Effects of *Kampo* Extracts on Drug Metabolism in Rat Liver Microsomes: *Rhei Rhizoma* Extract and *Glycyrrhizae Radix* Extract Inhibit Drug Oxidation

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ABSTRACT—In the present study, the effects on drug oxidations in rat liver microsomes in vitro using 126 *Kampo* extracts were investigated. Although the effects of inhibition on drug oxidations were dependent on the *Kampo* extracts and probe reactions studied, most of the *Kampo* extracts showed inhibitory effects on both N-demethylations of aminopyrine and erythromycin in rat liver microsomes. Among the *Kampo* extracts studied herein, Daio-kanzo-to exhibited the most remarkable inhibitory effect on both reactions. The *Rhei Rhizoma* extract inhibited not only aminopyrine and erythromycin N-demethylations, but also phenacetin O-deethylation, 7-ethoxycoumarin O-deethylation, ethanol oxidation and tolbutamide 4-hydroxylation in rat liver microsomes. The *Glycyrrhizae Radix* extract also showed a remarkable inhibitory effect on phenacetin O-deethylation as well as aminopyrine and erythromycin N-demethylations. In contrast, the *Glycyrrhizae Radix* extract virtually showed no effect on ethanol oxidation.

Keywords: *Kampo*, Japanese herbal medicine, Drug oxidation, Rat liver microsome

The desired pharmacological response of a patient to drug therapy is the result of the delivery of the drug to its site of action. Therefore, factors that alter the pharmacokinetic properties of a drug may alter the action of the drug. In fact, many drug interactions due to the inhibition by one drug of the metabolism of the other have been shown to cause clinically important events (1 – 4). Recently, clinically important interactions have been reported not only between Western drugs but also between a Western drug and natural compounds included in herbal medicines and foods. Several naturally occurring flavonoids have been reported to possess biological effects on hepatic monooxygenase (5, 6). In addition, since the first publications of the inhibitory effect of grapefruit juice on the metabolism of the dihydropyridine compounds, felodipine and nifedipine (7, 8), the plasma concentration of an increasing number of drugs such as terfenadine, cyclosporine, nitrendipine and nisoldipine have been found to be increased by simultaneous ingestion of grapefruit juice with these drugs (9 – 11). More recently, St John’s wort extracts containing flavonoids, xanthones and naphtodiantrons have been reported to have a potential risk when drugs metabolized by cytochrome P450 (CYP) are taken concomitantly (12, 13).

*Kampo* extracts (Japanese herbal medicines) consist of many herbs containing many low- and high-molecular compounds such as flavonoids, polyphenols and others. Furthermore, *Kampo* extracts are widely used for treatment of many diseases and are frequently taken together with Western drugs. Therefore, it is likely that *Kampo* extracts may affect the pharmacokinetic behavior of a drug concomitantly used.

Several reports have shown that natural herbs contain many compounds inhibiting the activity of CYPs (14 – 17). However, the information concerning pharmacokinetic interactions between *Kampo* extracts and Western medicines is limited at present, and there is no systematic studies with respect to these interactions. Therefore, in the present study, we investigated the effects on drug oxidations in rat liver microsomes in vitro using 126 *Kampo* extracts. The final goal of this project is to clarify a potential of *Kampo* extracts to affect drug metabolism.

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MATERIALS AND METHODS

Materials

The Kampo extracts and herbal extracts were kindly donated from Tsumura Co. (Tokyo). Nicotinamide adenine dinucleotide phosphate (NADP), glucose 6-phosphate and glucose 6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Tokyo). Aminopyrine, umbelliferone, and monomethylol dimethylhydantoin were obtained from Wako Pure Chemical Industries Ltd. (Osaka). Erythromycin and 7-ethoxycoumarin were from Merck (Darmstadt, Germany), Adrich Chemical Co., Inc. (Milwaukee, WI, USA), Kanto Chemical Co., Inc. (Tokyo), and Sigma Chemical Co. (St. Louis, MO, USA), respectively. Other chemicals were of the highest grade commercially available.

Preparation of liver microsomes

Male Sprague-Dawley rats (8-week-old) were fasted overnight prior to sacrifice. After perfusion with 1.15% KCl solution, the livers were excised and homogenized, and the liver homogenate was centrifuged at 9,000 × g for 20 min. The supernatant was ultracentrifuged at 105,000 × g for 60 min, and the resulting microsomal pellets were suspended with ice cold 1.15% KCl solution and ultracentrifuged at 105,000 × g for 30 min. The resulting pellets were homogenized with 50 mM potassium phosphate (pH 7.4) containing 0.1 mM EDTA at a protein concentration of about 20 mg/ml and stored at −80°C until use.

