Identification of Human P450 Isoforms Involved in the Metabolism of the Antiallergic Drug, Oxatomide, and Its Inhibitory Effect on Enzyme Activity

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Oxatomide is an antiallergic drug used for the treatment of diseases mediated by type I allergy. Recently, it has been reported that terfenadine and astemizole, which have antiallergic actions similar to those of oxatomide, show side effects on the cardiovascular system, such as QT prolongation, ventricular arrhythmia and cardiac arrest. This might be because concomitant drugs such as itraconazole inhibit cytochrome P450 3A4 (CYP3A4), the enzyme responsible for degradation of terfenadine and astemizole, and thus the blood concentrations of the drugs are abnormally increased. On the other hand, isoforms of P450 involved in the metabolism of oxatomide have not been clarified. Therefore, we attempted to identify these isoforms using microsome preparations of in vitro expression systems derived from a human lymphoblastoid cell line. Oxatomide was metabolized by CYP2D6-Val and CYP3A4, but not by CYP1A2, CYP2C9-Arg, CYP2C9-Cys or CYP2C19. We also examined whether oxatomide showed inhibitory effects on metabolic activity of individual P450 isoforms using model substrates for each isozyme. Oxatomide did not inhibit the metabolism of the model substrates for CYP1A2, CYP2C9-Arg, CYP2C9-Cys and CYP2C19, but inhibited the degradation of those for CYP2D6-Val and CYP3A4. It was found that oxatomide is metabolized by CYP2D6 and CYP3A4 in human liver microsomes, and simultaneously acts as an inhibitor for these isoforms, responsible for the metabolism of the drug itself.

Key words oxatomide; antiallergic drug; P450; cytochrome P450 2D6 (CYP2D6)-Val; cytochrome P450 3A4 (CYP3A4)

Oxatomide is an antiallergic drug used to treat diseases mainly mediated by type I allergic reaction. The drug is widely applied to skin diseases including chronic urticaria, skin itching and atopic dermatitis, allergic rhinitis and bronchial asthma in the clinical field. 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 the therapeutic effects of the drug.

It has been reported that terfenadine and astemizole, which have antiallergic effects similar to those of oxatomide, have side effects on the cardiovascular system, such as QT prolongation, ventricular arrhythmia and cardiac arrest. It is well known that drugs taken into organisms are transformed to metabolites, and that the major organ responsible for degrading such drugs is the liver, where there are a large number of enzymes involved in drug metabolism. Among them are a family of cytochrome P450 (CYP), which greatly contribute to drug metabolism, showing variable isofrom expression in the liver microsomes. Terfenadine and astemizole have been demonstrated to be metabolized mainly by two P450 isoforms, CYP2D6 and CYP3A4, the latter of which is known to be responsible for the metabolism of various drugs, including steroids (testosterone, estradiol, etc.), antibiotics (erythromycin, cyclosporin, etc.), plant alkaloids (lovastatin, benzphetamine, etc.) and quinidine, and to be markedly inhibited byazole antifungal agents such as itraconazole and ketoconazole. When itraconazole or ketoconazole is orally administered together with terfenadine or astemizole, drug interaction occurs, and the blood concentration of terfenadine or astemizole is increased, resulting in severe side effects. Although oxatomide is also antiallergic and shares a common partial chemical structure with terfenadine and astemizole, as shown in Fig. 1, there has been no report of the side effects of the drug on the cardiovascular system. However, it has been reported that extrapyramidal disorder is rarely induced by oxatomide, suggesting the possibility of side effects relating to extrapyramidal symptoms, if oxatomide undergoes a drug interaction. Nevertheless, there has been no available information on drug interaction of oxatomide, which leads to side effects, or on isoforms

Fig. 1. Chemical Structures of Oxatomide, Terfenadine and Astemizole
of P450 involved in oxatomide metabolism. Therefore, we attempted to identify P450 isoforms responsible for oxatomide metabolism using microsome preparations of in vitro expression systems derived from a human lymphoblastoid cell line. Furthermore, we examined the effects of oxatomide on selective substrate metabolism by various P450 isoforms, and discuss possible influences of oxatomide on the metabolism of concomitant drugs.

**MATERIALS AND METHODS**

**Reagents** Oxatomide and 3′-ethyloxatomide were synthesized at Kyowa Hakko Kogyo Co., Ltd. β-NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Ltd.; MgCl₂, from Wako Pure Chemical Industries, Ltd.; α-naphthoflavone, 7-ethoxyresorufin, resorufin sodium salt, diclofenac, phenacetin and alpranolol, from Sigma Chemical Co.; SKF-525A, S(-)-mephenytoin, (±)-4′-hydroxymephenytoin and 6β-hydroxytestosterone, from Ultrafine Chemicals; 4′-hydroxydiclofenac, (±)-bufuralol, and 1′-hydroxybufuralol, from Gentest; testosterone, from Nacalai Tesque, Inc.; and phenobarbital, from Tokyo Kasei Kogyo Co., Ltd. Other reagents were of special or HPLC grade.

