Effects of Japanese Herbal Medicine, *Kampo*, on Human UGT1A1 Activity

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Summary: *Kampo* is a traditional Japanese herbal medicine and widely used in clinical practice in Japan. Little is known about interactions between *Kampo* and other medicines. *Kampo* contains many aglycones, which can be conjugated by UDP-glucuronosyltransferase (UGT). Therefore, in the present study, the effects of *Kampo* on human UGT1A1 activity were investigated in vitro. Substrates of human UGT1A1, β-estradiol or 7-ethyl-10-hydroxycamptothecin (SN-38), were incubated with human liver microsomes in the presence of 51 *Kampos*, 14 medicinal herbs and their components. β-Estradiol 3-glucuronidation was strongly inhibited by some *Kampos* such as Bofu-tsusho-san, Mashinin-gan and Otsuji-to. Medicinal herbs such as Daio (**Rhei Rhizoma**), Kanzo (**Glycyrrhizae Radix**), Keihi (**Cinnamomi Cortex**) and Ogon (**Scutellariae Radix**) exhibited potent inhibition on that activity. On β-estradiol 3-glucuronidation, the major component of Keihi (cinnamaldehyde) and Ogon (wogonin) exhibited mixed-type inhibition of $K_i$ with values of 0.7 μM and 2.8 μM, respectively. On SN-38 glucuronidation, the inhibitory potencies of *Kampos*, medicinal herbs and their components tended to be similar to those on β-estradiol 3-glucuronidation. In the present study, *Kampo* was clarified to inhibit β-estradiol and SN-38 glucuronidation mainly catalyzed by UGT1A1.

Keywords: UGT; inhibition; *Kampo*; herbal medicine; phase II metabolism; drug interaction

Introduction

*Kampo* is a mixture of several medicinal herbs and widely used in clinical practice in Japan. *Kampo* is a traditional medicine of Chinese origin and was uniquely developed in Japan. Many components in *Kampo* may have pharmacological effects that could lead to improvement of disease and the maintenance of homeostasis. Since scientific research on its efficacy has advanced, prescriptions of *Kampo* have been increasing. Recently, clinical trials on *Kampo* have been performed in the US. However, many components of the herbs in *Kampo* resulted in complex pharmacological and toxicological effects.¹⁻⁴

Several adverse reactions caused by *Kampo* have been reported.⁵ Warnings concerning Sho-saiko-to and interstitial pneumonia were given by the Japanese Ministry of Health, Labour, and Welfare. Especially, when co-administered with interferons α/β, Sho-saiko-to was suggested to induce interstitial pneumonia. One of the most famous medicinal herbs, Kanzo (**Glycyrrhizae Radix**), represents more than 70% of the prescribed *Kampo*. A high intake of glycyrrhizic acid, a major component of Kanzo, causes hypermineralocorticoidism.⁶ Irinotecan hydrochloride-induced intestinal toxicity is prevented by co-administration of a *Kampo*, Hange-shasin-to,⁷ which is therefore used in clinical practice in Japan. Since several *Kampos* and medicinal herbs can be obtained over the counter, it is possible that co-administration with prescribed medicines often occurs. *Kampo* is often prescribed in individuals suffering from chronic disease and therefore may be frequently co-administered with other medicines. However, information on interactions between *Kampo* and medicines is limited. Interactions with herbs via cytochrome P450 (P450) have been reported and have received attention.⁹ Interactions of St. John’s wart or grapefruit juice via P450 are well known. Inhibitory effects of *Kampo* on P450 activity have been
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Materials and methods

