver microsomes. Morphine 3-glucuronidation was inhibited by more than 50% by 9 of 51 Kampo medicines such as Ryo-kei-jutsu-kan-to. AZT glucuronidation was inhibited by more than 50% by 24 of 51 Kampo medicines such as Jumihaidoku-to. Medicinal herbs such as Daio (Rhei Rhizoma), Kanzo (Glycyrrhizae Radix) and Keihi (Cinnamomomi Cortex) exhibited more than 80% inhibition on both glucuronidations. The major ingredients of these medicinal herbs inhibited UGT2B7 activity with low K_i. Kampo medicines were found to inhibit the UGT2B7 activity and may cause drug interactions via the inhibition of UGT.

Keywords: UGT; inhibition; Kampo medicine; human liver microsomes; drug interactions

Introduction

Kampo is a unique type of medicine developed from traditional Chinese medicine in Japan approximately 1,500 years ago,1) and is a mixture of several medicinal herbs. Medicinal herbs contain many pharmacologically active components, such as saponins, glycosides, and flavonoids.2) Since scientific research on its efficacy has advanced, prescriptions of Kampo medicines have been increasing in Japan. Recently, clinical trials on Kampo medicines have been performed in the United States. Since Kampo medicines are used widely for many people, drug interactions between Kampo medicines and other drugs have been reported. Co-administration of interferon with Shosaiko-to for the treatment of hepatitis induces interstitial pneumonia.3) The Japanese Ministry of Health, Labour, and Welfare gave a warning concerning this interaction. However, Hangeshashin-to prevents severe diarrhea induced by the anti-cancer drug irinotecan hydrochloride.4) Herb-drug interactions have also been reported. For example, St. John’s wort, used for mild to moderate depression, induces cytochrome P450 3A4 leading to decreased efficacy of several CYP3A4 substrates.5) Recently, in vitro studies have shown that some Kampo medicines and medicinal herbs affect CYP activity.2,6) UDP-glucuronosyltransferase (UGT) is one of the major conjugative enzymes that mainly detoxify endogenous and xenobiotic compounds. In humans, UGTs are classified into UGT1 and UGT2 families. The UGT2 family is divided into UGT2A and UGT2B subfamilies.7) One of the isoforms, UGT2B7, is the most important isoform because 35% of the top 200 drugs prescribed in the United States in 2002 that have glucuronidation as a clearance mechanism are conjugated by this isoform.8) UGT2B7 conjugates endogenous compounds such as bile acid and retinoids and xenobiotics such as morphine and 3'-azido-3'-deoxythymidine (AZT).9,10)
Kampo Medicine Inhibits Human UGT2B7 Activity

Some glycosides in Kampo medicines are hydrolyzed to their aglycons by intestinal bacterial flora and absorbed into the body. Aglycons are metabolized to glycoside by UGT in liver and small intestine. Therefore, it is surmised that glucuronidation may be responsible for some drug interactions caused by combination of Kampo medicines and other drugs. In our laboratory, the inhibitory effects of Kampo medicines, medicinal herbs and their ingredients on human UGT1A1 activity were previously investigated.\(^1\)\(^1\) The present study was conducted to clarify the inhibitory effects of Kampo medicines on human UGT2B7 activity.

