

## Effect of Benifuuki Tea on Cytochrome P450-mediated Metabolic Activity in Rats

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**Abstract.** *Background/Aim: Benifuuki tea has recently been used as an alternative therapy for pollinosis, and it may be consumed with pharmaceutical drugs. This study aimed to examine cytochrome P450 (CYP)-mediated food-drug interactions with Benifuuki tea in rats. Materials and Methods: The inhibitory effects of Benifuuki tea and (-)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3''Me) on CYP activities were evaluated in vitro. Midazolam pharmacokinetics was investigated after two treatments with Benifuuki tea. In an ex vivo study, CYP activities were determined after 1-week-treatment with the tea. Results: Benifuuki tea and EGCG3''Me inhibited CYP2D and CYP3A activities in a concentration-dependent manner in vitro. However, MDZ metabolism did not change by Benifuuki treatment in vivo and ex vivo. In contrast, CYP2D activity was decreased ex vivo. Conclusion: Normal intake of Benifuuki tea is not likely to cause food-drug interactions by CYP3A inhibition or induction. In contrast, Benifuuki tea consumption may lead to food-drug interactions through the inhibition of CYP2D.*

Pollinosis is one of the most common allergic diseases in Japan, with an estimated morbidity of more than 20% (1-3). Patients with pollinosis experience symptoms such as sneezing and nasal discharge, which require long-term symptomatic treatment with antihistamines,  $\alpha_1$  agonists, and steroids. However, the side-effects of these pharmaceutical

products may reduce patients' quality of life. For this reason, patients often use health foods together with, or instead of, pharmaceutical drugs to treat pollinosis.

Benifuuki tea, a green tea cultivar (*Camellia sinensis* L.) has recently attracted attention as an alternative therapy for pollinosis. The major ingredients in green tea are known to be catechins. One of the main catechins in Yabukita tea, which is commonly consumed in Japan, is (-)-epigallocatechin gallate (EGCG). In addition to EGCG, Benifuuki tea contains another major catechin, namely (-)-epigallocatechin 3-(3''-O-methyl) gallate (EGCG3''Me) (4). EGCG3''Me has been shown to inhibit mast cell activation through the suppression of tyrosine phosphorylation of cellular proteins (5). Furthermore, it has been reported that EGCG3''Me inhibits histamine release from murine bone marrow mast cells more potently compared with EGCG (6). In fact, compared with Yabukita tea, Benifuuki tea has been shown to significantly relieve the symptoms of pollinosis in a double-blinded clinical trial (7). Therefore, in recent years, Benifuuki tea containing EGCG3''Me has become increasingly popular among patients with pollinosis.

Individuals with pollinosis may easily obtain Benifuuki tea at drug stores, grocery stores, or on the internet, and consume this tea over long periods; therefore, it is likely that its consumption is combined with regularly used medicines. Moreover, drug interactions between medicines and beverages such as herbal tea or fruit juice have been previously reported; for example, it has been shown that grapefruit juice elevates the blood levels of medicines such as nifedipine and simvastatin through the inhibition of cytochrome P450 (CYP) 3A activity in the small intestine (8). Conversely, we showed that the ingestion of tenryocha tea or rooibos tea for 2 weeks reduced the area under the blood concentration-time curve (AUC) and the maximum serum concentration ( $C_{\max}$ ) of midazolam (MDZ) by more than 60% through intestinal CYP3A induction in rats (9). Although most beverages are likely consumed together with other medicines, there are few reports on possible food-drug interactions, especially *in vivo*.

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Previous studies have described the effect of green tea on CYP3A activity: green tea extract (GTE) has been shown to inhibit CYP3A activity in the human liver and intestinal microsomes (10, 11). Furthermore, an *in vivo* study showed that 1 week of treatment with GTE in rats reduced intestinal CYP3A activity but increased hepatic CYP3A protein levels (11). GTE therefore shows both inhibitory and inducible effects on CYP3A activity, and EGCG has been suggested to be responsible for some of these effects (10-12).

As the degree of absorption of EGCG from the gastrointestinal tract is low, drug interactions mediated by GTE *via* CYP3A modulation are thought to occur mainly in the small intestine. The plasma EGCG level is likely low after drinking a normal quantity of green tea; therefore, it would be difficult to achieve to a level that would influence hepatic CYP3A activity. Conversely, the intake of EGCG 3''Me has been shown to result in higher AUC and bioavailability compared with orally administered EGCG in rats (13). Furthermore, a study of healthy human volunteers showed that the AUC of EGCG 3''Me was higher than that of EGCG after Benifuuki tea ingestion (14). Therefore, if EGCG 3''Me as well as EGCG influence CYP3A, Benifuuki tea, which contains both, is likely to have more potent inhibitory or inducible effects on CYP3A than Yabukita tea. However, there have been no reports on drug interactions mediated by Benifuuki tea *via* CYP3A modulation.

