Identification of Human P450 Isoforms Involved in the Metabolism of the Antiallergic Drug, Oxatomide, and Its Kinetic Parameters and Inhibition Constants

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Oxatomide is an antiallergic drug used for the treatment of diseases mediated by type I allergic reaction. Recently, terfenadine and astemizole, which have antiallergic actions similar to those of oxatomide, showed side effects on the cardiovascular system. This might be because concomitant drugs such as itraconazole inhibit cytochrome P450 3A4 (CYP3A4), the enzyme responsible for the degradation of terfenadine and astemizole, and thus the blood concentrations of the drugs are abnormally increased. In another article of this issue, we have reported that oxatomide is metabolized by CYP2D6-Val and CYP3A4, and simultaneously inhibits the metabolism of the model substrates for these enzymes. In this study, we performed the kinetic analysis of oxatomide metabolism using microsomes prepared from human liver, and found that the \( K_m \) and \( V_{max} \) values were 26.1 \( \mu M \) and 1254.4 pmol/mg protein/min, respectively. Ketocazole, one of the representative inhibitors for CYP3A4, potently inhibited the metabolism of oxatomide, but other well-known CYP inhibitors did not show significant inhibition. These results suggest that the metabolism of oxatomide is principally catalyzed by CYP3A4. Furthermore, oxatomide inhibited the metabolism of \( \pm \)bufuralol and testosterone, model substrates for CYP2D6 and CYP3A4, respectively, in a dose-dependent manner with the \( K_i \) values of 57.4 and 24.3 \( \mu M \), respectively. These observations, together with the finding that the putative highest concentration of oxatomide in blood was \( \approx 40 \) ng/ml \( (\approx 93 \) nm) at 4 h after each dosage during consecutive 6-d administration, encouraged us to conclude that oxatomide won’t inhibit CYP2D6 or CYP3A4 at clinical doses.

Key words oxatomide; antiallergic drug; cytochrome P450 2D6 (CYP2D6); CYP3A4; metabolism

Oxatomide is an antiallergic drug used for the treatment of diseases mainly mediated by a type I allergic reaction. Pharmacological studies have demonstrated that oxatomide acts as an antagonist for various chemical mediators such as histamine, leukotriene and platelet-activating factor, as well as inhibits the release of these substances, and that all these actions contribute to therapeutic effects of the drug. 1–3 Oxatomide is widely applied to skin diseases, including chronic urticaria, 4 skin itching, 5 atopic dermatitis, 6 allergic rhinitis 7) and bronchial asthma 8) in the clinical field.

In a previous article, we showed that the P450 isoforms responsible for the metabolism of oxatomide were cytochrome P450 2D6 (CYP2D6) and CYP3A4, using microsome preparations of the \emph{in vitro} expression systems derived from a human lymphoblastoid cell line. 9) We also demonstrated that oxatomide inhibited the enzyme activity of CPY2D6-Val and CYP3A4 in a dose-dependent manner, using model substrates for these isoforms. A similar action has been reported for the antiallergic drugs, terfenadine and astemizole, which inhibit both CYP2D6-Val and CYP3A4. 10–12 It has been shown that terfenadine and astemizole have side effects on the cardiovascular system, such as QT prolongation, ventricular arrhythmia and cardiac arrest. 13–18 Since the principal metabolic pathway for the two drugs is mediated by CYP3A4, the blood concentration of the drugs is increased when CYP3A4 is strongly inhibited by concomitant drugs such as itraconazole and ketoconazole. 19–22 On the other hand, there has been no report of side effects of oxatomide on the cardiovascular system, such as QT prolongation, but the drug has been shown to induce catalepsy at high doses in experimental animals, 23 and infrequently to cause extrapyramidal disorder by clinical usage. 24 Our results in the former article raised the possibility that oxatomide might inhibit the same enzymes as those inhibited by terfenadine and astemizole, which suggests we should investigate the mode of action of oxatomide in greater detail. Therefore, to clarify whether oxatomide brings about any drug interaction or is affected by the interaction, we examined kinetic parameters \( (K_m \) and \( V_{max} \)) for the oxatomide metabolism and inhibition constants \( (K_i) \) of oxatomide in metabolism of the model substrates for CYP2D6 and CYP3A4, and possible drug interaction is discussed.