Assay

A typical reaction mixture consisted of 100 mM potassium phosphate (pH 7.4), 0.1 mM EDTA, NADPH-generating system (0.33 mM NADP, 8 mM glucose 6-phosphate, 0.1 unit of glucose 6-phosphate dehydrogenase and 6 mM MgCl₂), microsomes and substrates, and Kampo or herbal extracts were added, if necessary, in a final volume of 1 ml. A reaction mixture without substrates was used as a blank. The reaction was initiated by the addition of the NADPH-generating system and was carried out aerobically with shaking. The concentrations of aminopyrine, erythromycin, 7-ethoxycoumarin, phenacetin, tolbutamide and ethanol were 5 mM, 1 mM, 500 μM, 10 μM, 1 mM and 50 nM, respectively. The Kampo extracts and herbal extracts suspended with distilled water were added to the reaction mixture at a concentration up to 10 mg per ml in order to determine the activity of 7-ethoxycoumarin O-deethylase was determined by essentially the same method previously reported (19). Briefly, the fluorescence intensity due to metabolite was immediately measured at 380 nm (excitation wavelength) and 460 nm (emission wavelength). Umbelliferone (0.2 μM) was used as the standard. Phenacetin O-deethylase activity was measured by HPLC according to the method of Tassaneeyakul et al. (20) using 2-acetamidophenol as an internal standard. The determination of the activity of tolbutamide hydroxylase was carried out according to the HPLC method previously described (21). Ethanol oxidation was measured by the method of Ingelman-Sundberg and Johansson (22). Microsomal protein was determined by the method of Lowry et al. (23) using bovine serum albumin as a standard.

RESULTS

The effects of Kampo extracts on N-demethylations of aminopyrine and erythromycin in rat liver microsomes are shown in Fig. 1. Daio-kanzo-to (TJ-84) inhibited both N-demethylations of aminopyrine and erythromycin in a dose-dependent manner. In contrast, Sho-hange-ka-bukuryo-to (TJ-21) did not show any significant effect on both reactions. From these results, it was suggested that the effects on drug oxidations are dependent on Kampo extracts. Then, Kampo extracts were added to the incubation mixture at a concentration up to 10 mg per ml in order to determine the figure of Sho-bukuryo-to and Dai-kanzo-to on N-demethylations of aminopyrine and erythromycin in rat liver microsomes. Control activities of aminopyrine (closed symbols) and erythromycin (open symbols) in the absence of Kampo extracts were 7.7 and 2.2 nmol/mg per min, respectively. The circle and square symbols represent the activities in the presence of Sho-bukuryo-to and Dai-kanzo-to, respectively. Each plot represents the mean of duplicate determinations.
IC₅₀ value of each Kampo extract against both N-demethylase activities. 38 of 126 Kampo extracts and 28 of them did not have any remarkable effects on N-demethylations of aminopyrine and erythromycin, respectively. However, as shown in Table 1, many of Kampo extracts tested were found to possess inhibitory effects on both N-demethylase activities in varying extents under the experimental conditions used here. The Dai-kozan-to (TJ-84) was found to be one of the Kampo extract containing the components having relatively strong inhibitory effects on both aminopyrine and erythromycin N-demethylations. In contrast, both reactions were virtually unaffected by Anchu-san (TJ-5), Hachimi-jio-gan (TJ-7), Saiko-keishi-to (TJ-10), Hange-koboku-to (TJ-16), Sho-hange-baku-kyuro-to (TJ-21), Bakumondo-to (TJ-29), Shimbu-to (TJ-30), Byakko-ka-ninjin-to (TJ-34), Moku-boi-to (TJ-36), Bukuryo-in (TJ-69), Koso-san (TJ-70), Shimosu-to (TJ-71), Shikunshi-to (TJ-75), Nichin-to (TJ-81), Ogi-kenchu-to (TJ-98),

<table>
<thead>
<tr>
<th>IC₅₀ (mg/ml)</th>
<th>Aminopyrine N-demethylation</th>
<th>Erythromycin N-demethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td>Dai-kozan-to,玛仕応,流川-若草-2</td>
<td>Dai-kozan-to</td>
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<tr>
<td>1.0 – 2.0</td>
<td>Bofu-tsubo-san, 坂沢-若草, 朝日-若草</td>
<td>Dai-kozan-to, 坂沢-若草, 朝日-若草</td>
</tr>
<tr>
<td></td>
<td>Bofu-tsubo-san, 坂沢-若草</td>
<td>Dai-kozan-to, 坂沢-若草</td>
</tr>
<tr>
<td>2.1 – 3.0</td>
<td>Dai-saiko-to, 還元-若草, 紅花-若草, 唐草-若草, 唐草</td>
<td>Dai-kozan-to, 坂沢-若草</td>
</tr>
<tr>
<td>3.1 – 4.0</td>
<td>Chiko-untan-to, 四ツ、紅花-若草</td>
<td>Dai-kozan-to, 坂沢-若草, 透日-若草</td>
</tr>
<tr>
<td></td>
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<td>Dai-kozan-to, 坂沢-若草, 透日-若草</td>
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<tr>
<td>4.1 – 5.0</td>
<td>Boi-ogi-to, 紅花-若草, 紅花-若草</td>
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<td>Dai-kozan-to, 坂沢-若草, 透日-若草</td>
</tr>
<tr>
<td>5.0&lt;</td>
<td>Anchu-san, 柿木-若草</td>
<td>Dai-kozan-to, 坂沢-若草</td>
</tr>
</tbody>
</table>

