Full Paper

In Vivo Inhibition of CYP3A-Mediated Midazolam Metabolism by Anchusan in Rats

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Abstract. Cytochrome P450 (CYP)-mediated drug interactions caused by Kampo medicine have not been investigated sufficiently. The current study was conducted to reveal the effect of anchusan, a commonly used Kampo formula for gastrointestinal disease, on CYP3A-mediated drug metabolism in rats. The pharmacokinetics of midazolam (MDZ) was investigated after the single or one-week administration of anchusan (500 mg/kg) to evaluate its inhibitory and inducible effect on CYP3A, respectively. MDZ was administrated 16 h after the last anchusan treatment in the multiple dose study, while their intervals were 2 or 16 h in the single dose study. Unexpectedly, the multiple-pretreatment of anchusan increased the AUC of MDZ by 2.4-fold rather than decreasing it, and the CYP3A contents and activities were unchanged in hepatic and intestinal microsomes of these rats. In contrast, no significant inhibitory effects on MDZ metabolism were observed by the single anchusan pretreatment. In vitro study showed that the preincubation of anchusan and some of its component extracts with rat liver microsomes reduced CYP3A activity in a time- and NADPH-dependent manner. These results suggested that anchusan increased the serum MDZ concentration in rats, at least in part, by the time-dependent inhibition of CYP3A.

Keywords: CYP3A, drug interaction, time-dependent inhibition, Kampo medicine, anchusan

Introduction

Kampo medicines, the traditional Japanese medicines, are widely used in Japan. Kampo is a general term for the unique system of traditional medicine developed in Japan from Chinese origins. Now 120 kinds of crude drugs (almost all of plant origin, with some of animal or mineral origin) are listed in the Japanese pharmacopeia, and they are used as the source of Kampo medicines (1). These are prescribed for various types of acute or chronic disease, so it is quite often that patients are prescribed both Kampo and western medicines at once by their physician. Furthermore, in terms of self-medication, Kampo formulation is also common as an over-the-counter (OTC) drug and their usefulness has been reported (2). Therefore, in many cases, co-administration of Kampo medicines with western medicines is predicted during the medication.

When the multiple drugs are taken at the same time, it is necessary to consider the drug–drug interaction. This results from pharmacodynamic and pharmacokinetic modification of the drug response by the existence of concomitant drugs. Pharmacokinetic interaction is usually difficult to predict and cause unexpected outcomes. In many cases the induction or inhibition of the drug-metabolizing enzyme cytochrome P450 (CYP) is known to participate in this type of interaction. 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 such as erythromycin, cyclosporine, and nifedipine (3 – 5).

There have been many reports concerning CYP3A-mediated drug interactions. In addition, recent studies revealed that not only chemical drugs but also natural products such as foods or herbs may affect CYP3A activity. As a typical example, grapefruit juice (GFJ) is well
known to increase the bioavailability of CYP3A-mediated drug metabolism through the inhibition of intestinal CYP3A (6). In contrast, St. John’s wort (SJW) reduces the oral bioavailability of these drugs by inducing the intestinal and hepatic CYP3A (7). Because Kampo medicines include many kinds of herbs, Kampo–western drug interaction mediated by this enzyme was anticipated. In fact, among a number of Kampo medicines the high dose of saireito was suggested to have the ability to enhance and inhibit the metabolism of nifedipine in rats (8); meanwhile, rikkunshito, yokukansan, and boiogito have minimal effect on the metabolism of triazolam in mice (9). However, these kinds of investigations are quite limited and there is no information concerning CYP3A-mediated drug interaction for most of the Kampo medicines, even for those that are used frequently.