MATERIALS AND METHODS

\textbf{Reagents} Oxatomide and 3’-ethylxoxatome were synthesized at Kyowa Hakko Kogyo Co., Ltd. \( \beta \)-NAD\(^+\), \( \beta \)-NADP\(^+\), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Ltd.; MgCl\(_2\), from Wako Pure Chemicals Industries, Ltd.; \( \alpha \)-naphthoflavone, sulfaphenazole, tranylcypromine hydrochloride, quinidine and alpenrolol hydrochloride, from Sigma Chemical Co.; 6\( \beta \)-hydroxytestosterone, from Ultrafine Chemicals; \( \pm \)-bufuralol hydrochloride and 1’-hydroxybufuralol maleate, from Gentest; testosterone and phenacetin, from Nacalai Tesque, Inc.; and ketoconazole, from ICN Pharmaceuticals, Inc. Other reagents were of special or HPLC grade.

\textbf{Microsome Preparations} Pooled human liver microsomes, prepared from ten subjects, were purchased from Gentest. The microsomes were stored at around \( -80 ^\circ C \), and…

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freezing and thawing was allowed less than three times.

Methods. 1) Determination of Kinetic Parameters ($K_{m}$ and $V_{max}$) for Oxatomide Metabolism. a. Analysis of Oxatomide Oxatomide was analyzed by HPLC (LC10-AD series, Shimadzu Corporation) using an analytical column (YMC-Pack AM-312, 6×150 mm, YMC). Ammonium acetate (10 mm, pH 4.8, titrated by acetic acid) and methanol were used as the mobile phase. Flow velocity was 1 ml/min, and the gradient condition was 3:7 for 20 min and 3:7 to 1:9 for 5 min, and 1:9 for the next 5 min. The column temperature was 50°C. Elution time and peak forms were analyzed for oxatomide and its internal standard, 3′-ethoxyoxatomide. Fluorescence detection was performed at an excitation wavelength (Ex) of 280 nm and an emission wavelength (Em) of 309 nm.

b. Recovery of Oxatomide after the Reaction with Heat-Inactivated Microsomes Oxatomide (final concentration, 1 and 10 μM) was incubated with heat-inactivated (100°C, 10 min) human liver microsomes. After the addition of 3′-ethoxyoxatomide (3 μg/ml), the mixture was centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4°C for 5 min, and the supernatant was analyzed by HPLC using the same method as 1)a. Theoretical 100% recovery was determined by the HPLC peak area of unreacted oxatomide and its internal standard. The recovery after the microsomal reaction was calculated from the peak area of the oxatomide compared to the theoretical value.

c. Standard Curves (Validation of Linearity) Two series of oxatomide solutions (0.1, 0.5, 1, 5, 10 μM and 5, 10, 50, 100, 600 μM; n = 3) were incubated with heat-inactivated (100°C, 10 min) human liver microsomes. After the addition of 3′-ethoxyoxatomide, the mixture was centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4°C for 5 min, and the supernatant was analyzed by HPLC using the same method as 1)a. The standard curves were made by summation linear regression (weight, 1/concentration) for concentrations of 0.01—1.2 μM, and by non-summation linear regression for concentrations of 0.5—60 μM. The concentrations of standard substances were calculated from these curves, and the linearity was confirmed by mean trueness (RE%) and precision (CV%), which were calculated as follows:

\[
\text{trueness (RE%)} = \frac{(\text{measured concentration}) - (\text{prepared concentration})}{\text{prepared concentration}} \times 100
\]