**Microsome Preparations** Microsomes expressing cloned P450 isoforms (CYP1A2, CYP2C9-Arg, CYP2C9-Cys, CYP2C9, CYP2D6-Val and CYP3A4), control (vector), and human P450 reductase expressed control microsomes, all derived from a human lymphoblastoid cell line, were purchased from Gentest.

**Methods. 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 rate 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′-ethyloxatomide.

**Identification of P450 Isoforms Involved in Oxatomide Metabolism Using Microsomes of in Vitro Expression Systems** Microsomes expressing individual P450 isoforms were incubated with 0.1, 1 and 10 M of oxatomide in 500 µl (final concentration, 1.0 mg protein/ml) at 37°C for 10 or 30 min. The reaction was initiated by the addition of microsomes after 5-min preincubation, and stopped by adding the equivalent volume of the internal standard, a methanol solution of 3′-ethyloxatomide. For the reactions with CYP1A2, CYP2C9, CYP2D6-Val and CYP3A4, 0.1 mM phosphate buffer (pH 7.4) containing 1.3 mM β-NADP⁺, 3.3 mM glucose-6-phosphate, 3.3 mM MgCl₂ and 0.4 unit/ml glucose-6-phosphate dehydrogenase (NADPH G.S.) was used. In the cases of CYP2C9 and CYP2C9-Cys, phosphate buffer was replaced by Tris–HCl buffer (pH 7.4). The reaction mixture was centrifuged at 12000 rpm for 4°C for 5 min, and the supernatant was analyzed by HPLC under the conditions described above. The flow rate was 1 ml/min, and the column temperature was 50°C. Fluorescence detection was performed at an excitation wavelength (Ex) of 280 nm and an emission wavelength (Em) of 309 nm.

**Metabolic Inhibition Tests Using Microsomes Expressing P450 Isoforms** Inhibitory effects of oxatomide on the metabolic enzymes were examined at 0.1, 1, and 10 M. As a positive control for CYP1A2, α-naphthoflavone (5 µg/ml) was used, and SKF-525A (300 µg/ml) was used as positive controls for CYP2C9-Arg, CYP2C9-Cys, CYP2C19, CYP2D6-Val and CYP3A4.

Inhibition of CYP1A2 (7-Ethoxyresorufin Deethylation Activity)⁶: 7-Ethoxyresorufin (4 µg/ml) and the CYP1A2-expressing microsomes (0.25 mg protein/ml) were incubated in 2 ml of 0.1 M phosphate buffer (pH 7.4) containing NADPH G.S. at 37°C for 60 min. The reaction was initiated by the addition of the microsomes after 5-min preincubation, then stopped by adding 100 µl of 17% potassium carbonate solution. The concentration of the product, resorufin, was determined by fluorescence (Ex. 550 nm, Em. 586 nm) according to the method by Burke et al. ²⁷

Inhibition of CYP2C9-Arg (Diclofenac 4′-Hydroxylase Activity)⁸: Diclofenac (100 µg/ml) and the CYP2C9-Arg-expressing microsomes (0.4 mg protein/ml) were incubated in 250 µl of 0.1 M Tris–HCl buffer (pH 7.4), including NADPH G.S. at 37°C for 30 min. The reaction was initiated by the addition of the microsomes after 5-min preincubation, and stopped by adding 100 µl of 25 µg/ml phenacetin (the internal standard) methanol solution. The reaction mixture was centrifuged at 10000 rpm for 4°C for 3 min. The concentration of the product, 4′-hydroxydiclofenac, was determined by HPLC analysis using an Inertsil ODS-3 column (5 µm, 4.6×250 mm). Solvents were H₂O : acetic acid (1000 : 5, v/v) and methanol : acetic acid (1000 : 5, v/v). Flow rate was 1 ml/min, and the gradient condition was 3:2 to 1:1:9 in 30 min and 1:9 for an additional 5 min, and the column temperature was 50°C. Detection was performed by UV absorption at 280 nm.²⁹

Inhibition of CYP2C9-Cys (Diclofenac 4′-Hydroxylase Activity)⁸: Diclofenac (100 µg/ml) and the CYP2C9-Cys-expressing microsomes (2.0 mg protein/ml) were incubated in 250 µl of 0.1 M Tris–HCl buffer (pH 7.4), including NADPH G.S., at 37°C for 60 min. The reaction was initiated by the addition of the microsomes after 5-min preincubation, and stopped by adding 100 µl of 25 µg/ml phenacetin (the internal standard) methanol solution. The reaction mixture was centrifuged at 10000 rpm at 4°C for 3 min. The concentration of the product, 4′-hydroxydiclofenac, was determined by HPLC analysis under the same conditions as in the case of CYP2C9-Arg.