CYP isozymes other than CYP3A also play an important role in drug metabolism. It has been shown that GTE and EGCG inhibit CYP1A, CYP2C9, CYP2C19, and CYP2D6 activities *in vitro* (10-12). On the other hand, GTE has been shown to increase mRNA and protein levels of CYP1A and CYP2C in HepG2 cells (15). These CYP isoforms are expressed in the small intestine at very low levels, and drug interactions mediated by these enzymes mainly occur in the liver. Therefore, these *in vitro* drug interactions are less likely to occur *in vivo* when consuming green tea containing EGCG as its main ingredient, because of its low absorption from the intestinal tract. However, the effect of Benifuuki tea, which is rich in EGCG 3''Me, on these CYPs is unknown.

In this context, the current study was conducted on rats to determine whether the intake of a normal quantity of Benifuuki tea influences the metabolism of a co-administered medicine through the inhibition or induction of the main drug-metabolizing enzyme, CYP3A. Furthermore, we examined the effect of multiple treatments with Benifuuki tea on CYP1A, CYP2C, and CYP2D, which also play important roles in drug metabolism.

## Materials and Methods

**Chemicals and Benifuuki tea.** MDZ maleate was kindly donated by Nippon Roche Co. Ltd. (Tokyo, Japan); 4-hydroxymidazolam and 7-hydroxywarfarin were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Bufuralol and 1'-hydroxybufuralol were purchased from Gentest Corp. (Woburn, MA, USA), S-warfarin was

from purchased Toronto Research Chemicals Inc. (Toronto, ON, Canada), resorufin and ethoxyresorufin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and nitrazepam and diazepam were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). EGCG was obtained from Mitsui Norin Co. Ltd. (Shizuoka, Japan), and EGCG 3''Me was purchased from Nagara Science Co. Ltd. (Gifu, Japan). All other chemicals were of analytical grade quality.

Polyethylene terephthalate (PET)-bottled Benifuuki tea containing 17.5 mg/500 mL EGCG 3''Me was a kind gift from Akahori Shoten (Shizuoka, Japan).

**Treatment of animals and sampling.** Male Sprague-Dawley rats (Japan Laboratory Animals, Inc., Tokyo, Japan), weighing 220-250 g, were used in this study. The rats were housed in a temperature-controlled room with a 12-h light and dark cycle and fed standard laboratory chow. The rats were fasted overnight before the day of sampling. The procedures for this study were reviewed and approved by the Showa University Ethics Committee for Animal Care and Use, and conducted in accordance with guidelines established by the National Institute of Health guide for the care and use of Laboratory animals (8th edition).

In the two-treatment study, 10 ml/kg Benifuuki tea or water (control) was orally administered to rats (n=5) at 0.5 and 2 h before oral administration of MDZ (20 mg/kg). Blood samples (200  $\mu$ l) were collected from the jugular vein before and 15, 30, 45, 60, 90, 120, 180, and 240 min after MDZ administration. The samples were centrifuged at  $7,500 \times g$  for 10 min at 4°C, and each serum sample was stored at -80°C until analysis.

In the 1-week-treatment study, rats (n=6) were administered 10 ml/kg Benifuuki tea or water (control) orally once a day for 1 week. One day after the last Benifuuki tea treatment, the rats were sacrificed, and the liver and small intestine were excised. Liver microsomes were prepared based on a conventional fractional centrifugation method, and were suspended in 100 mM potassium phosphate buffer (pH 7.4) (16). Microsomal fractions were frozen in liquid nitrogen and stored at -80°C until use. Protein concentrations were measured by the method of Lowry *et al.* (17).

**Determination of serum midazolam concentration.** After the two treatments with Benifuuki tea, MDZ serum concentrations were measured by an HPLC method previously reported by our laboratory (18, 19). Briefly, 100  $\mu$ l of each serum sample was alkalized and extracted with dichloromethane: pentane (1:1) for 1 min in the presence of diazepam as an internal standard. After the evaporation of the organic phase, the dried residue was dissolved in the mobile phase and injected onto an HPLC apparatus equipped with a CAPCELLPAK C18 MGII column (2.0 mm  $\times$  250 mm, Shiseido, Tokyo, Japan). The mobile phase consisted of 10 mM potassium phosphate buffer (pH 5.0): acetonitrile (50:50), and the flow rate was 0.2 ml/min. Peaks were detected using a UV detector at 220 nm.