\[
\text{precision (CV%)} = \frac{\text{standard deviation}}{\text{mean of measured concentrations}} \times 100
\]

d. Determination of Kinetic Parameters ($K_{m}$ and $V_{max}$) for Oxatomide Metabolism i) Experiment 1: Human liver microsomes (1.0 mg protein/ml) and oxatomide (1 μM) were incubated in 500 μl of 80 mM Tris–HCl buffer (pH 7.4) containing 0.8 mM β-NADH, 0.8 mM β-NADPH, 8.0 mM glucose-6-phosphate, 30 mM MgCl₂, and 10 units/ml glucose-6-phosphate dehydrogenase (NADPH G.S.) at 37°C. The reaction was initiated by the addition of microsomes after 5 min preincubation, and stopped by adding an equivalent volume of the methanol solution of the internal standard substance, 3′-ethoxyoxatomide. Samples were collected at 0, 5, 10, 20 and 30 min, and centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4°C for 5 min. The supernatant was analyzed by HPLC using the same method as 1)a.

ii) Experiment 2: Human liver microsomes (1.0 mg protein/ml) and oxatomide (0.1—50 μM) were incubated in 500 μl of 80 mM Tris–HCl buffer (pH 7.4) containing NADPH G.S. at 37°C for 10 min. The reaction was initiated by the addition of microsomes after 5 min preincubation, and stopped by adding the equivalent volume of methanol solution of the internal standard substance, 3′-ethoxyoxatomide. The reaction mixture was centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4°C for 5 min, and the supernatant was analyzed by HPLC using the same method as 1)a.

Analysis: The reaction mixture to which the internal standard solution had been added was centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4°C for 5 min to remove protein. An aliquot (50 μl) of the supernatant was analyzed by HPLC in the conditions described above, and the concentration of unchanged compound was calculated from the standard curve using the range of 0.01—1.2 μM for 0.1—1 μM oxatomide, and the range of 0.5—60 μM for 5—50 μM oxatomide in Experiment 2.

2) Identification of P450 Isomers Involved in Oxatomide Metabolism Using Human Liver Microsomes Oxatomide (10 μM) was incubated with human liver microsomes (1.0 mg protein/ml) in 500 μl of 80 mM Tris–HCl buffer (pH 7.4) containing NADPH G.S. at 37°C for 10 or 30 min. The inhibitors used were 1 μM α-naphthoflavone (CYP1A), 10 μM sulfaphenazole (CYP2C9), 20 μM trimlycypryone (CYP2C19), 4 μM quinidine (CYP2D6), and 3 μM ketoconazole (CYP3A4). The reaction was initiated by the addition of microsomes after 5 min preincubation, and stopped by adding the equivalent volume of the internal standard substance, 3′-ethoxyoxatomide, dissolved in methanol. The reaction mixture was centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4°C for 5 min, and the supernatant was analyzed by HPLC using the same method as 1)a. All experiments were done in duplicate.

3) Determination of Inhibition Constants ($K_{i}$) of Oxatomide Using Human Liver Microsomes. i) Inhibition of CYP2D6 Bufuralol (5, 10, 20, 30, 40 μM) and the microsomes (0.5 mg protein/ml) were incubated in the presence of 0, 1, 10 and 50 μM oxatomide, in 500 μl of 80 mM Tris–HCl buffer (pH 7.4) containing NADPH G.S. at 37°C for 15 min. Quinidine (4 μM) was used as a positive control inhibitor. The reaction was initiated by the addition of microsomes after 5 min preincubation, and stopped by adding 200 μl of the internal standard substance, alpranolol (5 μg/ml), dissolved in methanol. The reaction mixture was centrifuged by a high-speed micro refrigerated centrifuge...
(CF15D2, Hitachi Koki Co., Ltd.) with a T15A12 rotor (Hitachi Koki Co., Ltd.) at 12000 rpm at 4 °C for 5 min, and the concentration of the product, 1'-hydroxybufuralol, was analyzed by HPLC. The column was Inertsil ODS-3 (5 μm, 4.6×250 mm, GL science), and the solvents were 1 mM perchloric acid and acetonitrile. Flow velocity was 1 ml/min, and the gradient condition was 9:1 to 1:1 for 20 min, and 1:1 for the 1 min addition, and the column temperature was 50 °C. Fluorescence detection was performed at an excitation wavelength (Ex) of 252 nm and emission wavelength (Em) of 302 nm.25

