Effects of Continuous Ingestion of Herbal Teas on Intestinal CYP3A in the Rat

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Abstract. Tenryocha, rooibos, and guava teas are widely consumed as herbal beverages, especially as a therapy against pollen allergy. To investigate the possible herbal tea-drug interaction the effect of continuous ingestion of these teas on cytochrome P450 (CYP) 3A were studied. Rats (n = 6) were allowed free access to either tea (experimental groups) or water (control) for two weeks. Midazolam (MDZ) (20 mg/kg) was orally administered and the serum concentration was determined. The area under the serum concentration-time curve (AUC₀-∞) and the maximum serum concentrations (Cmax) of MDZ were reduced by more than 60% after the treatment of tenryocha and rooibos tea (P < 0.05). Intestinal MDZ 1'- and 4-hydroxylation activities mediated by CYP3A were increased in tenryocha and rooibos tea-treated group by 50% compared to the control group, although the results were not statistically significant. Furthermore, the Western blot analysis showed that CYP3A content was significantly increased in the intestine after the treatment of these teas (P < 0.05). Hepatic MDZ hydroxylation and CYP3A content were slightly increased by these teas. The results suggested that two weeks ingestion of tenryocha and rooibos tea reduced serum concentration of MDZ by the induction of intestinal CYP3A. The possible interaction between tenryocha or rooibos tea and medicines mediated by CYP3A was suggested.

Keywords: CYP3A, herbal tea, herb-drug interaction, tenryocha, rooibos

Introduction

Cytochrome P450 (CYP) is well known as one of the most important drug-metabolizing enzymes. Among various CYP isoforms, CYP3A4 is expressed predominantly in human liver and small intestine and is involved in the metabolism of more than 50% of clinically important drugs, including immunosuppressive agents, antibiotics, calcium-channel blockers, and HIV protease inhibitors (1 – 3). Therefore, the activity of CYP3A4 can determine the efficacy of various therapeutic drugs in patients, making it very important to investigate the drug-drug interaction mediated by CYP3A4. The inhibition of CYP3A4 may result in enhanced blood and tissue concentrations of these drugs, leading to toxicity, while the induction of this enzyme may cause reduced drug concentrations, leading to decreased efficacy and treatment failure. In fact, it has been reported that the activity of CYP3A4 is modulated by inducers such as rifampicin, dexamethasone, and phenobarbital or by inhibitors such as ketoconazole, itraconazole, and cimetidine; and severe drug-drug interactions were caused by co-administration of these drugs and CYP3A4 substrates (4).

Recent studies indicated that substances causing these interactions are not limited to chemical drugs, natural products such as foods or herbal supplements may also change the CYP3A4 activity (5). Herbal supplements have been noticed as an alternative to clinical drugs lately, and its consumption has been increasing rapidly not only in the United States but also in European and Asian countries.

Some reported studies on herb-drug interactions revealed that the extract of herbal supplements including ginkgo leaf, echinacea, and milkthistle inhibited CYP3A4 activity in human liver microsomes (6 – 8).
In fact, the modulation of CYP3A-mediated metabolism in human subjects was observed by echinacea, while milkthistle and ginkgo showed minimal risk for herb-drug interaction via CYP3A in human studies (9–11). However, such experiments have been done using only a few of herbs and currently there is insufficient data for predicting herb-drug interactions. In addition, although herbal teas are one of the popular forms to intake herbs all over the world, there have been few reports concerning the effects of herbal teas on CYPs.

Tenryocha, rooibos, and guava tea, which are made from the dried leaves of Rubus suavissimus (RS), Aspalathus linearis (AL), and Psidium guajava (PG), respectively, are common in Japan as herbal beverages. These teas are reported to suppress the release of the histamine from mast cells and therefore expected to relieve pollen allergy, asthma, and other chronic allergies (12–15). The pollen allergy raging throughout Japan these days affects one in five persons in the center of Tokyo. Under this situation, these herbal teas, which are easily available at the drug store, supermarket, and also through the internet, might be popularly consumed to prevent and improve allergic status. Therefore, it is a big concern that some patients are taking these herbal teas along with therapeutic medicines without being aware of possible herb-drug interactions. Because CYP3A4 is the key enzyme in the metabolism of a large number of clinically used drugs, it is important to clarify the influence of these herbal teas on CYP3A4-mediated drug metabolism.

