Inhibitory Effects of Gastrointestinal Drugs on CYP Activities in Human Liver Microsomes

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OTC drugs have an important role in self-medication. However, the pharmacokinetic properties of some OTC drugs have not been fully investigated and reports concerning their drug interactions are insufficient. Several gastrointestinal drugs are available as OTC drugs. Because of their pharmacological properties, these drugs are often used concomitantly with other drugs. Therefore, it is important to predict the possible drug interactions among these drugs. In the current study, we investigated the inhibitory effects of five gastrointestinal drugs, namely loperamide, oxethazaine, papaverine, pirenzepine, and trimebutine, on CYP activities in human liver microsomes. Furthermore, we calculated the ratio of the intrinsic clearance of each CYP substrate in the presence or absence of the gastrointestinal drugs. The possibility of drug interactions in vivo was predicted by cut-off criteria. CYP3A4 activity was markedly inhibited by trimebutine, papaverine, and oxethazaine. Their inhibitory properties were competitive and the Ki values were 6.56, 12.8, and 3.08 µM, respectively. Alternative R values of CYP3A4 exceeded the cut-off level. These results suggested that drug interactions mediated by CYP3A4 may occur during treatment with these gastrointestinal drugs, necessitating the confirmation of the clinical significance of these drug interactions to prevent unexpected adverse effects.

Key words gastrointestinal drug; OTC drug; drug interaction; CYP

In recent years, people have become increasingly interested in their own health due to a rapidly aging society and/or the increase of lifestyle-related diseases. As a result, the concept of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention. Self-medication is defined as the selection of self-care, which includes self-medication, has attracted popular attention.

Many drug interactions affect specific metabolic processes, especially those mediated by CYP. CYP-mediated drug interactions are roughly classified into enzyme inhibition and enzyme induction. Most are based on enzyme inhibition, with many documented clinically significant cases. Therefore, it is important to examine whether OTC drugs have inhibitory effects on CYP activity.

A recent study reported that diphenhydramine interacted with metoprolol via CYP2D6 inhibition.3) Diphenhydramine potently inhibited metoprolol a-hydroxylation in vitro. Moreover, a clinical study found that diphenhydramine decreased metoprolol metabolic clearance 2.5-fold in CYP2D6 extensive metabolizers.3) Diphenhydramine, a first generation antihistamine, had long been widely used both as a non-prescription and an ethical drug. This interaction has been overlooked so far. Therefore, this potent inhibitory effect on CYP2D6 by diphenhydramine suggested the necessity of examining drug interactions among OTC drugs.

Several gastrointestinal drugs are available as both OTC and ethical drugs. They have been used for a long time; however, few cases of drug interactions have been reported. There have been some reports investigating the inhibitory effect of loperamide,5–7) an anti-diarrheal agent, on CYP3A4 activity. However, the results were varied and have not been conclusive as to its CYP3A4 inhibitory effect. Meanwhile, trimebutine is also widely used as a gastrointestinal agent to treat functional bowel disorders. This drug is metabolized in liver microsomes and the contribution of CYP3A4 has been considered; therefore, its inhibitory effect on CYP activity is concerning. However, an appropriate study has not been conducted, and it is still unknown whether these drugs cause drug interactions. Because of their pharmacological properties, gastrointestinal drugs are often used concomitantly with other therapeutic agents. Therefore, it is important to investigate the possible drug interactions of these drugs to avoid the risk of adverse reaction and to promote safe self-medication.

In the current study, we investigated the inhibitory effects of five gastrointestinal drugs, namely loperamide, oxethazaine, papaverine, pirenzepine, and trimebutine, on CYP activities in human liver microsomes. Furthermore, we calculated the ratio of the intrinsic clearance of each CYP substrate in the presence or absence of these drugs, and the possibility of in vivo drug interaction was predicted using cut-off criteria.

MATERIALS AND METHODS

Chemicals Loperamide hydrochloride, oxethazaine, pa-
Paverine hydrochloride, pirenzepine hydrochloride hydrate, and trimebutine maleate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bufuralol and 1'-hydroxybufuralol were obtained from Gentest Co. (Waburn, MA, U.S.A.). S-Warfarin, S-mephenytoin, and midazolam were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada), ENZO Life Sciences Inc. (New York, NY, U.S.A.), and Nippon Roche K.K. Inc. (Osaka, Japan), respectively. 7-Hydroxywarfarin, 1'-hydroxymidazolam and 4-hydroxymephenytoin were obtained from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Ethoxyresorufin and resorfin were provided by Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). All other chemicals and reagents used were of the highest, commercially available quality.