ii) Inhibition of CYP3A4 Testosterone (20, 50, 100, 150, 200 μM) and the microsomes (0.1 mg protein/ml) were incubated in the presence of 0, 1, 10 and 50 μM oxatomide, in 500 μl of 80 mM Tris–HCl buffer (pH 7.4) containing NADPH G.S. at 37 °C for 30 min. Ketoconazole (1 μM) was used as a positive control inhibitor. The reaction was initiated by the addition of microsomes after 5 min preincubation, and stopped by adding 200 μl of the internal standard substance, phenacetin (5 μg/ml), dissolved in methanol. The reaction mixture was centrifuged by a high-speed micro refrigerated centrifuge (CF15D2, Hitachi Koki Co., Ltd.) at 12000 rpm at 4 °C for 10 min, and the concentration of the product, 6β-hydroxytestosterone, was analyzed by HPLC. The column was Cosmosil 5C18-AR (4.6×150 mm, Nacalai Tesque), and the solvents were H2O: tetrahydrofuran (5:1, v/v) and methanol. Flow velocity was 1 ml/min, the gradient condition was 4:1 to 7:3 for 10 min and 3:7 for the 5 min addition, and the column temperature was 40 °C. Detection was performed by UV absorption at 240 nm.26

Data Analyses Standard Curve in Method 1): Two series of oxatomide solutions (0.01, 0.05, 0.1, 0.5, 1.2 μM and 0.5, 1, 5, 10, 60 μM) were incubated with heat-inactivated (100 °C, 10 min) human liver microsomes. After addition of the internal standard, the mixture was centrifuged at 12000 rpm at 4 °C for 5 min, and the supernatant was analyzed by HPLC. The regression equation was obtained from the measured values by using JMP 3.1 (SAS, Inc.). Kinetic Parameters (Km and Vmax) for Oxatomide Metabolism (Experiment 1): The concentration of oxatomide was calculated from the regression equation for a standard curve using Excel 97. The enzyme activity was determined by the decreased amount of drug compared to the control (the sample collected at time 0). The 1/[S]0−1/V plot was created using by the enzyme activity and the initial concentration of oxatomide to obtain Km and Vmax (t[S]0, initial concentration of oxatomide; V, enzyme activity).

Rate of Unchanged Compound (Experiment 2): The concentrations of oxatomide in the presence and absence of typical inhibitors were obtained from the regression equation for the standard curve using Excel 97. The rate of unchanged compound was calculated as follows:

\[
\text{rate of unchanged compound (％ of control)} = \frac{\text{concentration of oxatomide in the presence of the inhibitor}}{\text{concentration of oxatomide in the absence of the inhibitor}} \times 100
\]

Standard Curve in Method 3): Standard substances at a given concentration were incubated with heat-inactivated human liver microsomes and NADPH with coenzymes for NADPH reaction. After the reaction, the concentration of each substance was determined according to the corresponding method. A single-point standard curve passing through the origin was drawn, and the regression equation was obtained by Excel 97.

Inhibition Constants (Ki) of Oxatomide: The concentration of each subject substance was determined from the regression equation for each standard curve using Excel 97, and the enzyme activity was calculated. The slope of the 1/[S]0−1/V plot was obtained from the enzyme activity and the initial concentration of each model substrate. The inhibition constant (Ki) was determined from secondary plotting against t[S]0 (t[S]0, initial concentration of model substrate; V, enzyme activity; t, initial concentration of oxatomide).