**Pharmacokinetic analysis.** The pharmacokinetic parameters of MDZ were obtained by non-compartmental analysis using MOMENT (Excel®) based on the moment analytic method (20, 21).  $AUC_{0-\infty}$  was calculated according to the trapezoidal rule. The apparent elimination rate constant ( $K_e$ ) was determined from the gradient of the elimination phase, and the elimination half-life ( $t_{1/2}$ ) was calculated as  $\ln 2/K_e$ .  $C_{max}$  was obtained from the actual data.

**Assay for CYP activities.** Liver microsomes, prepared 1 day after the last treatment in the 1-week Benifuuki tea administration experiment, were used in the *ex vivo* study. Ethoxyresorufin O-deethylation (EROD), S-warfarin 7-hydroxylation (S-WF 7-OH), bufuralol 1'-hydroxylation (BF 1'-OH), and MDZ 4-hydroxylation (MDZ 4-OH) activities were assayed as the oxidation markers for CYP1A, CYP2C, CYP2D, and CYP3A, respectively. EROD was assayed fluorometrically according to methods described previously (22). S-WF 7-OH, BF 1'-OH, and MDZ 4-OH were assayed, and each metabolite was determined by HPLC as described previously (23-25).

An *in vitro* study was also carried out to investigate the inhibitory effects of Benifuuki tea, EGCG3''Me, or EGCG on CYP2D and CYP3A activities using pooled rat liver microsomes, which were prepared from 10 non-treated male Sprague-Dawley rats. The PET-bottled Benifuuki tea was established as 100% tea and was used after dilution to 1-10% as the final concentration. EGCG3''Me and EGCG were used at a concentration of 6-600  $\mu$ M. Each control activity was also determined under the same conditions, but with the addition of water instead of the tea or catechins. Data are expressed as the percentage of the remaining activities in the control experiments. IC<sub>50</sub> values were estimated through the nonlinear regression of the relative reaction velocities at a single substrate concentration in the presence of varying EGCG3''Me or EGCG concentrations.

In addition, preincubation experiments were performed to determine whether any produced metabolic intermediates inactivated CYP2D and CYP3A. Benifuuki tea (CYP2D: 0.5%, CYP3A: 5%) was preincubated with rat liver microsomes at 37°C for 5-15 min for BF 1'-OH, and for 10-30 min for MDZ 4-OH, in the presence or absence of NADPH. After the preincubation step, each substrate was added, and the activities were assayed as described above.

**Measurement of mRNA expression.** A total of 20-30 mg of liver and intestinal tissues, excised 1 day after the last treatment in the 1-week Benifuuki tea administration study, were used to determine the expression of CYP mRNAs. Total RNA was extracted from these samples using an RNeasy Mini kit (Qiagen Inc., CA, USA) according to the protocol provided by the manufacturer. The concentration of RNA was determined by spectrophotometry at 260 nm. RNA samples (900 ng) were reverse-transcribed using the High Capacity RNA-to-cDNA master Mix (Applied Biosystems Inc., CA, USA) to obtain cDNA, according to the manufacturer's recommendations. cDNA (2  $\mu$ l) was amplified using CYP2D2-, CYP3A2-, or CYP3A9-specific TaqMan primer and probe sets (TaqMan Gene Expression Assays, Applied Biosystems Inc., CA, USA) in a total volume of 20  $\mu$ l. Real-time PCR analysis was performed to quantify the relative CYP2D2, CYP3A2, or CYP3A9 mRNA expression using Applied Biosystems StepOne™. GAPDH levels were monitored as an endogenous control. PCR reactions included an initial denaturing cycle at 95°C for 10 min, followed by 40 amplification cycles of 95°C for 15 s and 60°C for 1 min. Relative-fold mRNA expression was determined for each sample using the 2<sup>- $\Delta\Delta$ CT</sup> method.

**Statistical analyses.** *In vivo* and *ex vivo* data are presented as the mean $\pm$ standard deviation (S.D.). *In vitro* data are expressed as the mean $\pm$ standard error (S.E.) of duplicate examinations. The significance was determined by applying Mann-Whitney *U*-test, or one-way analysis of variance with Dunnett *post hoc* test, as appropriate. A *p*-value with <0.05 was considered to be significant.

## Results

**CYP3A activity in vitro.** Benifuuki tea (1-10%) inhibited CYP3A-mediated MDZ 4-OH activity in pooled rat liver and small intestinal microsomes in a concentration-dependent manner (Figure 1A and B). Furthermore, EGCG3''Me, which is a major catechin present in Benifuuki tea, inhibited hepatic and small intestinal CYP3A activities, with IC<sub>50</sub> values of 69  $\mu$ M and 72  $\mu$ M, respectively (Figure 1D and E). The potency of the inhibition was similar to that of EGCG on liver CYP3A activity (IC<sub>50</sub>=74  $\mu$ M) (Figure 1F).