In our previous study, the short period multiple ingestion of tenryocha tea did not cause any remarkable change in the pharmacokinetic parameter of simvastatin in healthy human subjects (16). However, because these teas are usually used for a long time period, it is important to investigate the effects of long-term ingestion of the teas on CYP3A4-mediated activity.

The present study was conducted to investigate the effect of two-week ingestion of tenryocha, rooibos, and guava tea on CYP3A4-mediated drug metabolism in rats, using MDZ as a CYP3A probe substrate. The CYP3A activity and content were also determined in the liver and intestine after the continuous ingestion of the teas.

Materials and Methods

Chemicals

MDZ maleate was kindly donated by Nippon Roche Co., Ltd. (Tokyo). The following drugs were obtained from the indicated commercial sources: 1'-hydroxy-midazolam and 4-hydroxymidazolam (Daichi Pure Chemicals Co., Ltd., Tokyo); glucose-6-phosphate dehydrogenase (G-6-P-DH) and nicotinamido adenine dinucleotide phosphate (NADPH) (Oriental Yeast Co., Ltd., Tokyo); glucose-6-phosphate (G-6-P) (Sigma Chemical Co., St. Louis, MO, USA); nitrazepam and diazepam (Wako Pure Chemical, Osaka). The anti-CYP2C11 and anti-CYP3A2 antibodies were purchased from Daichi Pure Chemicals Co., Ltd. All other chemicals were of analytical grade.

Herbal tea preparations

The dried leaves of tenryocha (Rubus suavissimus S. Lee), rooibos (Aspalathus linearis), and guava (Psidium guajava L.) were purchased from Hondi-en Co., Ltd. (Okayama). Herbal tea solutions were prepared every second day in the usual way of brewing by decoction; the tea leaves were added to boiled tap water and simmered gently (tenryocha: 3 g/L for 5 min, rooibos: 4 g/L for 5 min, guava: 3 g/L for 3 min).

For the in vitro experiment, the teas were prepared the same way except 3 g of tea leaves were simmered in 200 mL of boiled tap water for 10 min. These prepared teas were established as 100% herbal tea and each tea was used after the dilution to 2.5% or 10% as the final concentration.

Treatment of animals with herbal teas

Male Sprague-Dawley rats (Japan Laboratory Animals, Inc., Tokyo), weighing 220–230 g, were used in this study. Twenty-four rats were divided into four groups of six rats each. They were housed in a temperature-controlled room with a 12-h light and dark cycle and fed the standard laboratory chow. Rats had free access to one of the herbal teas or water: tenryocha (RS group), rooibos (AL group), guava (PG group), or water (control group) for two weeks. The teas were freshly prepared every second day. The fluid intake and body weights were monitored every other day. The rats were fasted overnight before the experiments.

The procedures of this study have been reviewed and approved by the Showa University Ethics Committee for Animal Care and Use.

Determination of serum midazolam concentration

MDZ was orally administrated to rats at the dose of 20 mg/kg after 2 weeks of herbal tea treatment. A 200-µL sample of blood was collected from the jugular vein at 0, 15, 30, 45, 60, 90, 180, 240, and 360 min after MDZ administration. The blood samples were centrifuged at 7,500 × g for 10 min at 4°C and each separated serum was stored at −80°C until analysis.