Chemicals: The extracts of Kampo (a mixture of several medicinal herbs) and medicinal herbs clinically used in Japan were kindly supplied by Tsumura & Co. (Tokyo, Japan). According to the method previously described,9 Kampo and medicinal herbs were extracted with purified water. We chose 51 Kampo prescribed frequently in Japan, 14 medicinal herbs and 11 components. The fifty one Kampo were as follows: Bukumondo-to, Bofu-tsusho-san, Boi-ogi-to, Byakko-ka-ninjin-to, Choreito, Cho-to-san, Dai-kenchu-to, Gorei-san, Goshajingan, Goshuyu-to, Hachimi-jio-to, Hange-byakujutsu-temma-to, Hange-koboku-to, Hange-shashin-to, Hochuekki-to, Jumi-haidoku-to, Juncho-to, Juzen-taiho-to, Kakkon-to, Kami-kihi-to, Kami-shoyo-san, Keiga-rengyo-to, Keishi-bukuroy-gan, Keishi-bukuroy-gan-ka-yokuinin, Keishi-ka-jutsu-bu-to, Keishi-ka-shakuyaku-dai-to, Kikyoto, Mao-bushi-saishin-to, Mashinjin-gan, Ninjin-yoei-to, Oren-gedoku-to, Otsui-to, Rikkunshito, Ryo-kei-jutsukan-to, Saiko-ka-ryukotsu-borei-to, Saiko-keishi-kankyoto, Sairei-to, Seihai-to, Seijo-bofu-to, Shakuyaku-kanzo-to, Shimbu-to, Shokenchu-to, Sho-saiko-to, Sho-seiryu-to, Sokke-kakketsu-to, Tokaku-joki-to, Toki-shakuyaku-san, Toki-shigyaku-ka-goshuyu-syokyo-to, Unkei-to, Unsei-in and Yoku-kan-san. The fourteen medicinal herbs were as follows: Bukuroy (Hoelen), Daio (Rhei Rhizoma), Hange (Pinelliae Tuber), Kanzo (Glycyrrhizae Radix), Keihi (Cinnamomi Cortex), Kikyo (Platycodi Radix), Ninjin (Ginseng Radix), Ogon (Scutellariae Radix), Saiko (Bupleuri Radix), Shakuyaku (Paoniaeae Radix), Shokyo (Zingiberis Rhizoma), Sojutsu (Atractylodis lanceae Rhizoma), Taisei (Zizyphi Fructus) and Toki (Angelicae Radix). The twelve components were as follows: the major components of Daio: emodin, rhein, sennidine A, and sennoside A; the major components of Kanzo: glycyrrhetinic acid and glycyrrhizic acid; the major components of Keihi: cinnamaldehyde and cinnamic acid; the major components of Ogon: baicalein, baicalin, and wogonin. Baicalein, baicalin, cinnamaldehyde, cinnamic acid, glycyrrhetinic acid, glycyrrhizic acid, sennoside A, and wogonin were obtained from Wako Pure Chemicals (Osaka, Japan). Emodin and rhein were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sennidine A was obtained from ChromaDex (Santa Ana, CA, USA). UDP-glucuronic acid (UDP-GA), alamethicin, β-estradiol, and β-estradiol 3-glucuronide were purchased from Sigma-Aldrich. SN-38 was purchased from Toronto Research Chemicals (North York, Canada). SN-38 glucuronide was kindly provided by Yakult Honsha (Tokyo, Japan). Pooled human liver microsomes and pooled human jejunum microsomes were purchased from BD Gentest (Woburn, MA, USA) and KAC (Kyoto, Japan), respectively. All other chemicals and solvents were of analytical or the highest grade commercially available.

Inhibition analyses of β-estradiol 3-glucuronidation: A typical incubation mixture (total volume, 0.2 mL) contained 20 μM β-estradiol, 50 mM Tris-HCl buffer (pH 7.4), 10 mM MgCl2, 120 μg of alamethicin/mg microsomal protein, 2 mM UDP-GA, 0.2 mg/mL human liver microsomes or human jejunum microsomes, and Kampo, medicinal herb or component. In the preliminary study, the rate of this activity was linear with respect to the microsomal protein concentration and incubation time under the present experimental conditions. β-Estradiol and alamethicin were dissolved with dimethyl sulfoxide and 30% methanol, respectively. The Kampo and medicinal herbs were dissolved with distilled water. All components except glycyrrhizic acid (distilled water) were dissolved with dimethyl sulfoxide. The final concentration of the organic solvent in the reaction mixture was <1% (v/v) for the Kampo and herbal medicine and <2% (v/v) for the components. In the preliminary study, organic solvent concentration did not affect enzyme activity up to 2% (v/v). The reaction mixture was incubated for 10 min at 37°C. The concentration of Kampo was 1 mg/mL. The concentrations of medicinal herbs were as follows: Shokyo, 0.1 mg/mL; Hange and Toki, 0.5 mg/mL; Bukuroy, Daio, Kanzo, Keihi, Kikyo, Ninjin, Ougon, Saiko, Shakuyaku, Sojutsu, and Taisei, 1.0 mg/mL. The concentrations of components in liver microsomes were 50 and 100 μM except for cinnamalde-
hyde (10 μM) and those in jejunum microsomes were 1 mM except for cinnamaldehyde (10 μM) and rhein (250 μM). The concentrations of cinnamaldehyde and rhein were maxima in the present experimental conditions.