To identify the possible contribution of active intermediates to the inhibitory effect of Benifuuki tea on CYP3A activity, another inhibition study that included a preincubation step was conducted. The results indicated that the inhibition potency of Benifuuki tea on CYP3A activity did not depend on NADPH or the preincubation time (Figure 1C).

**MDZ pharmacokinetics.** Benifuuki tea was administered twice at 0.5 h and 2 h before MDZ administration; then, MDZ pharmacokinetics were compared with those of the control group treated with water. The serum MDZ concentration-time profiles and the pharmacokinetic parameters are shown in Figure 2 and Table I. No significant differences in serum MDZ level and its AUC<sub>0- $\infty$</sub> , C<sub>max</sub>, and t<sub>1/2</sub> values were found between the two groups.

**CYP activities and mRNA expression ex vivo.** After 1 week of treatment with Benifuuki tea, liver and intestinal tissues were excised, and CYP activities were analyzed. Benifuuki tea treatment did not increase hepatic CYP activities through the induction of these enzymes (Figure 3A, B and C, 3D). In contrast, CYP2D-mediated BFL 1'-OH was significantly inhibited by 20% in the Benifuuki tea treatment group (*p*<0.05) (Figure 3C). On the other hand, the mRNA expression levels of liver CYP2D2, liver CYP3A2, and intestinal CYP3A9 after Benifuuki tea treatment were similar to those of the controls (Figure 3E, F and G).

**CYP2D activity in vitro.** Benifuuki tea (1-10%) inhibited hepatic BFL 1'-OH activity in a concentration-dependent manner (Figure 4A). Furthermore, EGCG 3''Me and EGCG both inhibited BFL 1'-OH activity, with IC<sub>50</sub> values of 65  $\mu$ M for each (Figure 4B and C). Meanwhile, in the preincubation study, there was no remarkable change in the potency of inhibition of Benifuuki tea on BFL 1'-OH activity following the preincubation step (Figure 4D).

## Discussion

In the current study, we examined the effect of Benifuuki tea, which is used to relieve the symptoms of pollinosis, on CYP-mediated food-drug interactions.