Human Liver Samples Human liver microsomes (a pooled fraction from 10 patients) were obtained from the non-profit Human and Animal Bridging Research Organization (Chiba, Japan). The study protocol was approved by the Ethics Committee of Showa University School of Medicine.

Assay of CYP Activities The inhibitory effects of OTC drugs on five different CYP isoform-specific activities were studied: ethoxyresorufin O-deethylation (EROD) for CYP1A2, S-warfarin 7-hydroxylation (S-WF 7-OH) for CYP2C9, S-mephenytoin 4'-hydroxylation (S-MP 4'-OH) for CYP2C19, bufuralol 1'-hydroxylation (BF 1'-OH) for CYP2D6, and midazolam 1'-hydroxylation (MDZ 1'-OH) for CYP3A4.

EROD was assayed fluorometrically, according to methods described previously.9,10 Resorfin was determined using a fluorescence spectrophotometer (F-2000, Hitachi, Tokyo, Japan). S-WF 7-OH, S-MP 4'-OH, BF 1'-OH, and MDZ 1'-OH activities were assayed by HPLC according to the method reported previously.9,10 All of the HPLC analyses were carried out using the Shimadzu Prominence UFLC™ system, equipped with a CBM-20A Communication bus module, LC-20AD pump, a SIL-20A automated sample injector, and a SBM-20A UV detector (Shimadzu, Kyoto, Japan). The detection of 7-hydroxywarfarin and 1'-hydroxybufuralol were performed using an L-7480 fluorescence detector (Hitachi). Data were processed with LC solution software (Shimadzu).

Direct Inhibition Study The inhibitory effects of gastrointestinal drugs on CYP activities were determined in the presence of various concentrations of gastrointestinal drugs. Trimebutine maleate and loperamide hydrochloride were dissolved in methanol. Acetic acid was used as solvent for pirenzepine hydrochloride and oxethazaine. The final concentration of these solvents in the reaction mixture was 0.01%. The concentration of gastrointestinal drugs used in this study was 1 to 100 μM.

Concerning the OTC drugs that showed marked inhibitory effects, further kinetic studies were undertaken to determine
the inhibition mechanism and to calculate the apparent inhibition constant ($K_i$). $K_i$ was calculated using nonlinear regression analysis using the Statistical Analysis System® (SAS) version 9.1.

**Preincubation-Dependent Inhibition Study** To evaluate the possibility of mechanism-based inactivation of CYP, the gastrointestinal drugs were preincubated with human liver microsomes and a reduced nicotinamide adenine dinucleotide...
phosphate (NADPH)–regeneration system in the absence of probe substrates. If additional inhibition was observed during the preincubation step with NADPH, the possibility of mechanism-based inhibition is suggested. The preincubation time for each activity was as follows: EROD, 10 min; S-WF 7-OH, 60 min; S-MP 4′-OH, 60 min; BF 1′-OH, 10 min; and MDZ 1′-OH, 10 min. Following preincubation, each probe substrate was added and the activities were analyzed as described above.

**Prediction of Drug Interactions in Vivo** The possibility of drug interaction between gastrointestinal drugs in vivo was estimated using cut-off criteria as recommended by the U.S. Food and Drug Administration (FDA) and Ministry of Health, Labour and Welfare (MHLW).\(^{11-13}\) The ratio of intrinsic clear-
ance value of the substrate for specific CYP reaction in the presence or absence of the gastrointestinal drug (R value) was calculated as follows:

\[ R = 1 + \frac{[I]}{K_i} \]

Where \([I]\) is the total maximal plasma concentration \((C_{\text{max}})\) of the gastrointestinal drug after oral administration. The \(K_i\) is the value obtained in this study. Because CYP3A is highly expressed in the gastrointestinal (GI) tract, possibility of a drug interaction occurring in the GI tract was also estimated using alternative R value as follows:

Alternative R value \((R = 1 + \frac{[I]_g}{K_i})\)

As the maximum concentration in GI tract \(([I]_g)\) dose, (molar dose)/250mL was used. Using \([I]_g\) is considered to more appropriately reflect the total intestinal luminal concentration of the OTC drug than systemic blood concentration.