Anchusan is one of the popular Kampo formulas, which has cholagogic and anti-ulcerative effect, for the treatment of upper gastrointestinal diseases such as chronic gastritis and functional dyspepsia (10, 11). Anchusan comprises the extract of the following 7 crude drugs: 4 parts of Cinnamomi Cortex, 3 parts of Corydalis Tuber, 3 parts of Ostrea Testa, 1.5 parts of Foeniculi Fructus, 1 parts of Glycerhizae Radix, 1 parts of Amomi Semen, and 0.5 parts of Alpiniae Officinaris Rhizoma. These crude drugs include many chemical constituents and some of them are reported to be an inhibitor or inducer of CYP3A. For example, the inhibition of CYP3A-mediated metabolism has been shown by glabridin, kaempferol, and oleic acid in human liver microsomes and by putrescine and quercetin in rats in vivo (12 – 16), whereas the induction of this enzyme was reported by glycyrrhizin, 1,8-cineole, and cadinene in rats and by quercetin in human cell lines (17 – 20). There is a strong possibility that Anchusan may contain these chemical ingredients in its formula; therefore, there is a concern that there may be more potent inhibition or induction of CYP3A compared to the effect of a single compound. However, the effect of anchusan on the metabolism of co-administered drugs was predicted to be complicated because of the opposing effects of those chemicals against CYP3A. Rengelshausen et al. has shown that the opposite effect of SJW on voriconazole pharmacokinetics depends on the length of its intake; short-term administration of SJW resulted in a marked increase in voriconazole exposure, whereas its long-term intake caused a significant reduction in voriconazole bioavailability (21). This report demonstrated the ability of SJW to both inhibit and induce voriconazole metabolism. The similar effect was considerable during medication with anchusan, resulting in clinically important interaction by the co-administration of CYP3A substrates.

Therefore, the current study was conducted to investi-
viewed and approved by the Showa University Ethics Committee for Animal Care and Use.

Drug administration and sampling
In the single pretreatment studies, anchusan suspension (500 mg/kg) or water (control) was orally administered to rats, followed 2 or 16 h later by oral administration of MDZ (20 mg/kg). In the multiple-pretreatment study, the rats were treated with anchusan suspension (500 mg/kg) or water once a day for one week, and then study, the rats were treated with MDZ (20 mg/kg). In the multiple-pretreatment study, the rats were treated with anchusan suspension (500 mg/kg) or water (control) was orally administered to rats, followed 2 or 16 h later by oral administration of MDZ (20 mg/kg). In the multiple-pretreatment study, the rats were treated with anchusan suspension (500 mg/kg) or water (control) was orally administered to rats, followed 2 or 16 h later by oral administration of MDZ (20 mg/kg). In the multiple-pretreatment study, the rats were treated with anchusan suspension (500 mg/kg) or water (control) was orally administered to rats, followed 2 or 16 h later by oral administration of MDZ (20 mg/kg).

Blood samples (200 μL) were collected from the jugular vein before and at 15, 30, 45, 60, 90, 120, 180, and 240 min after MDZ administration. The samples were centrifuged at 7,500 × g for 10 min at 4°C and each separated serum was stored at −80°C until the analysis.

After multiple treatment of anchusan for a week, rats were sacrificed at 22 h after the last dose to excise the liver and small intestine. The time setting is considered to be proper for investigating if the induction of CYP3A enzyme was caused by anchusan treatment. This condition is supported by the report showing that the CYP3A activity at least up to 24 h after single treatment of bilobalide, an ingredient of ginkgo biloba (22). The liver microsomes were prepared by a conventional fractional centrifugation method (23). The preparation of intestinal microsomes was performed according to the method of Bonkovsky et al. and Koudriakova et al. with minor modifications as described previously (24 – 26). All microsomal fractions were frozen by liquid nitrogen and kept at −80°C until use. Protein concentrations were measured by the method of Lowry et al. (27).

Determination of serum midazolam concentration
The serum concentration of MDZ was determined according to the method of Mandema et al. with slight modifications (26, 28). Briefly, 100 μL of each serum was diluted with 500 μL of 0.1 N NaOH, and 100 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 C18 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 a SCL-10A VP system controller, VC-10AD VP pump, DGU-14-A degasser, SIL-10AD VP auto sampler, a SPD-10A VP detector (set at 220 nm) (Shimadzu, Kyoto), and a Fujitsu computer running Shimadzu software version CLASS-VP.

Pharmacokinetic analysis
The serum concentration–time data of orally administered MDZ of each rat was assessed by non-compartment analysis using MOMENT (Excel®) based on the moment analytic method (29, 30). The area under the serum concentration–time curve (AUC0–∞) was calculated according to the trapezoidal rule. The elimination half-life (t1/2) was calculated, dividing ln2 by λ, where λ is the terminal elimination rate constant calculated by a linear regression of at least three data points from the terminal portion. The maximum serum concentration (Cmax) was obtained from the actual data.