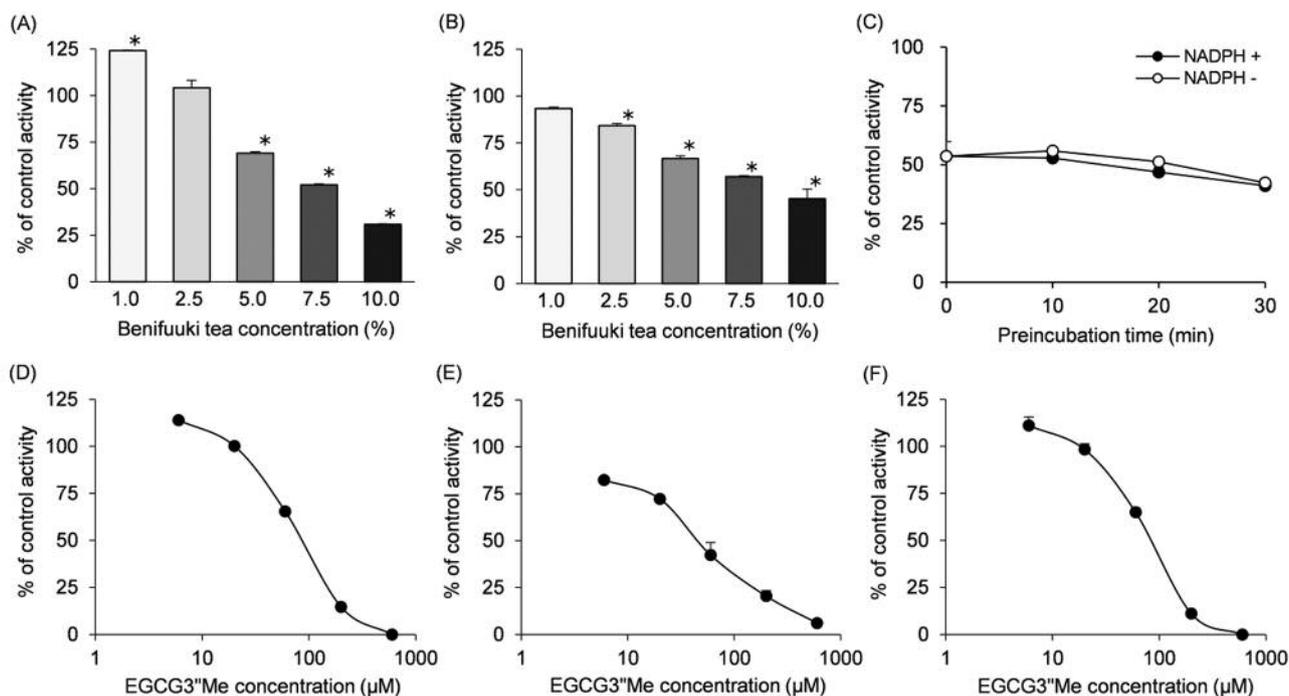


Figure 1. Inhibitory effect of Benifuuki tea, EGCG3''Me, and EGCG on MDZ 4-OH activity *in vitro*. A direct inhibition study with Benifuuki tea was performed in pooled rat liver (A) or intestinal (B) microsomes. The effect of EGCG3''Me in pooled rat liver (D) or intestinal (E) microsomes, and that of EGCG in pooled rat liver microsomes (F), was also studied. Pre-incubation effect of Benifuuki tea (5%) on MDZ 4-OH activity was studied using pooled rat liver microsomes in the presence (closed circles) or absence (opened circles) of NADPH (C). The mean $\pm$ S.E. values of duplicate examinations are shown as a percentage of the control activity. \* $p$ <0.05 compared to control.

First, the inhibitory effects of Benifuuki tea and its main ingredient, EGCG3''Me, on CYP3A activity were investigated *in vitro* using MDZ 4-OH as a marker of CYP3A-mediated metabolism. The results indicated that Benifuuki tea and EGCG3''Me inhibited MDZ-4OH activity in rat liver and intestinal microsomes in a concentration-dependent manner (Figure 1). The potency of the inhibition of hepatic CYP3A activity by EGCG3''Me ( $IC_{50}$ =69  $\mu$ M) was similar to that by EGCG ( $IC_{50}$ =74  $\mu$ M) (Figure 1D and F). It has been reported that EGCG is the most abundant of the catechins in green tea and the most effective inhibitor of CYPs *in vitro* (26). Our results showed that, like common green tea (Yabukita tea), Benifuuki tea inhibited CYP3A activity. In addition to EGCG, Benifuuki tea contains a high level of EGCG3''Me, an ingredient considered to have an inhibitory effect on this enzyme.

The Benifuuki tea we used in this study contained 35  $\mu$ g/ml (74  $\mu$ M) EGCG3''Me and 17.5  $\mu$ g/ml (38  $\mu$ M) EGCG (data not shown). Accordingly, 10% Benifuuki tea, which inhibited hepatic CYP3A activity by more than 50% *in vitro*, contained 7.4  $\mu$ M EGCG3''Me and 3.8  $\mu$ M EGCG. These concentrations of EGCG3''Me and EGCG did not have any inhibitory effects on CYP3A activity (Figure 1D and F). Thus, it was concluded

that, in addition to EGCG3''Me and EGCG, Benifuuki tea contains ingredients that inhibit CYP3A activity; all of these compounds were considered to be involved in the inhibitory effect.

We then investigated the effect of Benifuuki tea on MDZ pharmacokinetics in rats to determine whether the tea caused drug interactions through CYP3A inhibition *in vivo*. Two treatments with Benifuuki tea, at 0.5 h and 2 h, before MDZ administration did not cause any remarkable change in the MDZ pharmacokinetic parameters (Figure 2, Table I). Nishikawa *et al.* reported that a single oral treatment with 400 mg/kg GTE, which contains a considerably higher amount of EGCG than green tea, did not change blood MDZ concentrations (11). Compared with EGCG, EGCG3''Me is thought to reach at a high enough level to the liver; therefore, it was hypothesized that Benifuuki tea inhibits hepatic CYP3A activity. However, no significant increase in serum MDZ concentration was observed *in vivo*. Therefore, it was concluded that the quantities of these CYP3A inhibitors present in the liver and small intestine after two treatments with Benifuuki tea were insufficient to inhibit CYP3A. The quantity of Benifuuki tea administered to rats in this study (10 ml/kg) was estimated to be equal to the

Table I. Pharmacokinetic parameters of MDZ after two treatments with Benifuuki tea in rats.

	AUC <sub>0-∞</sub> (ng/mL*h)	C <sub>max</sub> (ng/mL)	t <sub>1/2</sub> (min)
Control	2,332±108	2,223±199	34±12
Benifuuki tea	2,773±830	2,365±644	33±14

Each value represents the mean±S.D. for 5 rats.

level humans generally consume in one serving. Thus, the results suggest that the food-drug interactions mediated by CYP3A inhibition likely do not occur as a result of the intake of one normal serving of Benifuuki tea.