**Materials and Methods**

**Materials:** Kampo medicines (extracts of several medicinal herbs) and medicinal herbs were kindly supplied by Tsumura & Co. (Tokyo, Japan). Kampo medicines and medicinal herbs were made by the extraction of several medicinal herbs with purified water.\(^1\)\(^2\) We chose 51 Kampo medicines frequently used in Japan, 14 medicinal herbs and 11 ingredients. The 51 Kampo medicines are as follows (Table 1): Bakumondo-to, Bofutosho-san, Boi-ogi-to, Byakko-ka-ninjin-to, Chorei-to, Cho-to-san, Dai-kenchu-to, Gorei-san, Gosha-jinki-gan, Goshuyu-to, Hachimi-jio-gan, Hange-byakujutsu-temma-to, Hange-koboku-to, Hange-shashin-to, Hochu-ekki-to, Juncho-to, Juzen-taiho-to, Kakkon-to, Kami-shoyo-san, Keigai-rengyo-to, Keishi-bukuryo-gan, Keishi-bukuryo-gan-ka-yokuinin, Keishi-ka-jutsu-bu-to, Keishi-ka-shakuyaku-daio-to, Kikyo-to, Mao-bushi-saishin-to, Mashinin-gan, Ninjin-yoei-to, Oren-gedoku-to, Otsujito, Rikkunshi-to, Ryo-kei-jutsu-kan-to, Saiko-ka-ryukotsu-borei-to, Saiko-keishi-kankyo-to, Sairei-to, Seihei-to, Seijo-bofu-to, Shaku-yaku-kanzo-to, Shimbu-to, Sho-kenchu-to, Sho-sai-to, Sho-seiryu-to, Sosei-kaketsu-to, Tokaku-joki-to, Toki-shaguyaku-san, Toki-shigaku-ka-goshuyu-syokyo-to, Unkei-to, Unsei-in and Yoku-kan-san. The 14 medicinal herb extracts frequently included in the 51 Kampo medicines are as follows: Bukuryo (scientific name of plant; *Hoelen*), Daio (*Rhei Rhizoma*), Hange (*Pineniae Tuber*), Kanzo (*Glycyrrhizae Radix*), Keihi (*Cinnamomi Cortex*), Kikyo (*Platyvodi Radix*), Ninjin (*Ginseng Radix*), Ogon (*Scutellariae Radix*), Saiko (*Bupleuri Radix*), Shakuyaku (*Paoniae Radix*), Shokyo (*Zingiberis Rhizoma*), Sojutsu (*Atractylodis Lanceae Rhizoma*), Taiso (*Zizyphi Fructus*), and Toki (*Angelicae Radix*). The 11 ingredients are as follows: the major ingredients of Daio: emodin, rhein, sennidine A, and sennoside A; the major ingredients of Kanzo: glycyrrhetinic acid and glycyrrhizic acid; the major ingredients of Keihi: cinnamaldehyde and cinnamic acid; the major ingredients of Ogon: baicalein, baicalin and wogonin. Emodin and rhein were purchased from Sigma-Aldrich (St. Louis, MO). Baicalein, baicalin, cinnamaldehyde, cinnamic acid, glycyrrhetinic acid, glycyrrhizic acid, sennidine A, sennoside A, and wogonin were obtained from Wako Pure Chemical Industries (Osaka, Japan). Morphine hydrochloride was purchased from Takeda Pharmaceutical Company (Osaka, Japan). Morphine 3-glucuronide was a generous gift from Dr. Kazuta Oguri (Kyushu University, Fukuoka, Japan). AZT and AZT glucuronide were obtained from Wako Pure Chemical Industries. Pooled human liver microsomes (HLM) from 20 donors and recombinant UGT2B7 microsomes expressed in baculovirus-infected insect cells were

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### Table 1. Kampo medicines used in this study

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<thead>
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<th>Number</th>
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purchased from BD Gentest (Woburn, MA). Uridine 5′-diphosphogluconic acid (UDP-GA) and alamethicin were from Sigma-Aldrich. All other chemicals and solvents were of analytical or the highest grade commercially available.