After removal of protein by centrifugation at 1,000 g for 10 min, a portion of the sample was subjected to high-performance liquid chromatography with a Develosil C30-UG 5-μm analytical column (4.6 × 150 mm, Nomura Chemical, Aichi, Japan) or TSK-GEL 80Ts 5-μm analytical column (4.6 × 250 mm, Tosoh, Tokyo, Japan).

Product formation was measured as described previously15 with slight modification. The mobile phase was 30% methanol: 3 mM sodium 1-octanesulfonate (pH 2.5) = 22:6:72 and the flow rate was 1.0 mL/min. The eluent was monitored fluorometrically (excitation, 370 nm; emission, 425 nm). For determination of Ki in human liver microsomes, the concentration of SN-38 ranged from 10 to 80 μM and the inhibitors ranged as follow: Kampoo: Bofu-tsusho-san, 50–200 μg/mL; Hange-shashinto, 40–250 μg/mL; Mashinin-gan, 50–200 μg/mL; Oren-gedoku-to, 100–400 μg/mL; Otsuji-to, 120–400 μg/mL; Tokaku-joki-to, 40–200 μg/mL.

Medicinal herb: Daio, 20–60 μg/mL; Kanzo, 40–160 μg/mL; Ogon, 30–90 μg/mL; Keihi, 40–120 μg/mL.

Component: baicalein, 20–80 μM; cinnamaldehyde, 1–3 mM; emodin, 50–150 μM; glycyrrhetinic acid, 20–80 μM; sennidine A, 20–80 μM; wogonin, 20–80 μM.

**Results**

**Inhibitory effects of Kampoo on β-estradiol 3-glucuronidation in human liver microsomes:** The inhibitory effects of 51 Kampoo, 14 medicinal herbs, and their components on β-estradiol 3-glucuronidation in human liver microsomes were determined. As shown in **Figure 1**, β-estradiol 3-glucuronidation in human liver microsomes was strongly inhibited (>90% inhibition) by 12 Kampoo, (Bofu-tsusho-san, Juncho-to, Kakkon-to, Keigai-rensyo-to, Keishi-ka-shakuyaku-daio-to, Mashinin-gan, Otsuji-to, Ryo-kei-jutsu-kan-to, Saiko-keishi-kankyo-to, Seijo-bofu-to, Shakuyaku-kanzo-to, and Tokaku-joki-to). All Kampoo except Chorei-to exhibited more than 50% inhibition on this activity. As shown in **Figure 2A**, the activity was strongly inhibited by 4 medicinal herbs, Daio, Kanzo, Keihi, and Ogon. The major components of Daio (emodin and sennidine A), Keihi (cinnamaldehyde) and Ogon (baicalein and wogonin) inhibited the β-estradiol 3-glucuronidation (**Fig. 2B**). The inhibitory potencies of aglycones (glycyrrhetinic acid and baicalein) were much stronger than those of glycosides (glycyrrhizic acid and baicalin).

**Inhibition constants for β-estradiol 3-glucuronidation in human liver microsomes:** K of the Kampoo, medicinal herbs and their components are shown in **Table 1** and **Figure 3**. The inhibition pattern of Bofu-tsusho-san was competitive but 2 other Kampoo exhibited mixed-type inhibition. The medicinal herbs exhibited similar or lower apparent K compared with Kampoo. In the case of the components, apparent K of cinnamaldehyde, emodin, glycyrrhetinic acid, sennidine A, and wogonin were 0.7, 8.8, 28.8, 23.4 and 2.8 μM, respectively.