However, Benifuuki tea is often consumed over a long period to relieve the symptoms of pollinosis. It has been reported that St. John's Wort induces CYP3A *in vivo*, despite inhibiting the activity of this enzyme *in vitro* (27, 28). There is little information on food-drug interactions involving CYP induction. In order to ensure the effective use of medicines, it is important to investigate the decrease in efficacy caused by the reduction in their concentrations in the blood. We examined the inducible effects of 1 week of treatment with Benifuuki tea on hepatic CYP activities and the intestinal mRNA level of CYP3A9, which is predominantly expressed in the rat small intestine. The results indicated no significant increase in hepatic CYP1A, CYP2C, CYP2D, and CYP3A activities or hepatic CYP2D2, hepatic CYP3A2, and intestinal CYP3A9 mRNA levels following the tea treatment (Figure 3). These results suggest that Benifuuki tea does not elicit the induction of CYPs mainly involved in drug metabolism in the liver and small intestine. Nishikawa *et al.* reported that, in rats, 1 week of treatment with GTE increased hepatic CYP3A protein level by 1.5 times but reduced intestinal CYP3A activity (11). Nishikawa *et al.* used GTE containing a high amount of EGCG, whose levels must have been sufficient to modulate hepatic CYP3A. Conversely, as shown in the present *in vivo* study, normal intake of Benifuuki tea for 1 week did not have an inducible effect on CYPs.

In contrast, a 20% decrease in hepatic CYP2D-mediated BFL 1'-OH activity unexpectedly occurred after Benifuuki tea treatment for 1 week (Figure 3). Therefore, we investigated the possibility of down-regulation of hepatic CYP2D by measuring its mRNA expression. The results indicated that the CYP2D2 mRNA level was the same as that of the control group. In other words, CYP2D activity was inhibited by 1 week of treatment with Benifuuki tea (even though this was measured 1 day after the last tea treatment) without a reduction in CYP2D2 mRNA expression. It was previously reported that GTE and EGCG have no or a minor inhibitory effect on CYP2D activity both *in vitro* and *in vivo*

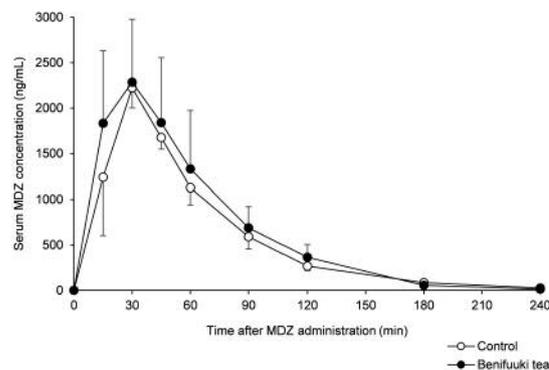


Figure 2. Serum concentration-time profiles of MDZ after two treatments with Benifuuki tea. Benifuuki tea (10 ml/kg) or water (control) was orally administered to rats twice at 0.5 and 2 h before oral administration of MDZ (20 mg/kg). Each point represents the mean±S.D. of 5 rats.

(10, 29). Therefore, it was hypothesized that an ingredient specific to Benifuuki tea, such as EGCG3''Me, was involved in the modulation of CYP2D activity. Accordingly, the inhibitory effects of Benifuuki tea and EGCG3''Me on CYP2D activity were then investigated *in vitro*. The results showed that Benifuuki tea strongly inhibited CYP2D activity in a concentration-dependent manner (Figure 4). However, the obtained IC<sub>50</sub> value of 65 μM for EGCG3''Me was equal to that for EGCG, suggesting that Benifuuki tea contains ingredients, other than these catechins, that inhibit CYP2D activity.

Mechanism-based inhibition (MBI) is an important process involved in drug interactions; in the present context, the inhibitory effect is considered to continue owing to the irreversible inactivation of CYP2D. The inhibition of CYP2D activity even 1 day after 1 week of tea treatment may be attributable to MBI of CYP2D by Benifuuki tea. Therefore, to clarify this issue, we conducted another *in vitro* study that included a preincubation step. The results showed no additional inhibition of BFL 1'-OH when Benifuuki tea was preincubated with rat liver microsomes and NADPH before the addition of BFL, indicating that MBI did not occur.

In this study, we were unable to clarify the mechanism by which Benifuuki tea inhibited CYP2D activity 1 day after 1 week of tea treatment. Although the inhibition observed in this experiment was not potent (20% inhibition compared with that in the control group), an *in vitro* study revealed the potent direct inhibitory effect of Benifuuki tea on CYP2D activity. This finding suggests that a drug interaction occurs, depending on the timing of combination with drugs metabolized by CYP2D. Although CYP2D has a low abundance ratio in the liver, it actively participates in the metabolism of medicines,