Assay of MDZ hydroxylation activity
Because MDZ is known to be metabolized predominantly to the 4-hydroxymidazolam and to a lesser extent to the 1′-hydroxymidazolam by CYP3A enzymes in rats (31), we analyzed MDZ 4-hydroxylation (MDZ 4-OH) activity using HPLC according to a previous report (32). Liver and intestinal microsomes, prepared at 22 h after the last dose of one week anchusan or water treatment, were used in the ex vivo study.

An in vitro study was also carried out to investigate the inhibitory effects of anchusan and its seven components on MDZ 4-OH using pooled rat liver microsomes, which was prepared from 10 non-treated male Sprague-Dawley rats. The powder extract of anchusan and the components were sonicated in distilled water for 5 min and added to the incubation mixture with the final concentration of 10 – 1,000 μg/mL. Control activity was also determined in the same conditions but with addition of water instead of the extracts.

In addition, the preincubation experiment was performed to determine the contribution of metabolic intermediates, which might be produced via CYP3A from the chemical constituents of anchusan, to the inactivation of this enzyme. Each of the extract was preincubated with rat liver microsomes and NADPH at 37°C for 10, 20, or 30 min. After the preincubation step, MDZ was added and the activity was assayed as described above. The concentrations of the extracts used in this experiment were those that had 10% – 20% inhibitory effects on the activity in the direct inhibition study (Fig. 4): anchusan, Corydalis Tuber, Ostreae Testa, Foeniculi Fructus and Anomi Semen: 300 μg/mL; Glycyrrhizae Radix: 200 μg/mL; Alpiniae Officinar Rhizoma: 100 μg/mL; Cinnamomi Cortex: 50 μg/mL.
Immunoblot analysis of liver and intestinal CYPs

To investigate the inducible effect of the multiple treatment of anchusan on CYP3A levels in liver and intestine, the Western blot analysis was carried out (33). Hepatic (5 μg) and intestinal (22.5 μg) microsomes derived from rats treated with anchusan or water for a week 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 CYP3A2 antibody according to the manufacturer’s instructions. Although CYP3A2 is known to be not expressed in intestine, this antibody was used in this study because we could not obtain antibodies against the intestine-expressed CYP3As, such as CYP3A9, CYP3A18, and CYP3A62. In addition, these CYP3A isozymes are known to have high similarity with CYP3A2. Therefore, we thought that it might be possible to detect total CYP3As in intestine using this antibody. 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 analysis

All tests were carried out using 3 – 5 rats for each group, and the values are presented as mean ± standard deviation (S.D.). Differences between the groups were analyzed by the Mann-Whitney test. A difference of P < 0.05 was considered to be statistically significant.

Results

Pharmacokinetics of MDZ

The serum MDZ concentration–time profiles and the pharmacokinetic parameters after 2 or 16 h of single pretreatment of anchusan or water (control) are shown in Fig. 1 and Table 1. No significant effect on the MDZ pharmacokinetics was observed by the single anchusan treatment.

Originally, the multiple-pretreatment study was conducted to evaluate the inducible effect of anchusan on CYP3A-mediated metabolism; therefore, the administration of MDZ was scheduled to 16 h after last dose of anchusan. However, the higher mean serum concentration of MDZ was observed in the anchusan-treated group compared to the control group, unexpectedly (Fig. 2). The mean AUC0–∞ was significantly increased from 76.25 ± 19.52 to 179.91 ± 102.54 μg/mL·h after one-week anchusan treatment (P < 0.05) (Table 2). In addi-

![Fig. 1.](image-url) Serum concentration–time profiles of MDZ 2 or 16 h after single pretreatment of anchusan in rats. Anchusan suspension (500 mg/kg) or water (control) was orally administered to rats, followed 2 h (A) or 16 h (B) later by oral administration of MDZ (20 mg/kg). Each point represent the mean ± S.D. of 3 – 5 rats.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Anchusan</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>Cmax (ng/mL)</td>
<td>AUC0–∞ (μg/mL·h)</td>
</tr>
<tr>
<td>Control</td>
<td>25.18 ± 5.82</td>
<td>1511.0 ± 346.9</td>
</tr>
<tr>
<td>Anchusan</td>
<td>33.27 ± 5.90</td>
<td>1508.0 ± 771.0</td>
</tr>
<tr>
<td>16 h</td>
<td>Cmax (ng/mL)</td>
<td>AUC0–∞ (μg/mL·h)</td>
</tr>
<tr>
<td>Control</td>
<td>43.86 ± 8.92</td>
<td>798.8 ± 303.5</td>
</tr>
<tr>
<td>Anchusan</td>
<td>47.24 ± 29.4</td>
<td>1529.0 ± 668.4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 3 – 5 rats.
Inhibitory Effect of Anchusan on CYP3A