Based on the R value, we predicted the possibility of clinical drug interaction. The R value and alternative R value exceeded 1.1 and 11, respectively, indicating a high possibility of drug interaction.

RESULTS

Direct Inhibitory Effects of Gastrointestinal Drugs on CYP Activities The inhibitory effects of gastrointestinal drugs on CYP activities are shown in Fig. 1. All of the gastrointestinal drugs, except pirenzepine, inhibited several CYP activities in a concentration-dependent manner. The gastrointestinal drugs which had IC50 values lower than 30 \(\mu M\), were further studied to determine the \(K_i\) values and their inhibitory characteristics (Fig. 2). Oxethazaine showed a potent competitive inhibitory effect on CYP3A4 activity, with a \(K_i\) value of 3.08 \(\mu M\). This activity was also competitively inhibited by papaverine and trimebutine \((K_i=12.8, 6.56 \, \mu M, \text{respectively})\), and non-competitively by loperamide \((K_i=14.5 \, \mu M)\). In addition, CYP2D6 activity was competitively inhibited by oxethazaine and trimebutine, with \(K_i\) values of 6.3 and 20.2 \(\mu M\), respectively, and non-competitively by loperamide \((K_i=5.9 \, \mu M)\).

Preincubation Effects of Gastrointestinal Drugs on CYP Activity To estimate the possibility of mechanism-based inhibition, the gastrointestinal drugs were preincubated with human liver microsomes in the presence or absence of NADPH. The result showed no remarkable differences in CYP activities with the preincubation step. Therefore, these drugs were considered less likely to cause mechanism-based inhibition.

Prediction of Risk of Drug Interaction R Values were calculated using \(K_i\) values obtained in the current study and evaluated whether these gastrointestinal drugs caused drug interaction in vivo (Table 1). The dose and \(C_{\text{max}}\) values of each gastrointestinal drug were obtained from their respective pharmaceutical drug package inserts. Calculated R values of loperamide and trimebutine for CYP2D6 and those of loperamide, papaverine, and trimebutine for CYP3A4 did not exceed the cut-off value of 1.1. We could not calculate the R value of oxethazaine for these enzymes, because there was no information about its \(C_{\text{max}}\) value. In contrast, alternative R values for loperamide, oxethazaine, papaverine, and trimebutine for the prediction of intestinal CYP3A4-mediated drug interactions were 1.54, 28.8, 25.9, and 122.1, respectively.

DISCUSSION

In the current study, we investigated the CYP inhibitory effects of several gastrointestinal drugs, which have long been used OTC and on prescription, using human liver microsomes. Our results showed that all the gastrointestinal drugs used in this study, except pirenzepine, had inhibitory effects on multiple CYPs.

Oxethazaine, papaverine, trimebutine, and loperamide showed potent inhibitory effects on CYP3A4 activity. Especially, oxethazaine indicated the lowest \(K_i\) value at 3.08 \(\mu M\). Oxethazaine has long been frequently used as both an OTC and ethical drug because of its efficacy against gastrointestinal pain. However, there is no information regarding its pharmacokinetics, including its metabolic pathway and pharmacokinetic properties. Therefore, we could not calculate the R value of CYP3A4 to predict its in vivo inhibition in the liver. However, if its plasma concentrations were high, the inhibition of hepatic CYP3A4 activity would occur in vivo because of its low \(K_i\) value. Incidentally, CYP3A4 is expressed not only in the liver but also in the small intestine, and is the most abundant CYP in these organs. CYP3A4 plays an important role in the first-pass effect of medicine. In our current study, oxethazaine exceeded the cut-off value for the alternative R value, suggesting the inhibition of CYP3A4 by oxethazaine in the small intestine at the absorption phase. Similarly, the alternative R value of trimebutine and papaverine exceeded the cut-off value, although these R values showed low levels. Therefore, it is considered that oxethazaine, trimebutine, and papaverine cause drug interactions mediated by CYP3A4 inhibition in the small intestine rather than the liver. CYP3A4 is responsible for the metabolism of more than 50% of therapeutic drugs such as nifedipine, triazolam, simvastaine, and vardenafil. Because of the pharmacological properties of gastrointestinal drugs, it is often used concomitantly with other therapeutic agents. Therefore, careful attention is needed to avoid drug interactions through CYP3A4 inhibition.