The serum concentration of MDZ was determined according to the method of Mandema et al. with slight modifications (17). Briefly, 100 µL of each serum was diluted with 500 µL of 0.1 N NaOH, and 25 ng of
Diazepam was added as an internal standard. The mixture was extracted with 5 mL of dichloromethane : pentane (1:1) for 30 s and centrifuged at 1,600 × g for 10 min. The upper organic phase was transferred into a clean glass tube and evaporated to dryness. The dried residue was dissolved in 100 µL of mobile phase, and 70 µL was injected onto an HPLC equipped with a CAPCELLPAK SG120 column (4.6 mm × 250 mm; Shiseido, Tokyo) at room temperature. The mobile phase consisted of 10 mM potassium phosphate buffer (pH 5.0) : acetonitrile (50:50) and the flow rate was 0.8 mL/min. The HPLC instrumentation included an SCL-10A VP system controller (Shimadzu, Kyoto), a CCPM-II pump (Tosoh, Tokyo), a DG-100 degasser (Eicom, Kyoto), an HPLC auto sampler 460 (Kontron Instruments, Zurich, Switzerland), a Shimadzu SPD-10A VP detector (set at 220 nm), and an Fujitsu computer running Shimadzu software version CLASS-VP.

**Pharmacokinetic analysis**

The area under the serum concentration-time curve (AUC$_{0-\infty}$) was calculated according to the trapezoidal rule. The apparent elimination rate constant (K$_e$) was estimated from the gradient of the elimination phase. The elimination half-life (t$_{1/2}$) was calculated from the following equation: t$_{1/2}$ = ln2/K$_e$. The maximum serum concentrations (C$_{max}$) and the time to reach C$_{max}$ (t$_{max}$) of MDZ were obtained from the actual data.

**Preparation of liver and intestinal microsomes**

Rats were sacrificed at 6 h after MDZ administration. Liver microsomal preparation was performed based on a conventional fractional centrifugation method and suspended in 100 mM Na$^+$-K$^+$ phosphate buffer (pH 7.4) containing 20% glycerol (18). Pooled liver microsomes were also prepared from 10 non-treated male Sprague-Dawley rats to investigate the effect of herbal teas on MDZ hydroxylation in vitro.

The intestinal microsomes were prepared by the method of Bondovsky et al. (19) and Koudriakova et al. (20) with minor modifications. The obtained intestinal microsomes were suspended in Solution D (100 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol and 10 mM EDTA).

All microsomal fractions were frozen by liquid nitrogen and kept at −80°C until used. Protein concentrations were measured by the method of Lowry et al. (21).

**Assay of MDZ hydroxylation activities**

MDZ 1′-hydroxylation (MDZ 1′-OH) and 4-hydroxylation (MDZ 4-OH) activities were determined as described previously (22). Liver and intestinal microsomes prepared at 6 h after MDZ treatment were used in the ex vivo study.

For the in vitro study, MDZ 4-OH activity was measured using pooled rat liver microsomes in the presence of herbal teas at the final concentration of 2.5% or 10%, as described above. Control activity was also determined in the same conditions but with addition of water instead of the herbal teas.

**Total hepatic CYP content**

The total P450 content in liver microsomes was determined by the methods described by Omura and Sato (23).

**Immunoblot analysis of liver and intestinal CYPs**

It has been reported that despite 4-hydroxymidazolam formation in rat liver being mediated almost exclusively by CYP3A1 and CYP3A2, MDZ 1′-OH was mediated by these CYP3As along with CYP2C isoforms (24, 25). However, CYP2Cs are known to not be expressed in rat intestine (26). Thus, Western blot analysis was carried out to detect the levels of hepatic CYP2C11 along with that of CYP3A isoforms in hepatic and intestinal microsomes (27). Liver (10 µg) and intestinal (50 µg) microsomes were electrophoresed in a 10% polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). Immunodetection was performed using anti-rat CYP2C11 and CYP3A2 antibodies according to the manufacturer’s instructions. The intensities of the immunoblots were estimated using a Gel-Pro Analyzer (Media Cybernetics, Inc., Silver Spring, MD, USA) adapted for the Microsoft computer.

**Statistical analyses**

Differences between the groups were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett t-test. A difference of P<0.05 was considered to be statistically significant.

**Results**

**In vitro study**

To examine whether the herbal teas inhibit CYP3A, the effect of the teas on MDZ 4-OH activity was investigated using pooled rat liver microsomes.