CYP3A contents and activity

There was no remarkable change in the expression of hepatic and intestinal CYP3A protein after multiple pretreatment of anchusan (Fig. 3: A, B). Because we used polyclonal CYP3A2 antibody for this study as mentioned above, the observed results by the intestinal microsomes should be the total amounts including CYP3A9, CYP3A18, and CYP3A62, which are mainly expressed in rat intestine.

No significant differences were observed in CYP3A-mediated MDZ 4-OH activity between the multiple anchusan–pretreated group and the control group in the liver (761.24 ± 215.17 vs. 898.84 ± 129.68 pmol·mg protein⁻¹·min⁻¹) and in the intestine (22.03 ± 12.53 vs. 32.61 ± 11.44 pmol·mg protein⁻¹·min⁻¹) (Fig. 3C).

In vitro studies

We first examined the direct inhibitory effects of anchusan and its components on MDZ 4-OH activity. Cinnamomi Cortex, Amomi Semen, and Alpiniae Officinar Rhizoma showed concentration-dependent inhibition on MDZ 4-OH activity with IC₅₀ values of 118, 470, and 255 μg/mL, respectively (Fig. 4). Anchusan showed weak inhibition on this activity with comparatively higher IC₅₀ value of 696 μg/mL.

To determine if it is possible for anchusan and its components to form the metabolic intermediates that inhibit CYP3A activity during incubation, MDZ 4-OH activity was assayed with the inclusion of 10–30-min preincubation step. Preincubation of rat liver microsomes with anchusan, Glycyrrhizae Radix, or Alpiniae Officinar Rhizoma in the presence of NADPH, caused a time-dependent inhibition of MDZ 4-OH activity: the remaining activities after 30 min of preincubation were 63%, 28%, and 59% of the control activity, respectively (Fig. 5).
However, these effects were not observed in the absence of NADPH.

Discussion

In the current study, we investigated the effect of anchusan, a Kampo formula commonly used for upper gastrointestinal disease, on CYP3A-mediated metabolism. Anchusan is composed of 7 crude drugs containing chemical constituents that can inhibit or induce CYP3A activity (12–20); accordingly, the effect of their mixed formula anchusan was a target of interest on considering drug interaction. Therefore, we conducted this study from the view of both aspects, inhibition and induction.

To clarify the inhibitory effect of anchusan on CYP3A-mediated metabolism, single pretreatment studies were scheduled using MDZ as a probe drug. MDZ is a short-acting benzodiazepine derivative that is metabolized predominately to 1′-hydroxymidazolam by CYP3A4 in humans and to 4-hydroxymidazolam by CYP3A2 in rats, and it has been recognized as a sensitive prove to investigate CYP3A function (31, 34). 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. MDZ was administered at 2 or 16 h after single treatment of anchusan because the time interval of two treatments might become a factor to modulate serum MDZ concentration. As a result, MDZ pharmacokinetics did not change by the pretreatment before 2 or 16 h after administration of anchusan (Fig. 1). Therefore, the clinically important inhibition of MDZ metabolism seemed not to be caused by the single pretreatment of anchusan. Our in vitro data using rat liver microsomes also showed that the inhibitory effect of anchusan on MDZ 4-OH was negligible, while its components Cinnamomi Cortex, Alpiniae Officinari Rhizoma, and Amomi Semen showed weak but concentration-dependent inhibition on this activity (Fig. 4). It has been suggested that even if one crude drug has

![Fig. 4. Inhibitory effect of anchusan and its components on MDZ 4-OH activity in vitro. The extract of anchusan and its components are added to the incubation mixture with the final concentration of 10–1,000 μg/mL. Control activity was determined with addition of water instead of the extracts (868.5 pmol·mg protein−1·min−1). Data are expressed as the mean of duplicate examinations. CC: Cinnamomi Cortex, CT: Corydalis Tuber, OT: Ostreae Testa, FF: Foeniculi Fructus, GR: Glycyrrhizae Radix, AS: Amomi Semen, AR: Alpiniae Officinari Rhizoma.](image-url)