**Inhibitory effects of Kampoo on β-estradiol 3-glucuronidation in human jejunum microsomes:**
Fig. 1. Inhibitory effects of Kampo on β-estradiol 3-glucuronidation in human liver microsomes

The concentrations of β-estradiol and Kampo were 20 μM and 1 mg/mL, respectively. Each data point represents the mean of duplicate determinations. Control activity was 1.5 nmol/min/mg protein.

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The medicinal herbs and their components, which exhibited strong inhibition on β-estradiol 3-glucuronidation in human liver microsomes, were also investigated for their inhibitory potencies in human jejunum microsomes (Fig. 4). The inhibitory effects of medicinal herbs and their components in human jejunum microsomes tended to be similar to those in liver microsomes. However, sennoside A and wogonin exhibited strong inhibition only in human jejunum and liver microsomes, respectively.

Inhibitory effects of Kampo on SN-38 glucuronidation in human liver microsomes: The inhibitory effects of 51 Kampos, 14 medicinal herbs and their components on SN-38 glucuronidation in human liver microsomes were determined. As shown in Figure 5, SN-38 glucuronidation in human liver microsomes was strongly inhibited by 7 Kampos (Bofu-tussho-san, Mashin-in-gan, Oren-gedoku-to, Otsuji-to, Saiko-keishi-kankyo-to, Shakuyaku-kanzo-to, and Tokaku-joki-to). As shown in Figure 6A, the activity was strongly inhibited by 4 medicinal herbs, Daio, Kanzo, Keihi, and Ogon. These 4 medicinal herbs exhibited strong inhibition on both SN-38 and β-estradiol glucuronidation. The major components of Daio (emodin and sennidine A), Kanzo (glycyrrhetic acid), Keihi (cinnamaldehyde), and Ogon (baicalein, baicalin, and wogonin) inhibited the SN-38 glucuronidation (Fig. 6B).

Inhibition constants for SN-38 glucuronidation in human liver microsomes: Apparent K_i of the Kampos, medicinal herbs and their components are shown in Table 2 and Figure 7. The Kampos and medicinal herbs except Hange-shasin-to exhibited mixed-type inhibition. Cinnamaldehyde, a major component of Keihi, was the most potent inhibitor among all the com-
ponents examined. Apparent $K_i$ of the Kampo, medicinal herbs and their components on SN-38 glucuronidation were slightly higher than those on the $\beta$-estradiol 3-glucuronidation.

**Inhibitory effects of Kampo on SN-38 glucuronidation in human jejunum microsomes:** The medicinal herbs and their components, which exhibited strong inhibition on SN-38 glucuronidation in human liver microsomes, were also investigated for their inhibitory potencies in human jejunum microsomes (Fig. 8). The inhibitory effects of the medicinal herbs and their components in human jejunum microsomes were similar to those in liver microsomes.

### Discussion

UGT1A1 is expressed mainly in liver and intestine in humans and plays an important role in the glucuronidation of $\beta$-estradiol and SN-38. In the present study, many Kampo exhibited strong inhibition toward $\beta$-estradiol and SN-38 glucuronidation. Kampo with low $K_i$ for $\beta$-estradiol 3-glucuronidation also strongly inhibited SN-38 glucuronidation. The inhibitory potencies on $\beta$-estradiol 3-glucuronidation were well correlated with those on SN-38 glucuronidation ($r = 0.87$) in the 51 Kampo. In the Kampo that exhibited more than 80% inhibition of both $\beta$-estradiol and SN-38 glucuronidation, Kanzo, Kei-

**Table 1.** Inhibition constants of $\beta$-estradiol 3-glucuronidation by Kampo, medicinal herb and their components in human liver microsomes