All of the 10% teas showed potent inhibition on MDZ 4-OH activity by more than 80% compared to the control group: 459 pmol · mg protein$^{-1}·$min$^{-1}$ in the AL group versus control group (2084 pmol · mg protein$^{-1}·$min$^{-1}$) and the values were under the detection limit in the RS and PG groups. Tenryocha tea showed 80% inhibition of this activity even at the concentration of 2.5%:
437 pmol·mg protein\(^{-1}\)·min\(^{-1}\) in the RS group compared to the control group (2084 pmol·mg protein\(^{-1}\)·min\(^{-1}\)).

**In vivo study**

There were no significant differences in the body weight of rats between the control group (308 ± 13 g) and RS (307 ± 20 g), AL (299 ± 10 g), and PG (297 ± 14 g) groups on the day of the experiment. The amounts of fluid intake in each group during the last 24 h of tea or water treatment were similar: 330, 325, 285, and 345 mL in the control, RS, AL, and PG group, respectively.

**MDZ pharmacokinetics**

The serum MDZ concentration-time profiles after two-week treatment of herbal teas are shown in Fig. 1. The serum concentrations of MDZ were significantly lower in the RS and AL groups than in the control group. The pharmacokinetic parameters are summarized in Table 1. Two-week ingestion of tenryocha tea significantly reduced AUC\(_{0-\infty}\) and C\(_{\text{max}}\) of MDZ by 60% compared to the control group. Rooibos tea also caused a significant reduction in AUC\(_{0-\infty}\) and C\(_{\text{max}}\) of MDZ by 70%. On the other hand, ingestion of guava tea had little or no effect on MDZ pharmacokinetics. There was no significant change of the t\(_{1/2}\) value of MDZ in any herbal tea-treated group.

**Total hepatic CYP contents**

No significant differences were observed in total hepatic CYP contents between the control group (582.89 ± 59.54 pmol·mg protein\(^{-1}\)) and herbal tea-treated groups (555.29 ± 89.05, 547.54 ± 99.26, and 498.12 ± 84.36 pmol·mg protein\(^{-1}\)) in RS, AL, and PG group, respectively.

**MDZ hydroxylation activity**

Hepatic MDZ 4-OH activity tended to increase in the RS and AL groups, by 40% and 25%, respectively, compared to the control group, but differences were not statistically different (Table 2). There was no remarkable change in hepatic MDZ 1'-OH activity in any tea-treated group.

In contrast, the treatment of tenryocha and rooibos tea resulted in the increase of both MDZ 1'-OH and MDZ 4-OH activities in intestinal microsomes, each by approximately 50% compared to control group, although these differences were not statistically significant.

**Immunoblot analysis**

The expression of hepatic CYP3A2 was slightly increased in the RS and AL groups, by about 25% and 15%, respectively, but they were negligible (Fig. 2A). There was no change in the expression of the band of hepatic CYP2C11 between any herbal tea-treated group and the control group (Fig. 2B).

In the analysis using intestinal microsomes, several bands were found at slightly different positions than CYP3A2. It has been reported that CYP3A9, CYP3A18, and CYP3A62 are mainly expressed instead of CYP3A1 and CYP3A2 in rat intestine (28, 29). In this study, we could not isolate these CYP3A isoforms because we used polyclonal CYP3A2 antibody that might have cross-reactivity to other CYP3A isoforms. However, the density of the total intestinal CYP3A bands were significantly increased by 78% in the RS group and by 62% in the AL group compared to the control group (Fig. 3).

**Discussion**

In recent years, herbal teas such as tenryocha, rooibos, and guava tea are widely used to prevent and relieve
pollen allergy, asthma, and other chronic allergies in Japan. The current study was conducted to clarify whether there may be a herbal teas-drug interaction via CYP3A. Our results showed that two weeks ingestion of tenryocha and rooibos tea caused significant declines in AUC and $C_{\text{max}}$ of MDZ while there was no change in the elimination half life (Table 1).