Table 1. Estimated IC₅₀ values for all studied Kampo extracts against N-demethylation of aminopyrine and erythromycin
Effect of Kampo on Drug Oxidation

Sho-kenchu-to (TJ-99), Dai-kenchu-to (TJ-100), Shomakakkon-to (TJ-101), Rikko-san (TJ-110), Chorei-togoshimotsu-to (TJ-112) and Kikyo-to (TJ-138) did not show any effects on both $N$-demethylations of aminopyrine and erythromycin. On the other hand, Chorei-to (TJ-40) more strongly inhibited of erythromycin $N$-demethylation, than aminopyrine $N$-demethylation. These results indicated that drug oxidations in liver microsomes are differentially influenced by Kampo extracts in varying extents.

Daio-kanzo-to (TJ-84) is a Kampo extract consisting of Rhei Rhizoma extract and Glycyrrhizae Radix extract. Therefore, in order to clarify which herbal medicine is predominantly associated with the inhibitory effect on $N$-demethylations of aminopyrine and erythromycin, the inhibitory effects on both reactions of Kampo extracts containing Rhei Rhizoma extract were compared with those of Kampo extracts that do not contain of Rhei Rhizoma extract (Fig. 2). Each Kampo medicine was added to the reaction mixture at a concentration of 2 mg/ml. The Kampo extracts containing Rhei Rhizoma extract tended to inhibit both reactions more strongly than did the Kampo extracts that do not contain Rhei Rhizoma extract, suggesting that Rhei Rhizoma is, at least in part, responsible for the inhibitory effects of Daio-kanzo-to (TJ-84) on both $N$-demethylations of aminopyrine and erythromycin. In contrast, no significant difference in the inhibitory effects on both reactions was observed among Kampo extracts regardless of the presence of Glycyrrhizae Radix extract. From these results, it was indicated that Rhei Rhizoma extract may be more inhibitory compared to Glycyrrhizae Radix extract against both reactions in rat liver microsomes. Then, to get a better understanding of whether the effects of the extract of Rhei Rhizoma and Glycyrrhizae Radix are dependent on the form of CYPs, the effects of both herbal extracts on the oxidations of probe substrates for CYPs were investigated.

The Rhei Rhizoma extract was found to inhibit not only aminopyrine and erythromycin $N$-demethylations, but also phenacetin $O$-deethylation, 7-ethoxycoumarin $O$-deethylation, ethanol oxidation and tolbutamide 4-hydroxylation in rat liver microsomes. As can be seen in Fig. 3A, $O$-deethylation of phenacetin was most strongly inhibited, followed by 4-hydroxylation of tolbutamide, $N$-demethylation of aminopyrine, $N$-demethylation of erythromycin, $O$-deethylation of 7-ethoxycoumarin and oxidation of ethanol. On the other hand, the Glycyrrhizae Radix extract also showed a remarkable inhibitory effect on phenacetin $O$-deethylation (Fig. 3B). In addition, 7-ethoxycoumarin $O$-deethylation was also decreased by the addition of Glycyrrhizae Radix extract, but the extent of the reduction was smaller than that observed in aminopyrine and erythromycin $N$-demethylations. Ethanol oxidation was virtually unaffected by Glycyrrhizae Radix extract in the range of concentrations investigated. These results indicated that the effects of Rhei Rhizoma and Glycyrrhizae Radix extracts

![Fig. 2. Comparison of inhibitory effects of Kampo extracts on aminopyrine and erythromycin $N$-demethylations. The activities of aminopyrine (A) and erythromycin (B) $N$-demethylases were measured in the presence of Kampo extracts at a concentration of 2 mg/ml. Each value represents percent of control.](image-url)
A. Hasegawa et al. may be dependent on the form of CYPs in rat liver microsomes and that both extracts, at least, may contain ingredients that inhibit CYP1A enzymes. The IC$_{50}$ of Rhei Rhizoma and Glycyrrhizae Radix against phenacetin O-deethylase were estimated to be 40 and 80 µg/ml, respectively.