**Morphine 3-glucuronidation:** Morphine 3-glucuronidation was determined as described previously with slight modification. A typical incubation mixture (200 µL total volume) contained 50 mM tris(hydroxymethyl)aminomethane-HCl buffer (pH 7.4), 5 mM MgCl₂, 3 mM UDP-GA, 0.5 mg/mL HLM or recombinant UGT2B7 microsomes, 12.5 µg/mL alamethicin, 0.5–2 mM morphine and Kampo medicines, medicinal herbs, or ingredients. Kampo medicines and medicinal herbs were dissolved in distilled water. The concentrations of the Kampo medicines and medicinal herbs were 1 mg/mL except Shokyo (0.1 mg/mL), Hange (0.5 mg/mL) and Toki (0.5 mg/mL) due to low solubility. The ingredients were dissolved with dimethyl sulfoxide without glycyrrhetic acid (distilled water). The final concentration of the organic solvents was less than 1.5%. In the preliminary study, the organic solvents decreased morphine glucuronidation by 6%, but the concentration of the organic solvent in the control was the same as in the sample or ingredients. Kampo medicines and medicinal herbs were dissolved in distilled water. The concentrations of the Kampo medicines and medicinal herbs were 1 mg/mL except Shokyo (0.1 mg/mL), Hange (0.5 mg/mL) and Toki (0.5 mg/mL) due to low solubility. The ingredients were dissolved with dimethyl sulfoxide without glycyrrhetic acid (distilled water). The final concentration of the organic solvents was less than 1.5%. In the preliminary study, the organic solvents decreased morphine glucuronidation by 6%, but the concentration of the organic solvent in the control was the same as in the sample with an ingredient. The concentration of the ingredient was 1 mM except for cinnamaldehyde (10 µM) and rhein (250 µM) due to low solubility. After a 1-min pre-incubation at 37°C, the reaction was initiated by the addition of UDP-GA and the mixture was incubated at 37°C for 30 min. The reaction was terminated by boiling for 5 min. Morphine 3-glucuronide was stable after boiling. After removal of the protein by centrifugation at 7,000 g for 10 min, a 20-µL portion of the sample was subjected to high-performance liquid chromatography (HPLC) with a Develosil C-30 UG-5 analytical column (4.6 × 150 mm, 5 µm, Nomura Chemical, Aichi, Japan). The flow rate was 1.2 mL/min and the column temperature was 35°C. Morphine 3-glucuronide was detected fluorometrically (excitation, 210 nm; emission, 350 nm). The mobile phase was 50 mM sodium dihydrogen phosphate (pH 4.5). The retention times of morphine 3-glucuronide and morphine were 13 min and 26 min, respectively. All data were analyzed using the mean of duplicate determinations.

For determination of Kᵢ, the concentration of AZT ranged from 0.25 to 2 mM and those of the inhibitors as follows:
Kampo medicines: Jumi-haidoku-to (16) and Ryo-kei-jutsu-kan-to (34), 200–500 µg/mL; Tokaku-joki-to (46), 300–700 µg/mL.
medicinal herbs: Daio and Kanzo, 60–180 µg/mL; Keihi, 100–220 µg/mL.
ingredients: baicalein, 50–200 µM; cinnamaldehyde, 0.6–2 µM; glycyrrhetic acid 20–80 µM; sennidine A, 40–160 µM.

**Data Analyses:** The correlation between morphine 3- and AZT glucuronidation was determined by Spearman correlation analysis. Kᵢ and inhibition types were determined by fitting the kinetic data to a competitive, non-competitive, uncompetitive, or mixed inhibition model by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

**Results**

**Inhibitory effects of Kampo medicines on UGT2B7 activities:** The inhibitory effects of 51 Kampo medicines on morphine 3- and AZT glucuronidation in HLM were determined. In the preliminary study, the rates of these activities were linear with respect to microsomal protein concentrations and incubation time. As shown in Figure 1A, morphine 3-glucuronidation was strongly inhibited by Jumi-haidoku-to (16), Otsuji-to (32), Ryo-kei-jutsu-kan-to (34), and Shakuyaku-kanzo-to (40) (< 40% of control), and 9 of the 51 Kampo medi-

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Inhibitory effects of medicinal herbs on UGT2B7 activity: The inhibitory effects of 14 medicinal herbs on the UGT2B7 activity are shown in Figure 2. Daio, Kanzo, and Keihi strongly inhibited both morphine 3- and AZT glucuronidation (<20% of control). The activity was also inhibited more than 70% inhibition by Sojutsu and Ogon. Kikyo, Hange, and Shokyo exhibited no inhibition on morphine 3- and AZT glucuronidation. The inhibitory potencies of the medicinal herbs on morphine 3-glucuronidation were significantly correlated with those on AZT glucuronidation (Fig. 2B, \( r = 0.90, p < 0.001 \)).

Inhibitory effects of ingredients on UGT2B7 activity: The inhibitory effects of 11 ingredients of medicinal herbs on the UGT2B7 activity are shown in Figure 3. Sennidine A (a representative ingredient of Daio), glycyrrhetinic acid (Kanzo), and cinnamaldehyde (Keihi) also exhibited more than 90% inhibition on both morphine 3- and AZT glucuronidation. The activity was also inhibited (>80% inhibition) by emodin (Daio) and baicalein (Ogon). Glycosides such as sennidine A, glycyrrhetinic acid, and baicalein showed stronger inhibitions than their aglycons. The inhibitory potencies of the ingredients on morphine 3-glucuronidation were significantly correlated with those on AZT glucuronidation (Fig. 3B, \( r = 0.94, p < 0.001 \)). In recombinant UGT2B7 microsomes, cinnamaldehyde, sennidine A and glycyrrhetinic acid also showed stronger inhibitions than 80% of the control on both morphine 3- and AZT glucuronidation (data not shown).