Inhibition of CYP2C9 (S(-)-Mephénytoïn 4′-Hydroxylation Activity)⁸: S(-)-Mephénytoïn (100 µg/ml) and the CYP2C9-expressing microsomes (4.0 mg protein/ml) were incubated in 250 µl of 0.1 M phosphate buffer (pH 7.4), including NADPH G.S., at 37°C for 120 min. The reaction was initiated by the addition of the microsomes after 5-min preincubation, and stopped by adding 100 µl of 5 µg/ml phenobarbital sodium (the internal standard) methanol solution. The reaction mixture was centrifuged at 10000 rpm at 4°C for 3 min. The concentration of the product, (±)-4′-hydroxymephénytoïn, was determined by HPLC analysis using an Inertsil ODS-3 column (5 µm, 4.6×250 mm, GL science). The mobile phase used was 0.05 M phosphate buffer (pH 4.0) : acetonitrile (76:24, v/v). Flow rate was 1 ml/min, and the column temperature was 40°C. Detection was performed
by UV absorption at 204 nm.\textsuperscript{30}

Inhibition of CYP2D6-Val (\((\pm)\)-Bufuralol 1'-Hydroxylase Activity)\textsuperscript{31}: \((\pm)\)-Bufuralol (100 \textmu M) and the CYP2D6-Val-expressing microsomes (0.4 mg protein/ml) were incubated in 250 \textmu l of 0.1 M phosphate buffer (pH 7.4), including NADPH G.S., at 37 °C for 10 min. The reaction was initiated by the addition of the microsomes after 5-min preincubation, and stopped by adding 100 \textmu l of 5 \textmu g/ml alprenolol (the internal standard) methanol solution. The reaction mixture was centrifuged at 10000 rpm at 4 °C for 3 min. The concentration of the product, 1'-hydroxybufuralol, was determined by HPLC analysis using an Inertsil ODS-3 column (5 \mu m, 4.6×250 mm, GL science). The mobile phase used was 1 M perchloric acid and acetonitrile. Flow rate was 1 ml/min, and the gradient condition was 9:1 to 1:1 in 10 min and 1:1 for an additional 1 min, and the column temperature was 50 °C. Detection was performed by fluorescence (Ex. 252 nm, Em. 302 nm).\textsuperscript{31}

Inhibition of CYP3A4 (Testosterone 6\beta-Hydroxylase Activity)\textsuperscript{32}: Testosterone (200 \textmu M) and the CYP3A4-expressing microsomes (0.4 mg protein/ml) were incubated in 500 \textmu l of 0.1 M phosphate buffer (pH 7.4), including NADPH G.S., at 37 °C for 30 min. The reaction was initiated by the addition of the microsomes after 5-min preincubation, and stopped by adding 200 \textmu l of 10 \textmu g/ml phenacetin (the internal standard) methanol solution. The reaction mixture was centrifuged at 10000 rpm at 4 °C for 3 min. The concentration of the product, 6\beta-hydroxytestosterone, was determined by HPLC analysis using Cosmosil 5C18-AR (4.6×250 mm, Nacalai tesque). The mobile phase used was H\textsubscript{2}O : tetrahydrofuran (5:1, v/v) and methanol. Flow rate was 1 ml/min, and the gradient condition was 4:1 to 7:3 in 10 min and 3:7 for an additional 5 min, and the column temperature was 40 °C. Detection was performed by UV absorption at 240 nm.\textsuperscript{32}

Data Analyses. Identification of P450 Isoforms Involving in Oxatomide Metabolism Standard Curve: The test substance (oxatomide) and the control microsomes at a given concentration were incubated in the buffer. After the addition of the internal standard substance, the mixture was centrifuged at 12000 rpm at 4 °C for 5 min. The supernatant was analyzed by HPLC to obtain the concentration of oxatomide, which was used for drawing a single-point standard curve passing the origin. The regression equation was set up from the obtained measurement using Excel 97.

Rate of Unchanged Compound: Concentrations of oxatomide in each experiment were determined by the regression equation for the standard curve using Excel 97. The concentrations of oxatomide were measured when the substance was incubated with either the microsomes expressing P450 or the control microsomes, and the rate of unchanged compound was calculated as follows:

\[
\text{rate of unchanged compound (\% of control)} = \frac{\text{concentration of oxatomide when reacted with the microsomes expressing human P450}}{\text{concentration of oxatomide when reacted with the control microsomes}} \times 100
\]

Enzyme Inhibition Tests Standard Curve: A given amount of each model substrate was added to the reaction buffer, and the concentration of the substrate was determined by a corresponding analytical method. A single-point standard curve passing the origin was drawn, and the regression equation for each substrate was set up from the obtained measurement using Excel 97.