<table>
<thead>
<tr>
<th>Kampo</th>
<th>$K_i$ (μg/mL)</th>
<th>Inhibitory type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bofu-tusuo-san</td>
<td>27 ± 4</td>
<td>competitive</td>
</tr>
<tr>
<td>Mashinin-gan</td>
<td>116 ± 31</td>
<td>mixed</td>
</tr>
<tr>
<td>Otsuji-to</td>
<td>178 ± 18</td>
<td>mixed</td>
</tr>
<tr>
<td>Medicinal herb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daio</td>
<td>30 ± 19</td>
<td>mixed</td>
</tr>
<tr>
<td>Kanzo</td>
<td>27 ± 8</td>
<td>mixed</td>
</tr>
<tr>
<td>Keihi</td>
<td>33 ± 3</td>
<td>competitive</td>
</tr>
<tr>
<td>Ogon</td>
<td>23 ± 6</td>
<td>competitive</td>
</tr>
<tr>
<td>Component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baicalein</td>
<td>34.8 ± 1.7</td>
<td>mixed</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>0.7 ± 0.2’</td>
<td>mixed</td>
</tr>
<tr>
<td>Emodin</td>
<td>8.8 ± 2.4’</td>
<td>non-competitive</td>
</tr>
<tr>
<td>Glycyrrhetic acid</td>
<td>28.8 ± 6.3’</td>
<td>mixed</td>
</tr>
<tr>
<td>Sennidine A</td>
<td>23.4 ± 1.9’</td>
<td>non-competitive</td>
</tr>
<tr>
<td>Wogonin</td>
<td>2.8 ± 0.3’</td>
<td>mixed</td>
</tr>
</tbody>
</table>

Fig. 3. Inhibitory effects of Bofu-tusyo-san (A), Ogon (B) and cinnamaldehyde (C) on $\beta$-estradiol 3-glucuronidation in human liver microsomes

Human liver microsomes were incubated with $\beta$-estradiol in the absence or presence of Bofu-tusyo-san, Ogon and cinnamaldehyde as described under Materials and Methods. Each data point represents the mean of duplicate determinations.

Fig. 4. Inhibitory effects of the medicinal herb and its components on $\beta$-estradiol 3-glucuronidation in human jejunum microsomes

The concentration of $\beta$-estradiol was 20 μM. Each data point represents the mean of duplicate determinations. Control activity was 1.5 and 15 nmol/min/mg protein in liver and jejunum microsomes, respectively. ND, not detected.
Fig. 5. Inhibitory effects of Kampo on SN-38 glucuronidation in human liver microsomes
The concentrations of SN-38 and Kampo were 40 \( \mu \text{M} \) and 1 mg/mL, respectively. Each data point represents the mean of duplicate determinations. Control activity was 0.30 nmol/min/mg protein.

Fig. 6. Inhibitory effects of the medicinal herb (A) and its components (B) on SN-38 glucuronidation in human liver microsomes
The concentration of SN-38 was 40 \( \mu \text{M} \). The concentrations of the medicinal herbs were as follows: 0.1 mg/mL, Shokyo; 0.5 mg/mL, Hange and Toki; 1.0 mg/mL, Bukuryo, Daio, Kanzo, Keihi, Kikyo, Ninjin, Ogon, Saiko, Shakuyaku, Sojutsu, and Taiso. The concentration of the component was 1 mM except for cinnamaldehyde (10 \( \mu \text{M} \)) and rhein (250 \( \mu \text{M} \)). Each data point represents the mean of duplicate determinations. Differences in the two values were less than 2.5% from the mean. Control activity was 0.30 nmol/min/mg protein. ND, not detected.

Inhibition of UGT1A1 Activity by Kampo
hi, Ogon and Daio were contained at high frequency. However, the inhibitory potencies of the Kampo s did not depend on the content of these medicinal herbs, indicating that the Kampo s showed complicated inhibition on these activities. Although many components in the Kampo s and medicinal herbs may be involved in the inhibition of these activities, this cannot explain why competitive type inhibition occurred. SN-38 was mainly glucuronized by UGT1A1 in human liver but also by UGT1A6 and UGT1A9.19) \( \beta \)-estradiol was glucuronized mainly by UGT1A1 and slightly by UGT1A3.20) These activities were mainly catalyzed by UGT1A1 in liver, and therefore it is suggested that Kampo s and medicinal herbs inhibit UGT1A1 activity.