RESULTS

1) Kinetic Parameters (Km and Vmax) for Oxatomide Metabolism The analytical HPLC system was validated beforehand using 3’-ethyloxatomide as an internal standard. As the elution time and peak forms of oxatomide and 3’-ethyloxatomide were satisfactory (Fig. 1), the system was used in the analysis of oxatomide. Next, we confirmed the metabolism of oxatomide using human liver microsomes. Oxatomide (1 μM) was incubated with the microsomes, and the concentrations of oxatomide at 0, 5, 10, 20 and 30 min of reaction were determined. Figure 2 shows that the concentration of oxatomide was decreased in a time-dependent manner. When oxatomide was reacted with the heat-inactivated microsome for 30 min, the concentration of the drug was 0.9353 μM, showing no decrease in the rate of unchanged compound. Thus, the time-dependent decrease in oxatomide was confirmed to be due to microsomal metabolism. Since the metabolic rate for oxatomide was found to be linear within 0–10 min of reaction, the reaction time was performed for 10 min in the following experiments.

The metabolic activity for oxatomide at 0.1, 0.5, 1, 5, 10 and 50 μM was determined from the decreased amount of the drug after 10-min reaction (Fig. 3A), and reciprocal Lineweaver–Burk plotting was performed, from which Km and Vmax for oxatomide were determined to be 26.1 μM and 1254.4 pmol/mg protein/min, respectively (Fig. 3B).

![Typical Chromatogram of Oxatomide and Internal Standard Concentration of Oxatomide, 10 μM](image)
2) Identification of Human P450 Isoforms Involved in Oxatomide Metabolism

Isoforms of human P450 involved in the metabolism of oxatomide were identified using human liver microsomes. Oxatomide (10 μM) was incubated with the microsomes for 10 or 30 min in the presence of various representative inhibitors of P450 isoforms. A similar reaction was performed in the absence of the inhibitors, and the rate of unchanged compound was compared between the two conditions to identify the isoforms responsible for oxatomide metabolism. As summarized in Table 2, the rate of unchanged oxatomide was 92.2—109.0% and 84.2—113.3% at 10 and 30 min of reaction, respectively, in the presence of α-naphthoflavone, sulfaphenazole, tranylcypromine and quinidine, showing no inhibition of the metabolism by CYP1A, CYP2C9, CYP2C19 or CYP2D6. In contrast, the rate of unchanged compound was increased to 144.0 and 215.0% at 10 and 30 min, respectively, in the presence of ketoconazole, indicating that the degradation of oxatomide was markedly inhibited. These results suggested that oxatomide might be metabolized by CYP3A4.

3) Inhibition Constants (K_i) of Oxatomide in the Reaction with Human Liver Microsomes

i) Inhibition of CYP2D6

Inhibitory effects of oxatomide on the metabolism of the model substrate for CYP2D6 were investigated using human liver microsomes to obtain the inhibition constant (K_i). As shown in Fig. 4A, oxatomide showed dose-dependent inhibition of the metabolism of bufuralol, a model substrate for CYP2D6, with a K_i value of 57.4 μM (Fig. 4B).

ii) Inhibition of CYP3A4

Inhibitory effects of oxatomide on the metabolism of the model substrate for CYP3A4 were investigated using human liver microsomes to obtain the inhibition constant (K_i). As shown in Fig. 5A, oxatomide showed dose-dependent inhibition on the metabolism of testosterone, a model substrate for CYP3A4, with a K_i value of 27.4 μM (Fig. 5B).

DISCUSSION

In the former article, using human-lymphoblastoid-derived microsomes, we demonstrated that oxatomide might inhibit CYP2D6 and CYP3A4.9 These results suggested that more detailed examinations should be investigated using human liver microsomes to evaluate the metabolic activity and K_i value of oxatomide regarding CYP2D6 and CYP3A4.