Inhibition constant and inhibitory type on morphine 3-glucuronidation: Inhibition constants and inhibitory types of Kampo medicines, medicinal herbs and ingredients on morphine 3-glucuronidation are shown in Table 2 and Figure 4. Inhibitory types of Ryo-kei-jutsu-kan-to (34) and Shakuyaku-kanzou-to (40) were mixed-type, whereas that of Otsuji-to (32) was non-competitive inhibition. Kanzo and Keihi exhibited non-competitive inhibition whereas Daio exhibited competitive inhibition. Cinnamaldehyde, glycyrrhetinic acid and sennidine A indicated non-competitive inhibition.

Inhibition constant and inhibitory type on AZT glucuronidation: The inhibition constants and inhibitory types of the Kampo medicines, medicinal herbs and ingredients on AZT glucuronidation are shown in Table 3 and Figure 5. \( K_i \) of three medicinal herbs were similar to those of the Kampo medicines. The inhibitory types of Jumi-haidoku-to (16) and Shakuyaku-kanzou-to (40) were mixed-type, whereas that of Otsuji-to (32) was non-competitive inhibition. Kanzo showed competitive inhibition whereas Daio and Keihi exhibited non-competitive inhibition. The inhibitory types of cinnamaldehyde and glycyrrhetinic acid were competitive but those of baicalein and sennidine A were non-
Fig. 2. Inhibitory effects of 14 medicinal herbs on UGT2B7 activities in HLM
The inhibition on morphine 3- and AZT glucuronidation (A) and correlation of the inhibition between morphine 3- and AZT glucuronidation (B) were demonstrated. The concentrations of the substrates were 1 mM and that of the medicinal herbs was 1.0 mg/mL except Shokyo (0.1 mg/mL), Hange and Toki (0.5 mg/mL). Each column represents the mean of duplicate determinations. The control activities of morphine 3- and AZT glucuronidation were 1.8 nmol/min/mg and 1.3 nmol/min/mg, respectively. ND: not detected.

Fig. 3. Inhibitory effects of 11 ingredients on UGT2B7 activities in HLM
The inhibition on morphine 3- and AZT glucuronidation (A) and correlation of the inhibition between morphine 3- and AZT glucuronidation (B) were demonstrated. The concentrations of the substrates were 1 mM and that of the ingredients was 1 mM except cinnamaldehyde (10 μM) and rhein (250 μM). Each column represents the mean of duplicate determinations. The control activities of morphine 3- and AZT glucuronidation were 1.8 nmol/min/mg and 1.3 nmol/min/mg, respectively. ND: not detected.

Discussion
One hundred fifty two Kampo medicines were listed in the National Health Insurance reimbursement price list in Japan in 2008. Some Kampo medicines could be purchased as over-the-counter drugs in Japan. Kampo medicines are often prescribed for individuals suffering from chronic disease because it leads to gradual improvement of the disease and the maintenance of homeostasis. According to the datasheet supplied by Tsumura & Co., when a Kampo medicine is co-administered with other Kampo medicines, careful attention should be paid to the duplication of medicinal herbs present, especially Kanzo. Recently, Kampo medicines are frequently co-administered with drugs (synthesized drugs) as well as other Kampo medicines. Therefore, prediction of drug interactions between Kampo medicines and drugs is important for avoiding adverse reactions. Since it is difficult to identify the pharmacological and toxic components in Kampo medicines, there are few reports
on drug interactions between Kampo and other drugs. To date, the inhibitory effects of several Kampo medicines on CYP activity have been demonstrated, but no study has been performed on UGT activity except our previous study on human UGT1A1. The present study shows that Kampo medicines, medicinal herbs and their ingredients exhibit inhibition on human UGT2B7 activity.