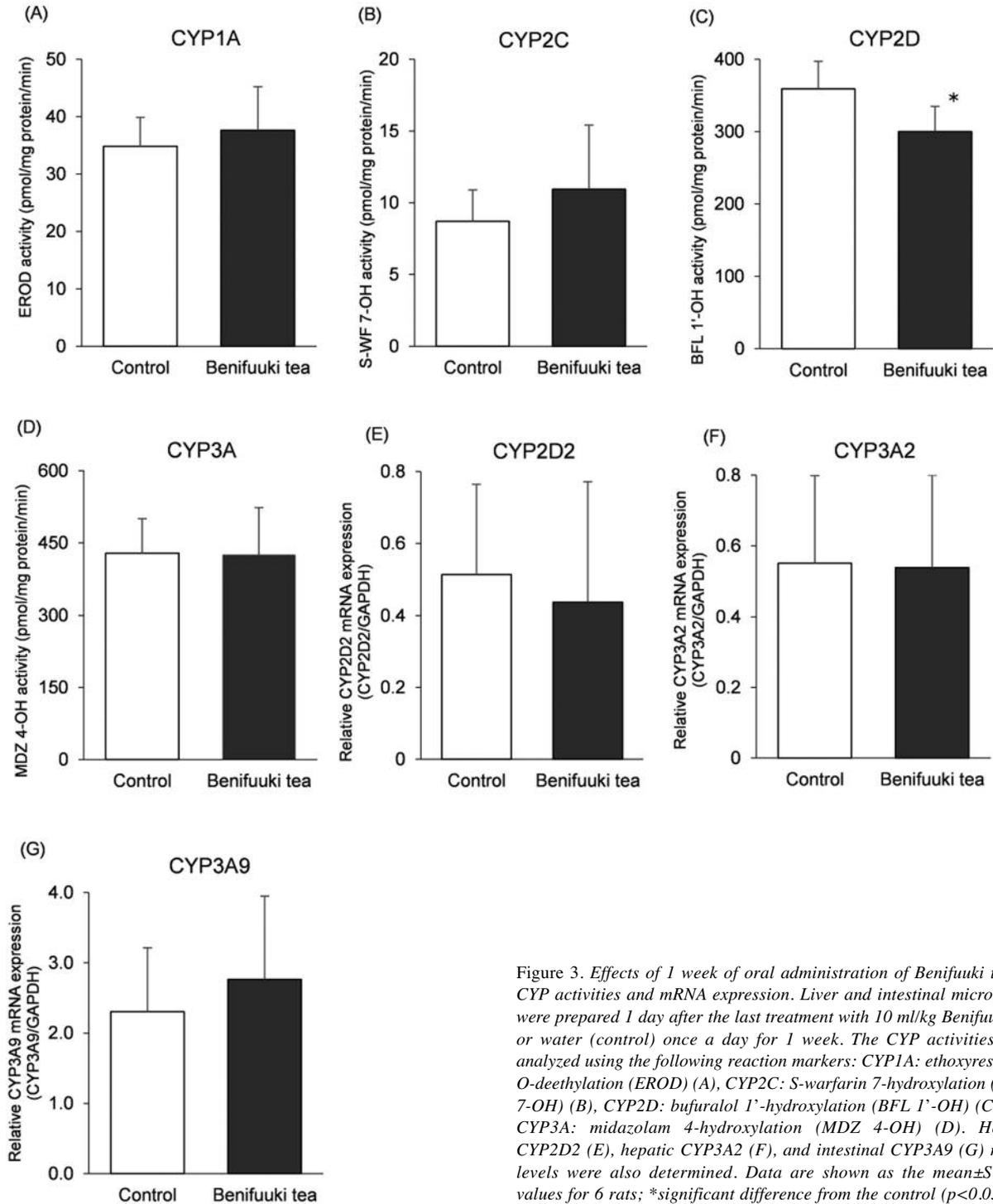


Figure 3. Effects of 1 week of oral administration of Benifuuki tea on CYP activities and mRNA expression. Liver and intestinal microsomes were prepared 1 day after the last treatment with 10 ml/kg Benifuuki tea or water (control) once a day for 1 week. The CYP activities were analyzed using the following reaction markers: CYP1A: ethoxyresorufin O-deethylation (EROD) (A), CYP2C: S-warfarin 7-hydroxylation (S-WF 7-OH) (B), CYP2D: bufuralol 1'-hydroxylation (BFL 1'-OH) (C), and CYP3A: midazolam 4-hydroxylation (MDZ 4-OH) (D). Hepatic CYP2D2 (E), hepatic CYP3A2 (F), and intestinal CYP3A9 (G) mRNA levels were also determined. Data are shown as the mean  $\pm$  S.D. of values for 6 rats; \*significant difference from the control ( $p < 0.05$ ).

following that by CYP3A, therefore, it is important to investigate drug interactions mediated by CYP2D inhibition. Further *in vivo* studies are needed to clarify drug interactions with Benifuuki tea mediated by CYP2D inhibition, using a probe drug for this enzyme.