![Fig. 5. Time-dependent inhibition of MDZ 4-OH activity by anchusan and its components in the presence or absence of NADPH. Anchusan, Corydalis Tuber, Ostreae Testa, Foeniculi Fructus, and Amomi Semen (300 μg/mL); Glycyrrhizae Radix (200 μg/mL); Alpiniae Officinari Rhizoma (100 μg/mL); or Cinnamomi Cortex (50 μg/mL) were preincubated with rat liver microsomes in the presence (closed circle) or absence (closed triangle) of NADPH at 37°C for 10, 20, and 30 min. After the preincubation step, MDZ was added and the activity was assayed. Data are shown as the mean of duplicate determinations. CC: Cinnamomi Cortex, CT: Corydalis Tuber, OT: Ostreae Testa, FF: Foeniculi Fructus, GR: Glycyrrhizae Radix, AS: Amomi Semen, AR: Alpiniae Officinari Rhizoma.](image-url)
a potent inhibitory effect on CYP enzyme, it does not always bring the effect to a Kampo prescription that includes this crude drug at the same level (35). Because Kampo medicines are quite complicated forms consisting of numerous chemicals, it is difficult to predict their effects on CYP activities from the results observed for a single component in the formulation.

Some of the crude drugs composing anchusan includes chemicals known to induce CYP3A enzyme (17 – 20); therefore, to investigate its inducing effect on CYP3A, we next conducted the multiple-pretreatment study. If the repeated administration of anchusan induced hepatic or intestinal CYP3A, the decrease in AUC and Cmax of MDZ would be observed. In our present study the timing of MDZ administration was set at 16 h after the last anchusan dose, which was considered enough to eliminate anchusan from the systemic circulation, to exclude the contribution of the possible direct effect of anchusan on CYP3A. However, the results demonstrated that in the rats given one-week pretreatment of anchusan (500 mg·kg⁻¹·day⁻¹), the serum concentration of MDZ was significantly higher compared to the control rats, with 2.4-fold higher AUC value (Table 2). This suggested that repeated dosing of anchusan inhibited CYP3A-mediated metabolism, rather than induced it. Furthermore, after this MDZ pharmacokinetic study we performed an ex vivo study using excised liver and intestine of these rats. In the hepatic and intestinal microsomes prepared after one-week treatment of anchusan, there were no remarkable changes of MDZ 4-OH activity between the control and anchusan-treated group (Fig. 3C). In addition, the observed CYP3A protein contents in liver and intestine were similar in both groups determined by the Western blot analysis (Fig. 3: A, B). These results also suggested that CYP3A was not induced by the multiple treatment of anchusan, even though some inducers are included in its original herbs.

There are some possible explanations for the increase of MDZ concentration level observed in the anchusan multiple-pretreatment study and not in the single-pretreatment study. At first, the single dose of anchusan, 500 mg/kg, might not be enough to inhibit CYP3A-mediated metabolism. However, the dose of anchusan used in the current study was 20-times higher than its clinical dose in humans and it was based on the dose at which anchusan was reported to inhibit ethanol-induced gastric hemorrhagic lesions in rats (10). Taking into account this report and species difference between human and rat, the dose was supposed to be appropriate. Therefore, clinically significant interaction mediated by CYP3A might not have occurred by the single dose of anchusan.