We performed kinetic analysis of oxatomide metabolism using microsomes prepared from human liver, and found that the K_m and V_max values were 26.1 μM and 1254.4 pmol/mg Protein/min, respectively. From this result, identification of the P450 isoforms involved in oxatomide metabolism and determination of inhibition constants of oxatomide were examined on concentrations up to 50 μM. We showed that one of the representative inhibitors for CYP3A4, ketoconazole, inhibited the degradation of oxatomide, indicating that the inhibitor affected the metabolism of oxatomide catalyzed by CYP3A4. In addition, the degradation of testosterone was inhibited by oxatomide, with a K_i value of 27.4 μM, suggesting that oxatomide also affects drug metabolism by CYP3A4 (testosterone 6β-hydroxylase), as indicated in the previous

Table 1. Inhibition of Oxatomide Metabolism in Pooled Human Liver Microsomes with Each Inhibitor

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (μM)</th>
<th>% of control (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>100.0 100.0</td>
</tr>
<tr>
<td>α-Naphthoflavone</td>
<td>1</td>
<td>103.8 98.3</td>
</tr>
<tr>
<td>Sulfaphenazole</td>
<td>10</td>
<td>92.2 84.2</td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>20</td>
<td>109.0 113.3</td>
</tr>
<tr>
<td>Quinidine</td>
<td>4</td>
<td>100.7 105.3</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>1</td>
<td>144.0 215.0</td>
</tr>
</tbody>
</table>

Oxatomide concentration was 10 μM and the control had no inhibitor in the incubation.
In contrast, quinidine, an inhibitor of CYP2D6, did not inhibit the metabolism of oxatomide. However, oxatomide inhibited the metabolism of bufuralol with a $K_i$ value of $57.4 \text{ mM}$, suggesting that oxatomide influences drug metabolism by CYP2D6 (bufuralol 1'-hydroxylase). The result of quinidine was unexpected, considering that our previous result showed that bufuralol metabolism was inhibited by oxatomide.9) From these results, oxatomide might be metabolized by CYP2D6 at a different site from the quinidine binding site. In addition, the $K_m$ value of the microsomes for oxatomide metabolism was $26.1 \text{ mM}$. It is reasonable that the $K_i$ value of oxatomide for CYP3A4 (27.4 $\mu M$) would be close to the $K_m$ value of the microsomes. However, the $K_i$ value of oxatomide for CYP2D6 (57.4 $\mu M$) is almost twice the $K_m$ value, and the reason is obscure.

It has been reported that terfenadine and astemizole, both of which have antiallergic actions similar to those of oxatomide, are metabolized by CYP2D6 and CYP3A4 in the same manner.10—12) It has also been demonstrated that the blood concentrations of these drugs are increased by such agents as itraconazole and ketoconazole, which strongly inhibit CYP3A4.19—22) On the other hand, it has been shown by Kishimoto et al. that epinastine is metabolized by CYP3A4 and CYP2D6, and also slightly by CYP2B6, but does not inhibit the metabolism of testosterone, a model substrate for CYP3A4, even at $100 \mu M$.27) This means that epinastine, different from terfenadine and astemizole, is metabolized by CYP3A4 but does not affect the metabolism of other drugs.
Therefore, there is the possibility that oxatomide, as well as epinastine, does not affect the metabolism of other drugs, which differs from the actions of terfenadine and astemizole. We showed that the metabolism of oxatomide was affected by inhibiting CYP3A4, while quinidine did not inhibit the CYP2D6-catalyzed metabolism of oxatomide, which raises the possibility that the metabolism of oxatomide could be compensated for by CYP2D6 when CYP3A4 is inhibited.