On the other hand, the \(K_i\) value of loperamide for CYP3A4 activity was 14.5 \(\mu M\). A previous study reported that loperamide is metabolized to N-demethylated loperamide mainly by CYP3A4 in human liver microsomes and that the metabolism is affected by CYP3A inhibition. In contrast, because it is not clear whether loperamide inhibits CYP3A enzyme, our study was designed to focus on loperamide as a CYP3A inhibitor. Marechal et al. showed that loperamide strongly inhibited CYP3A4-mediated 7-benzylxlo 4-trifluoromethylcoumarin with an IC50 value of 0.05 \(\mu M\), which is close to its therapeutic level. Therefore, it was considered that loperamide may cause a drug interaction mediated by CYP3A4. However, in the current study using a cut-off value for predicting CYP3A4 inhibition, we demonstrated that loperamide was less likely to cause a drug interaction in vivo. This could be due to the low clinical dose of loperamide used, which resulted in its low plasma concentration. This result is consistent with the report of Haaz et al. that loperamide inhibited the CYP3A4-mediated CPT-11 metabolism with a high IC50 value. They also concluded that significant drug interactions with loperamide may not occur in the clinical setting.

Loperamide, oxethazaine, and trimebutine also showed potent inhibition for CYP2D6 with \(K_i\) values of 5.9, 6.3, and
20.2 μM, respectively. However, R values of loperamide and trimebutine did not exceed cut-off values, making drug interactions mediated by CYP2D6 in vivo seem less likely. The likelihood of drug interactions with oxethazaine is thought to depend on its plasma concentration similar to CYP3A4, as discussed above.

In this study, we were not able to clarify the inhibitory effect of papaverine on CYP2C19 activity because measurement by HPLC was impossible. A previous report has shown that papaverine inhibited CYP2C19 activity with K_i values of 20 μM. Therefore, we tried calculating the R value for CYP2C19 using this K_i value; however, it did not exceed the cut-off level (R < 1.1). Therefore, we think that clinically significant drug interactions with papaverine would not occur.

This study also investigated the inhibitory effects of gastrointestinal drugs on CYP1A2, CYP2C9, and CYP2C19 activity. However, they had no remarkable effects on these CYP activities and are, therefore, less likely to cause drug interactions mediated by these CYP isofoms. Our study was designed to specifically focus on top five enzymes responsible for the biotransformation of drugs in clinical use. However, other CYP isoenzymes such as CYP2C8 and CYP2B6 are also important for drug interaction. Inhibition studies focusing on these isoenzymes should be performed in the future. Additionally, pirenzepine was not shown to have an inhibitory effect on any of the CYP activities in this study. We think that pirenzepine does not cause drug interactions through CYP.

In the present study, we did not use unbound drug concentration for the prediction of drug interactions in the liver. The method recommended by MHLW uses total plasma concentration. Our results suggested that drug interaction is less likely to occur in the liver even if total plasma concentration is used, which is considered to be comparable to unbound concentration. Therefore, no further prediction using unbound plasma concentration was performed.

In this study, we demonstrated that gastrointestinal drugs, which have been widely used as relatively safe OTC drugs, have potent inhibitory effects on CYP activities. Our results suggested that OTC drugs might cause drug interactions mediated by CYP inhibition when given in polypharmacy. Until now it was believed that OTC drugs were relatively safe and, even if drug interaction occurred, we might overlook them. As self-medication is increasingly promoted, the opportunity to use OTC drugs might increase for the treatment of digestive upset in patients with lifestyle-related diseases including hypertension and diabetes, conditions which require long-term medication. However, using OTC drugs, the safety of which has not been established, might cause unexpected adverse effects if used in polypharmacy. Therefore, it is important to clarify whether each OTC drug has the potential to cause a drug interaction in polypharmacy for safe self-medication.

In conclusion, oxethazaine, papaverine, and trimebutine showed potent inhibitory effects on CYP3A4 activity and high alternative R values. Therefore, they have the potential to cause drug interactions mediated by CYP3A4 inhibition in the small intestine. Further in vivo studies are required to determine the clinical relevance of these interactions.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