Apparent $K_m$ of UGT2B7 for morphine 3- and AZT glucuronidation were 0.46–1.0 mM$^{14,15}$ and 0.49–0.77 mM$^{14,16}$ respectively. In the present study, morphine and AZT concentrations were set at 1 mM taking $K_m$ into consideration. However, the plasma concentrations of morphine (0.1 $\mu$M$^{17}$) and AZT (6.6 $\mu$M$^{18}$) were lower than the concentrations of the substrates in the reaction mixtures. The liver concentration of the substrate should be much higher than the plasma concentration, although it is hard to measure. Therefore, we keep the liver concentration of the substrate in mind to extrapolate the

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<th>Inhibitor</th>
<th>$K_i$ ($\mu$g/mL)</th>
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<tr>
<td>Otsuji-to (32)</td>
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<tr>
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</tr>
<tr>
<td>Shakuyaku-kanzo-to (40)</td>
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<td>Daio</td>
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<tr>
<td>Kanzo</td>
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<td>Cinnamaldehyde</td>
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<tr>
<td>Glycyrrhetinic acid</td>
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<tr>
<td>Sennidine A</td>
<td>89.5 ± 6.3$^b$</td>
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$^a$ Number in parenthesis is the same number in Table 1
$^b$ $\mu$M

Fig. 4. Lineweaver-Burk plots of morphine 3-glucuronidation in the presence of the inhibitors in HLM
Ryo-kei-jutsu-kan-to (A, 34 in Fig. 1), Daio (B), cinnamaldehyde (C), and glycyrrhetinic acid (D). The concentration of morphine ranged from 0.5 to 2 mM. Data represent the mean of duplicate determinations.
result from the in vitro inhibition study to the in vivo situation in human. The usual dose of many Kampo medicines is 7.5–9.0 g/day orally in 2 or 3 divided doses before or between meals. As mentioned above, Kampo medicine (7.5–9.0 g) usually consists of 1.5–6.0 g of dried extract of mixed medicinal herbs. For instance, 7.5 g TSUMURA Ryo-kei-jutsu-kan-to (34) contains 1.5 g dried extract of the following mixed medicinal herbs: Bukuryo (6.0 g), Keihi (4.0 g), Sojutsu (3.0 g), and Kanzo (2.0 g). Kampo medicines are taken at 2.5–3.0 g/time (0.5–2.0 g of dried extract of mixed medicinal herbs/time), but we could not measure or estimate the concentration of each lot of medicinal herb. According to a report by Foti et al.,19) the estimated gut concentration of the herbal mixture was calculated as follows: a single tablet of herbal mixture (g) was divided by the intestinal volume (\(\approx 500 \text{ mL}\)). However, it is uncertain whether the calculation of the intestinal concentration of Kampo medicine by this method is appropriate.

Figures 1 and 2 show that some Kampo medicines and medicinal herbs inhibit UGT2B7 activity. The inhibition by Kampo medicines or medicinal herbs on the UGT2B7 activity means that their ingredients inhibit activity. In the present study, we investigated the inhibition by representative ingredients of medicinal herbs commercially available. Some ingredients such as cinnamaldehyde and glycyrrhetinic acid exhibited strong inhibition on both morphine 3- and AZT glucuronidation in HLM (Tables 2 and 3). Since there are many ingredients in medicinal herbs, other ingredients may also inhibit these activities strongly. However, the ingredients with low \(K_i\) contributed to the inhibitory potencies of Kampo medicines or medicinal herbs on UGT2B7 activity. The problem is that the plasma concentrations of the ingredients of the medicinal herb have not been investigated. It is difficult to predict the inhibitory potency of an ingredient on morphine 3- and AZT glucuronidation quantitatively. Further study is needed to predict the in vivo inhibition of UGT quantitatively from in vitro inhibition studies.