In the current study, we showed that Benifuuki tea was not likely to cause food-drug interactions mediated by CYP3A inhibition or induction when consuming a quantity obtained from normal drinking. However, weak inhibition of CYP2D activity was observed 1 day after 1 week of treatment with

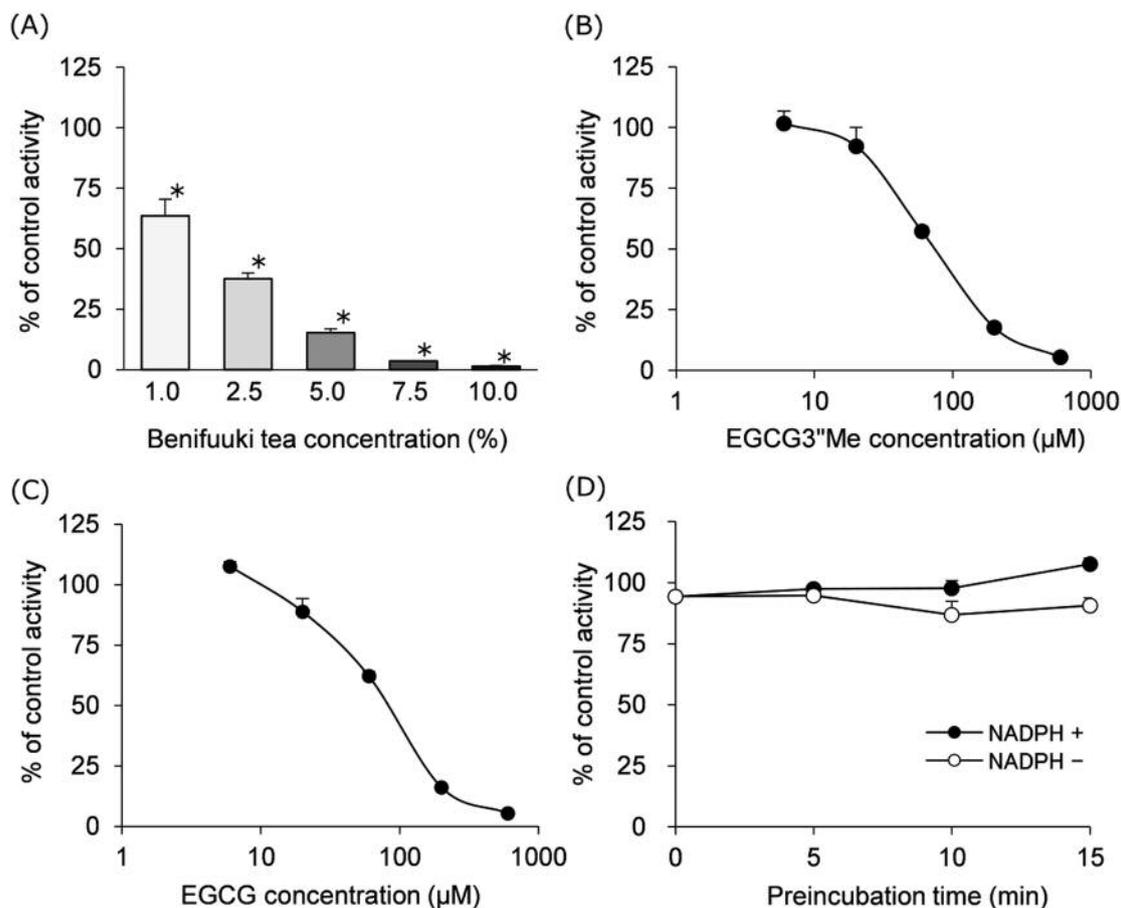


Figure 4. Inhibitory effects of Benifuuki tea, EGCG 3''Me, and EGCG on BFL 1'-OH activity in vitro. A direct inhibition study was performed with Benifuuki tea (A) (final concentration: 1-10%), EGCG 3''Me (B), or EGCG (C) (final concentration: 6-600 μM) in pooled rat liver microsomes. The preincubation effect of Benifuuki tea (0.5%) on the activity was studied using pooled rat liver microsomes in the presence (closed circles) or absence (open circles) of NADPH (D). The mean±S.E. values of duplicate examinations are shown as a % of the control activity. \* $p < 0.05$  compared with control.

Benifuuki tea. In addition, *in vitro* hepatic CYP2D activity was strongly inhibited by the tea, suggesting the possibility of a food-drug interaction mediated by Benifuuki tea through the direct inhibition of CYP2D.

### Conflicts of Interest

The Authors have no conflicts of interest to declare.

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