MDZ is a short-acting benzodiazepine derivative that is metabolized to 1'-hydroxymidazolam and 4-hydroxy-midazolam mainly by CYP3A4 in humans and by CYP3A2 in rats, and it has been recognized as a sensitive prove to investigate CYP3A function (30, 31). CYP3A enzyme is known to be expressed mainly in the liver and intestine, and both of them play important roles in drug metabolism. For this reason, either site can participate in the change of serum MDZ concentration. In our present study, we did not investigate the effects of the teas on MDZ metabolism by intravenous infusion, which usually reflects the contribution of hepatic metabolism. The reason is that the hepatic clearance of MDZ is blood-flow-limited and the modulation of hepatic CYP3A would not affect its total body clearance by the intravenous administration in rats (32, 33). When the hepatic CYP3A enzyme is induced, the oral administration of such a blood-flow-limited drug causes a decrease in its bioavailability, $C_{\text{max}}$, and AUC without changing its elimination half life. Therefore, even though there would be no change in the elimination half life of MDZ, the induction of hepatic and intestinal CYP3A by continuous ingestion of tenryocha and rooibos tea was considered.

To confirm the induction of CYP3A by these teas, we determined the CYP3A activity using MDZ 1'-OH and 4-OH in liver and intestinal microsomes prepared after two-week ingestion of the teas. The result showed that 4-hydroxylation, which is the main metabolic pathway of MDZ in rat liver, was increased by 40% in the RS group and by 25% in the AL group compared to the control in liver microsomes, but these differences did not reach statistical significance (Table 2). Also, no remarkable

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<th>Table 2. Effect of two-week ingestion of herbal tea on hepatic and intestinal midazolam hydroxylation in rats</th>
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<td><strong>Midazolam hydroxylation activity (pmol·min$^{-1}$·mg protein$^{-1}$)</strong></td>
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<td><strong>1'-hydroxylation</strong></td>
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Data represent the mean ± S.D. (n = 6). RS: *Rubus suavissimus* (tenryocha tea), AL: *Aspalathus linearis* (rooibos tea), PG: *Psidium guajava* (guava tea).

Fig. 2. Effect of two-week ingestion of herbal tea on the expression of CYP3A2 (A) and CYP2C11 (B) in rat liver. Data represent the mean % of control density ± S.D. (n = 6). RS: *Rubus suavissimus* (tenryocha tea), AL: *Aspalathus linearis* (rooibos tea), PG: *Psidium guajava* (guava tea).

Fig. 3. Effect of two-week ingestion of herbal tea on the expression of CYP3A in rat intestine. Data represent the mean % of control density ± S.D. (n = 6). *Significantly different from the control (P<0.05). RS: *Rubus suavissimus* (tenryocha tea), AL: *Aspalathus linearis* (rooibos tea), PG: *Psidium guajava* (guava tea).
change was observed in another metabolic pathway, hepatic MDZ 1'-OH. There have been some reports concerning the different contribution rate of CYP3A isoforms and the participation of other CYP isoforms in MDZ 1'-OH and MDZ 4-OH in rats (24, 25). These reports showed that MDZ 1'-OH is mediated by CYP3A1/3A2 along with CYP2C isoforms, especially CYP2C11, whereas MDZ 4-OH is almost mediated by these CYP3A isoforms. Then we performed Western blot analysis to detect both hepatic CYP3A2 and CYP2C11 protein content. The results showed only minor changes of CYP3A2 and CYP2C11 contents in the tea-treated groups (Fig. 2: A and B). This was suggested that hepatic CYP3A2 and CYP2C11 might not be changed by the teas. In contrast, two-week treatment of these teas resulted in the increase of intestinal MDZ 1'-OH and MDZ 4-OH activities by about 50% compared to the control group, even though the results did not reach statistical significance (Table 2). Western blot analysis was also performed to confirm if intestinal CYP3A protein was increased in these groups. In this analysis, several bands were found at slightly different positions than CYP3A2. It has been shown that CYP3A1 and CYP3A2 are not expressed in rat intestine; and instead, the mainly expressed forms are CYP3A9, CYP3A18, and CYP3A62 (28, 29). Although it was impossible to isolate these CYP3A isoforms in this study since we used a polyclonal CYP3A2 antibody, it seemed to cross-react with the other CYP3A isoforms and provided a band corresponding to the total CYP3As. The density of the total intestinal CYP3A band in the RS and AL groups was significantly increased more than 60% compared to that in the control group (Fig. 3). It has been reported that CYP2C isoforms were not detected in rat intestine, and therefore, MDZ 1'- and 4-OH might be both metabolized mostly by CYP3As in rat intestine (26). Considering these results, it was suggested that the two-week ingestion of tenryocha and rooibos tea induced intestinal CYP3A and resulted in the significant decrease of serum MDZ concentration.