DISCUSSION

In the present study, we investigated the effects on drug oxidations of 126 Kampo extracts in rat liver microsomes. Aminopyrine, which is known to be a substrate for several forms of CYPs, appeared to be one of the good probe substrates for investigating the effect of Kampo extracts on drug oxidation. Additionally, many drugs metabolized by CYPs are known to be a substrate for CYP3A enzymes. Therefore, aminopyrine and erythromycin were primarily used for screening in the present study.

In the range of concentrations studied, most of the Kampo extracts tested herein decreased the activity of aminopyrine and erythromycin N-demethylases in rat liver microsomes. Among the 126 Kampo extracts, judging from the IC$_{50}$ values, Daio-kanzo-to (TJ-84) was found to be one of the Kampo extracts showing the most strong inhibitory effect on both reactions. Since the Daio-kanzo-to (TJ-84) is a Kampo extract consisting of two herbal extracts, Rhei Rhizoma and Glycyrrhizae Radix (2:1), it was likely that Rhei Rhizoma and/or Glycyrrhizae Radix may inhibit these reactions in rat liver microsomes. Therefore, the inhibitory effect on aminopyrine and erythromycin N-demethylations of Kampo extracts containing Rhei Rhizoma was compared with that of Kampo extracts containing Glycyrrhizae Radix.

Among 16 Kampo extracts containing Rhei Rhizoma, 10 of those also contain Glycyrrhizae Radix. There was a significant difference in an inhibitory effect between Kampo extracts with and without Rhei Rhizoma. In contrast, there was no difference between Kampo extracts with and without Glycyrrhizae Radix, suggesting that Rhei Rhizoma may be more effective in inhibiting drug oxidations compared to Glycyrrhizae Radix. In fact, both Rhei Rhizoma and Glycyrrhizae Radix inhibited drug oxidations including phenacetin O-deethylation, ethanol oxidation and 7-ethoxycoumarin O-deethylation but the extents of the inhibition with Rhei Rhizoma were greater than with Glycyrrhizae Radix.

The possibility that Rhei Rhizoma, Glycyrrhizae Radix, or both also inhibit the activity of NADPH-cytochrome P450 reductase cannot be excluded. However, the inhibitory effects of these herbal medicines on drug oxidations were dependent on the probe reactions used, suggesting that a major part, if not all, of the inhibitory effects may be due to decreases in activities of CYPs. Rhei Rhizoma has been demonstrated to reduce the mutagenicity of mutagens including 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), aflatoxin B1 and benzo(a)pyrene (24, 25). In addition, it has been reported that emodin, one of the anthraquinone components of Rhei Rhizoma, inhibited the mutagenic activation of cooked food-derived compounds, Trp-P-2 and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) which are known to be substrates for CYP1A enzymes, and that emodin was also a substrate for CYP1A enzymes and was metabolically activated to a direct-acting mutagen (26–

![Fig. 3. Inhibitory effects of Rhei Rhizoma and Glycyrrhizae Radix extracts on drug oxidations in rat liver microsomes. The activities of aminopyrine N-demethylase (open square), erythromycin N-demethylase (closed circle), phenacetin O-deethylation (closed circle), 7-ethoxycoumarin O-deethylation (open circle) and tolbutamide 4-hydroxylase (open triangle) in the absence of Rhei Rhizoma extract (A) were 7.9, 2.3, 3.2, 0.1, 379.2 and 0.97 nmol/mg protein per min, respectively, and those of aminopyrine N-demethylase (open square), erythromycin N-demethylase (closed circle), phenacetin O-deethylation (open circle), and 7-ethoxycoumarin O-deethylation (open triangle) in the absence of Glycyrrhizae Radix extract (B) were 7.7, 2.2, 3.5, 0.1 and 401.4 nmol/mg protein per min, respectively.](image-url)
30). Furthermore, it has been demonstrated that the components of Rhei Rhizoma other than emodin, chrysophanol and rhein, also exerted antimutagenic activity against Trp-P-2 due to inhibition of the N-hydroxylation (31). Together with these results, it is likely that the inhibitory effect of Rhei Rhizoma on phenacetin and 7-ethoxycoumarin O-deethylations, which are considered to be probe reactions for CYP1A enzymes, may be partly attributable to anthraquinones in this herbal extract. However, it is unknown at present whether the anthraquinone components such as emodin, chrysophanol and rhein in Rhei Rhizoma are responsible for the inhibitory effects on ethanol oxidation, tolbutamide 4-hydroxylation and erythromycin N-demethylation, which are considered to be probe reactions for CYP2E, CYP2C and CYP3A enzymes, respectively. The study to clarify which ingredients of Rhei Rhizoma contribute to the inhibitory effects on drug oxidations is now in progress.

REFERENCES