It has been reported that when terfenadine (60 mg) is co-administered with ketoconazole (200 mg), the time of maximal concentration (T_{max}) and C_{max} are not significantly changed, while the area under the curve (AUC) is increased from 1951 to 3067 ng/h/ml, which results in QT prolongation.31) It has been also reported that the blood concentration of terfenadine is increased from 1.3 ng/ml to <20 and <80 ng/ml when the blood concentration of ketoconazole is increased from 0 to 1 and 5 μg/ml.29) Moreover, the blood concentration of astemizole has been reported to increase from 2.73—3.63 to 15.85 ng/ml by combined administration with cimetidine in a single normal subject.30) If oxatomide is affected by the agents inhibiting CYP3A4 and CYP2D6, like terfenadine and astemizole, the blood concentration of oxatomide might be increased, and side effects might occur. The maximal drug concentration (C_{max}) of oxatomide in clinical usage is 19.3 ng/ml (±45 nm) (unpublished observation), and when 30 mg is given to adult asthma patients twice a day continuously, the drug concentrations immediately before and 4 h after administration is 20—30 ng/ml and ±40 ng/ml (±93 nm, the putative highest concentration), respectively, on day 6 and later.31) But there has been no report of such a side effect, suggesting that oxatomide might not be affected by other concomitant drugs, and the concentration might not increase because the blood concentration of oxatomide is much lower than the K_{i} value of CYP2D6 and CYP3A4. Our results demonstrated that the apparent K_{m} values for both the P450 isoforms were increased with an increased concentration of oxatomide, whereas the V_{max} values were not changed, indicating that oxatomide inhibits CYP2D6 and CYP3A4 in a competitive manner. If this is the case, oxatomide might be dissociated from the bound enzyme, and the metabolism of oxatomide might be immediately compensated for by other isoforms, when other drugs bind with CYP3A4 and/or CYP2D6. Consequently, oxatomide would be degraded and disappear from the blood, without inducing any side effect.

There has been no report of side effects of oxatomide on the cardiovascular system by concomitant drugs. Iwamoto et al. investigated the effects of oxatomide and terfenadine on QT prolongation in a cardiogram in the presence and absence of the concomitant drug, itraconazole, using conscious dogs. They showed that a single administration (30 mg/kg), as well as concomitant administration of terfenadine (10 mg/kg) with itraconazole (100 mg/kg, administered 1 h before terfenadine), induced QT prolongation, while oxatomide did not exhibit such an effect by either single (30 mg/kg) or concomitant (10 mg/kg of oxatomide with itraconazole) administration.32) These results suggest that the increased blood concentration of oxatomide might not cause QT prolongation. However, oxatomide has infrequently been reported to induce extrapyramidal disorder.24) This action of oxatomide is thought to be due to the inhibition of dopamine D1 and D2 receptors in experiments using mice.33) In addition, oxatomide inhibits the uptake of dopamine.34) Therefore, it cannot be ruled out that oxatomide shows side effects on the central nervous system when both CYP2D6 and CYP3A4 are simultaneously inhibited to induce some drug interaction.

The major oxatomide metabolic pathways were oxidative N-dealkylations and also aromatic hydroxylation, as reported by Meuldermans et al.35) From the result of this article, CYP2D6 and CYP3A4 appear to be enzymes that metabolize oxatomide, so that the dealkylation and hydroxylation of oxatomide are dependent on CYP2D6 and CYP3A4. However, which enzyme will be related to the dealkylation and hydroxylation pathway is not yet understood. We determined that oxatomide degradation was not inhibited by quinidine, suggesting that CYP3A4 might be the main isoform of metabolism of oxatomide. In addition, Kikuchi et al. reported that in the rat model, the bioavailability of oxatomide was 6.74%.36) It is well known that CYP3A4 also exists in the small intestine, and many drugs that show low bioavailability were metabolized by small intestine CYP3A4 before the drugs reach the plasma. For all these reasons, we expect that oxatomide is mainly catalyzed by CYP3A4.

In conclusion, it is still important to pay attention to possible side effects of oxatomide due to concomitant administration with other drugs.

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