In general, when we consider the pharmacokinetics of orally administered drugs, it is important to note the effect of transporters such as P-glycoprotein (p-gp) in addition to that of CYPs. It has been shown that the herbal supplements or foods have an influence on p-gp, and it is one of the factors that can change the pharmacokinetic parameters of co-administered drugs. For example, the subchronic treatment of St. John’s Wort (SJW) is known to induce CYP3A and also intestinal p-gp, resulting in a decrease in the blood concentration of cyclosporine, indinavir, and digoxin (34). However, MDZ has been reported to be a substrate of CYP3A, but not of p-gp (35). These reports also support our data that the reduction of AUC and C_{max} in the RS and AL groups might be caused by the induction of intestinal CYP3A, not by induction of p-gp. However, the effects of the teas on the transporters other than p-gp, including organic anion transporting peptides (OATP) and multidrug resistance associated protein 2 (MRP2) are unknown. In addition, whether MDZ is a substrate of these transporters have not been clarified. Further studies on the effects of the teas on these transporters are needed to identify the detailed mechanism of the interaction between the teas and MDZ.

Herbal extracts usually contain a number of constituents, including essential oils, tannins, coumarins, saponins, glycosides, flavonoids, terpenoids, polyphenols, and alkaloids. There have been some data concerning the effect of these phytochemicals on CYP3A in human liver microsomes. The inhibition of CYP3A4 by some compounds, including furanocoumarin derivatives in grapefruit juice, silybin in milk thistle, hyperforin in SJW, and resveratrol in red wine, have been reported (36 – 39). Tenryocha tea includes abundant galloyl oxygen hexahydroxydiphenoyl (GOD)-ellagitannin and a sweet ingredient, rubusoside, and rooibos tea is rich in flavonoids such as aspalathin and quercetin (40 – 42). Pal and Mitra recently reported that quercetin in rooibos tea might contribute to the induction of CYP3A in our current study. However, it is unclear which phytochemicals in tenryocha tea affect CYP3As or CYP2C11. Further study is required to clamp the effect of the components of the tea on CYPs.

When studying a herb-drug interaction, it is important to consider the ingestion period of herbs. Depending on the period of ingestion, its effect on the pharmacokinetics of co-administered drug might be changeable. It has been reported that the multiple administration of SJW for two weeks induced hepatic CYP3A4, although this herbal extract might show the inhibitory effect in the single or short-term administration experiment because of its inhibitory effect in human liver microsomes (38, 44). As another example, grapefruit juice is reported to increase nifedipine bioavailability by short-term intraduodenal administration, whereas long-term administration of the juice increased the mean apparent clearance of nifedipine in rats (45). In the current study, we showed the potent inhibition of MDZ 4-OH by tenryocha tea in vitro using pooled rat liver microsomes. However, we previously showed that the
short-term ingestion of tenryocha tea did not show any significant change in CYP3A-mediated simvastatin metabolism in healthy human subjects (16). Considering these results, it was suggested that short-term ingestion of tenryocha tea might cause slight induction of CYP3A, and this effect might have masked the inhibition of CYP3A, resulting in no change of the simvastatin pharmacokinetics. The single ingestion study of this tea is needed to clarify this point.

In conclusion, we showed that continuous ingestion of tenryocha or rooibos tea for two weeks caused significant decline of AUC and Cmax of MDZ by the induction of intestinal CYP3As. In general, these teas are used for a long-term period to improve allergy status rather than single ingestion as a beverage. Our results showed the possible interaction between these teas and medicines metabolized by CYP3A. In contrast, guava tea does not seem to cause herb-drug interactions mediated by CYP3A.

References