In the present study, representative components of medicinal herbs were examined. The contents of major components in Kampo or medicinal herbs are highly variable according to the lot. Japanese Pharmacopoeia determined that Kanzo and Ogon contain more than 2.5% glycyrrhizic acid and 10.0% baicalin, respectively. The content of glycyrrhizic acid in Kanzo ranged from 0.60% to 7.0%.21) The relative content of cinnamaldehyde was 48.4–62.1% in Keihi.22) The content seems to vary depending on the herb. Lot-to-lot differences in Kampo may affect inhibitory effects on UGT activity. Many aglycones and glycosides are known as components of medicinal herbs. The major components of Kanzo were glycyrrhetic acid (aglycone) and glycyrrhizic acid (glycoside).23) and those of Ogon were baicalein (aglycone) and baicalin (glycoside).24) As shown in Figures 2B and 6B, aglycones exhibited stronger inhibition than glycosides. Yokoi et al.25) reported that SN-38 glucuronidation in human liver microsomes was inhibited by aglycones found in herbs, which is consistent with our results. Baicalein, a
substrate for UGT1A1, UGT1A8, and UGT1A9 in humans,26) exhibited mixed-type inhibition on SN-38 glucuronidation. Thus, aglycone in medicinal herbs may also be glucuronized by UGT, leading to inhibition of UGT activity. The major components of Daio, sennoside A and sennoside B,27) are well-known purgatives. One major metabolite of sennoside A is sennidine.28) Thus, in the case of sennoside, the metabolite exhibited stronger inhibition than the parent compound.

The inhibitory patterns of some components were different between β-estradiol and SN-38 glucuronidation. Rios and Tephly29) suggest that UGT1A1 has several binding sites. The substrates (SN-38 and β-estradiol) and/or the components may have different affinities toward each binding site. Sennoside A exhibited strong inhibition in jejenum microsomes but not liver microsomes. β-Estradiol 3- and SN-38 glucuronidation is mainly catalyzed by UGT1A1, but several UGT isoforms are also involved in these activities. One reason for this may be that UGT isoforms expressed in these tissues are different. However, further study is needed to clarify this point.

Concerning many Kampos, it remains unclear how components are absorbed and what their concentrations

Table 2. Inhibition constants of SN-38 glucuronidation by Kampo, medicinal herb and their components in human liver microsomes

<table>
<thead>
<tr>
<th>Kampo</th>
<th>$K_i$ (μg/mL)</th>
<th>Inhibitory type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bofu-tsusho-san</td>
<td>86 ± 49</td>
<td>mixed</td>
</tr>
<tr>
<td>Hange-shashin-to</td>
<td>208 ± 109</td>
<td>competitive</td>
</tr>
<tr>
<td>Mashinin-gan</td>
<td>247 ± 156</td>
<td>mixed</td>
</tr>
<tr>
<td>Oren-gedoku-to</td>
<td>165 ± 78</td>
<td>mixed</td>
</tr>
<tr>
<td>Otsuji-to</td>
<td>640 ± 417</td>
<td>mixed</td>
</tr>
<tr>
<td>Tokaku-joki-to</td>
<td>224 ± 103</td>
<td>mixed</td>
</tr>
<tr>
<td>Medicinal herb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daio</td>
<td>68 ± 7</td>
<td>mixed</td>
</tr>
<tr>
<td>Kanzo</td>
<td>95 ± 14</td>
<td>mixed</td>
</tr>
<tr>
<td>Keihi</td>
<td>105 ± 35</td>
<td>mixed</td>
</tr>
<tr>
<td>Ogon</td>
<td>80 ± 9</td>
<td>mixed</td>
</tr>
<tr>
<td>Component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baicalein</td>
<td>26.9 ± 3.4</td>
<td>mixed</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>3.0 ± 0.4</td>
<td>mixed</td>
</tr>
<tr>
<td>Emodin</td>
<td>60.8 ± 2.7</td>
<td>non-competitive</td>
</tr>
<tr>
<td>Glycyrrhetinic acid</td>
<td>25.4 ± 8.4</td>
<td>non-competitive</td>
</tr>
<tr>
<td>Sennidine A</td>
<td>40.1 ± 9.9</td>
<td>non-competitive</td>
</tr>
<tr>
<td>Wogonin</td>
<td>35.1 ± 8.8</td>
<td>non-competitive</td>
</tr>
</tbody>
</table>