Some Kampo medicines, medicinal herbs and their ingredients inhibited morphine 3- and AZT glucuronidation in HLM and their inhibitory potencies on both glucuronidations exhibited significant correlation (Figs. 1C and 2B). The ingredients that inhibited strongly the two glucuronidations in HLM also showed inhibition in recombinant UGT2B7 microsomes suggesting that they inhibit UGT2B7 activity. Although the concentration of AZT was the same as that of morphine, many Kampo medicines inhibited the AZT glucuronidation more strongly than the morphine 3-glucuronidation. One reason for this may be a difference in the affinity of the substrate for UGT2B7. Another reason may be different minor isoforms responsible for morphine 3-glucuronidation, which is also catalyzed by UGT1A1, UGT1A3, UGT1A6, UGT1A8, and UGT1A10, but their apparent \(K_m\) were higher than that of UGT2B7.20)
In the present study, the inhibitory types of some medicinal herbs and ingredients were different in morphine and AZT glucuronidation. The inhibitory type of ketoconazole was the mixed-type on morphine 3-glucuronidation\textsuperscript{21} but competitive on AZT glucuronidation.\textsuperscript{22} As Takeda \textit{et al.}\textsuperscript{21} suggest, the binding site of ketoconazole in UGT2B7 may be associated with this phenomenon. Uchaipichat \textit{et al.}\textsuperscript{23} suggest that the multiplicity of binding and effector sites results in complex kinetic interactions between UGT substrates, which may complicate inhibition studies. The differences may be explained by the occurrence of multiple substrate, inhibitor and allosteric binding sites within UGT2B7.\textsuperscript{23} Therefore, the inhibitory types may be dependent on the substrate and binding site of the inhibitors.

Morphine is mainly metabolized to 3-glucuronide (55\%) and 6-glucuronide (15\%) by UGT2B7 in humans.\textsuperscript{24} Therefore, the present study focused on the inhibition of morphine 3-glucuronidation. In an in\textit{vitro} study by Hara \textit{et al.},\textsuperscript{25} diclofenac, clomipramine, diazepam, lorazepam, and oxazepam strongly inhibited the morphine 3-glucuronidation. Diclofenac, clomipramine and oxazepam altered the pharmacokinetics of morphine in human \textit{in vivo}.\textsuperscript{26–28} To reduce the severe constipation caused by morphine, Daido-kenchu-to (7), Keishi-ka-shakuyaku-daiito (26), Mashinin-gan (29), and Sho-saiko-to (43) are co-administered.\textsuperscript{29} Mashinin-gan showed 52.8\% inhibition of morphine 3-glucuronidation in the present study. Thus, Kampo medicines may also inhibit morphine 3-glucuronidation and alter its pharmacokinetics \textit{in vivo}.

AZT is extensively metabolized to AZT-glucuronide which is not pharmacologically active.\textsuperscript{22} This causes serious hematologic toxicity such as anemia and leukopenia.\textsuperscript{30} In combination with fluconazole, the plasma concentration of AZT glucuronide is decreased, which results in a higher plasma concentration and prolonged half-life of AZT.\textsuperscript{31,32} Fluconazole inhibited AZT glucuronidation with 1.4 mM of \(K_i\) in HLM.\textsuperscript{33} Therefore, some Kampo medicines with low \(K_i\) could alter AZT pharmacokinetics, but further study is necessary to clarify the clinical impact.

It should be noted that the true extent of inhibition shown in in\textit{vitro} study may be almost certainly underestimated. Studies by Uchaipichat \textit{et al.}\textsuperscript{33} and Rowland \textit{et al.}\textsuperscript{34} have shown that endogenous fatty acids released during the course of an incubation act as potent competitive inhibitors of UGT2B7. This results in overestimation of \(K_i\) and underestimation of the likely magnitude of an inhibitory interaction \textit{in vivo}.

The present study shows that baicalein (Ogon), cinnamaldehyde (Kehii), emodin (Dai), glycyrrhetinic acid (Kanzo), and sennidine A (Dai) inhibit UGT2B7 activity. It has been reported that these ingredients also inhibit human UGT1A1 activity.\textsuperscript{11,35–37} Thus, baicalein, cinnamaldehyde, glycyrrhetinic acid and sennidine A may be inhibitors of both UGT1A1 and UGT2B7. Therefore, it is surmised that some potent inhibitors of UGT2B7 may also inhibit another UGT isofrom, but the mechanisms of UGT inhibition are not known in detail.

In conclusion, the present study clarifies that some Kampo medicines, medicinal herbs, and their ingredients exhibit strong inhibition on UGT2B7 activity \textit{in vitro}. Because UGT2B7 is an important isofrom that metabolizes many endogenous compounds and xenobiotics, we must keep in mind the possibility of drug interactions with Kampo medicines via UGT2B7.

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**References**


36) Nakajima, M., Sakata, N., Ohashi, N., Kume, T. and Yokoi, T.: Involvement of multiple UDP-glucuronosyltransferase 1A isoforms in glucuronidation of 5-(4′-hydroxyphenyl)-5-phenyl-