Even though the repeated dose could accumulate the prospective CYP3A inhibitory constituents included in anchusan, the time interval of 16 h set before MDZ administration in the multiple-pretreatment study was considered to be quite long to keep high concentrations of the constituents, if it directly inhibited CYP3A enzyme. One of the possibilities to resolve this issue is that the ingredients of anchusan might be transformed to the activated metabolites by CYPs. It has been demonstrated that an irreversible inhibition, which is called a mechanism-based inactivation, is one of the mechanisms of CYP inhibition (36). In that case, the inactivator binds to a target enzyme and then is catalytically activated to a reactive intermediate that covalently binds to heme and/ or protein in the enzyme active site, resulting in an irreversible loss of enzymatic activity. The mechanism-based inactivators can completely inactivate drug-metabolizing enzyme and cause serious adverse effects, which persist even after withdrawal of the inhibitors. The gene encoding the inactivated enzyme will produce the new enzyme, but this process must take several days or, at least, several hours to recover the enzyme activity to the sufficient level (37, 38). As an example, GFJ is known to inhibit intestinal CYP3A by a mechanism-based process, which resulted in 3-days lasting potent inhibitory effect. For the mechanism-based inactivation, in addition to NADPH, time is required for metabolism and activation of the inhibitor, which is indicated by time-dependent inhibition (37). Thus, to clarify the possibility of anchusan acting as a mechanism-based inactivator, the time and NADPH-dependent inhibition by anchusan and its components on CYP3A was examined with preincubation times of 0, 10, 20, and 30 min. In the presence of NADPH, anchusan showed a decrease of the residual MDZ 4-OH activity as the preincubation time was prolonged, whereas no inhibitory effects were observed in the absence of NADPH (Fig. 5). This result suggested that anchusan inhibited CYP3A activity in a time-dependent manner. Glycyrrhiza Radix and Alpiniae Officinar Rhizoma also had the similar but more potent effect against this enzyme. Mechanism-based inactivation by Glycyrrhiza Radix has also been shown by a previous report (12) that revealed that licorice root extract and glabridin, an isoflavon purified from licorice root extract, inactivated CYP3A4 activity in a time- and concentration-dependent manner, and this inactivation was NADPH-dependent and was not reversible. Our results newly revealed the inactivation effect of CYP3A by Alpiniae Officinar Rhizoma, in addition to that by Glycyrrhiza Radix. These data suggested that the reactive intermediate generated from the ingredients of anchusan via CYP3A metabolism during 16 h might contribute to the modulation of MDZ pharmacokinetics in the multiple-pretreatment study. The repeated treatments are considered to be necessary to increase serum MDZ concentration through
the accumulation of the reactive intermediate that inactivates this enzyme. Considering the small change in $t_{1/2}$, this effect was predicted to occur in intestine rather than in liver. More detailed studies are needed to conclude that anchusan is a mechanism-based inactivator of CYP3A enzyme.

Another idea is the involvement of the intestinal bacteria that has been revealed to participate in the metabolism of active Kampo ingredients. Many of the main active ingredients of Kampo medicines are glycosides, a molecular group accounting for more than 10% of all Kampo medicines. Orally administered glycosides reach the lower digestive tract without being absorbed and then are hydrolyzed by enteric bacteria to the aglycons, which are subsequently absorbed into the body and become activated (39). The $C_{\text{max}}$ of active ingredients are known to be about 6 – 12 h after administration; therefore, the elevated MDZ concentration by the multiple treatment of anchusan might be caused by the intestinal bacteria-generated metabolites, not by the original chemical constituents. This idea was also supported by the in vitro data that anchusan did not inhibit MDZ 4-OH activity in the situation where there is no contribution of intestinal bacteria (Fig. 4).

In general, when we consider the pharmacokinetics of orally administered drug, it is important to note the effect of transporters in addition to that of CYPs. It has been shown that herbal supplements or foods have an influence on transporters, and it is one of the factors that can change the pharmacokinetic parameters of co-administered drugs. For example, the subchronic treatment of SJW is known to induce CYP3A and also intestinal p-glycoprotein (p-gp), an efflux transporter, resulting in a decrease in the blood concentration of cyclosporine, indinavir, and digoxin (40). However, MDZ used in the current study has been reported to be a substrate of CYP3A, but not of p-gp (41), or an influx transporter organic anion-transporting polypeptide (OATP) (42). These reports also support our data that the increase of the serum MDZ concentration in the multiple-pretreatment study might be caused by the inhibition of CYP3A, neither by inhibition of p-gp nor by induction of OATP. However, there is little information concerning the effect of Kampo medicines on transporters, except a report showing Rikkunshito, Yokukansan, and Bioigito produced little inhibition of p-gp (9). Further studies are needed to identify the interaction between Kampo medicine and western drug via transporters in addition to that by CYPs.

In summary, the increase of serum MDZ concentration after one-week anchusan pretreatment was observed. The single-pretreatment study and in vitro study using rat liver microsomes suggested that this effect was caused by the inhibition of intestinal CYP3A, probably the time-dependent inhibition of this enzyme in part. Anchusan is often used for common gastrointestinal symptoms that imply the high frequency of co-administration with other medicines. Therefore, in the situation when a western drug is metabolized by CYP3A after an individual has taken multiple treatments of anchusan, the elevation of the blood concentration of the drug is a matter of concern. For the appropriate and safe use of Kampo medicines with western drugs, it is necessary to investigate their interactions in humans.

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