$\mu$M

**Fig. 7.** Inhibitory effects of Bofu-tsusho-san (A), Daio (B) and cinnamaldehyde (C) on SN-38 glucuronidation in human liver microsomes

Human liver microsomes were incubated with SN-38 in the absence or presence of Bofu-tsusho-san, Daio and cinnamaldehyde as described under Materials and Methods. Each data point represents the mean of duplicate determinations.

**Fig. 8.** Inhibitory effects of the medicinal herb and its components on SN-38 glucuronidation in human jejunum microsomes

The concentration of SN-38 was 40 μM. Each data point represents the mean of duplicate determinations. Control activity was 0.30 and 0.15 nmol/min/mg protein in liver and jejunum microsomes, respectively. ND, not detected.
are in plasma in humans, which makes it difficult to estimate drug interactions. According to a report by Foti et al., the estimated gut concentration of herbal mixture (g) was divided by intestinal volume (~500 mL). The dose of Kampo is usually 1.0–1.5 g three times daily in the form of Kampo extracts. Therefore, Kampo concentration in the gut is approximately 2.0–3.0 g/mL. However, the accurate concentration in intestine has not been clarified nor that in liver. The plasma concentration profiles of baicalin have been reported after oral administrations of Sho-saiko-to to humans. In this report, the maximum plasma concentration of baicalin was approximately 55 ng/mL (0.1 μM). Muto et al. suggest that the detected baicalin in plasma may result from glucuronidation of baicalein absorbed into the body. Although baicalein concentration in the liver and intestine may be higher than that in plasma, it is very difficult to compare liver and intestinal concentrations and K in humans due to lack of information. The pharmacokinetics of the major components of Kampo need to be clarified.

Takahashi et al. reported that phase I drug metabolizing enzymes such as CYP3A4 and CYP1A2 were inhibited by Hochu-ekki-to, Sairei-to and Syo-saiko-to in human liver microsomes. The major metabolic pathways of β-estradiol in humans are 2-hydroxylation by CYP3A4 and CYP1A2 and glucuronidation by UGT1A1. In the present study, Sairei-to inhibited β-estradiol 3-glucuronidation suggesting that the plasma concentration of β-estradiol in humans may be elevated by inhibition of phase I and II enzymes. Estrogen drugs are prescribed for menopausal syndrome and menoxenia. As described in a datasheet from Tsumura & Co., Kami-syo-san, Keishibukuro-gan, Keishibukuro-gan-ka-yokuinin, Tokaku-joki-to, Toki-shakuyaku-san, Unkei-to and Unsei-in are prescribed for menopausal syndrome and menoxenia in clinical practice. If β-estradiol is co-administered with Tokaku-joki-to, β-estradiol 3-glucuronidation may be inhibited, leading to elevation of estradiol concentration.

Bilirubin is a specific substrate for UGT1A1. Some flavonoids inhibit bilirubin glucuronidation. Since the present study clarifies the inhibition of UGT1A1 by Kampo, we should be careful about increase in bilirubin level in vivo in humans after treatment with Kampo. Further study on extrapolation from in vitro to in vivo is needed.

In conclusion, the present study clarifies that Kampo inhibits UGT1A1 activity. The components of Kampo, medicinal herbs and their component also demonstrated inhibition. The pharmacological and toxicological effects of Kampo have been studied recently, although the pharmacokinetics of Kampo, especially the components, are still unknown. Therefore, since it is extremely difficult to extrapolate inhibitory effect in vitro to in vivo, we may keep the possibility of drug interactions by Kampo in mind.

Acknowledgments: We acknowledge Tsumura & Co. for kindly providing all the extracts of Kampo and medicinal herbs and Mr. Brent Bell for reviewing the manuscript.

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