Uninhibition Rate: The metabolic activity of each P450 isozyme in the presence and absence of inhibitors was determined from the concentration of the corresponding model substrate, which was calculated from the regression equation for the standard curve using Excel 97. The uninhibited rates and inhibited rates for each model substrate were calculated as follows by comparing the metabolic activity in the presence of the test or a positive control substance with that in the absence of the test or a positive control substance:

\[
\text{uninhibition rate (\% of uninhibited activity)} = \frac{\text{amount metabolized in the presence of the test or a positive control substance}}{\text{amount metabolized in the absence of the test or a positive control substance}} \times 100
\]

\[
\text{inhibition rate (\% of inhibited activity)} = 100 - \text{uninhibition rate}
\]

RESULTS

Analysis of Oxatomide An analytical HPLC system was validated using 3'-ethyloxatomide as an internal standard for oxatomide. Figure 2 shows an HPLC profile of oxatomide and 3'-ethyloxatomide. Judging from the elution time and peak forms, it was concluded that the system was suitable for analysis of oxatomide.

Identification of Human P450 Isoforms Involved in Oxatomide Metabolism To identify P450 isoforms involved in oxatomide metabolism, human-lymphoblastoid-derived microsomes expressing six P450 isoforms (CYP1A2, CYP2C9-Arg, CYP2C9-Cys, CYP2C19, CYP2D6-Val and CYP3A4) were examined. The microsomes were reacted with both oxatomide and the NADPH-generating coenzyme at 37 °C for 10 or 30 min. The concentration of oxatomide after the enzyme reaction was compared to that reacted with the control microsomes to obtain the rate of unchanged compound. Table 1 shows that the rate of unchanged compound was decreased when the drug was reacted with CYP2D6-Val and CYP3A4 for 10 or 30 min. The rate of unchanged compound for oxatomide after the reaction with CYP2D6-Val for 10 min was 11.3, 19.3 and 76.0% at 0.1, 1 and 10 \mu M, respectively. At 30 min of reaction, the remaining rate of oxatomide at 0.1 \mu M was below the detection limit, while those at 1 and
In the case of CYP3A4, the rate of unchanged compound was 44.2, 54.8 and 74.3% at 0.1, 1 and 10 μM, respectively, at 10 min. At 30 min, the remaining rate of oxatomide was 22.3, 27.1 and 53.2% at 0.1, 1 and 10 μM, respectively.

Figure 3 shows the comparison of the oxatomide concentration with the remaining rate and reaction time by CYP2D6-Val and CYP3A4. The metabolism reaction showed that the tendency saturated in 30 min in all concentrations. Furthermore, the concentration dependency with the metabolism activity of CYP2D6-Val and CYP3A4 was shown in Fig 4. The metabolism activity of CYP3A4 increased almost linearly with the increase in oxatomide concentration, although CYP2D6-Val showed a little saturation on 10 μM of oxatomide.

No decrease in the rate of unchanged oxatomide was found for other isoforms, as shown in Table 1.

Table 1. Metabolism of Oxatomide with Microsomes Prepared from B-Lymphoblastoid Cell Lines Expressing cDNAs Encoding Human CYP Isoforms

<table>
<thead>
<tr>
<th>CYP isoforms</th>
<th>0.1 μM</th>
<th>1 μM</th>
<th>10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>30 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Control</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>1A2</td>
<td>88.7</td>
<td>109.5</td>
<td>97.1</td>
</tr>
<tr>
<td>2C9-Arg</td>
<td>95.7</td>
<td>95.9</td>
<td>97.2</td>
</tr>
<tr>
<td>2C9-Cys</td>
<td>97.4</td>
<td>93.7</td>
<td>94.6</td>
</tr>
<tr>
<td>2C19</td>
<td>94.4</td>
<td>110.5</td>
<td>97.5</td>
</tr>
<tr>
<td>2D6-Val</td>
<td>11.3</td>
<td>N.D.</td>
<td>9.8</td>
</tr>
<tr>
<td>3A4</td>
<td>44.2</td>
<td>22.3</td>
<td>54.8</td>
</tr>
</tbody>
</table>

Different types of control microsomes were used for this evaluation. Control microsomes including vector only were used for CYP2C9-Arg and 2C9-Cys, and human P450 reductase expressed control microsomes were used for the other isoforms. Control was examined with the control microsomes. N.D.: not detected.

Table 2. Inhibition of Each P450 Model Substrate Metabolism in Microsomes Prepared from B-Lymphoblastoid Cell Lines Expressing cDNAs Encoding Each Human CYP Isoform with Oxatomide

<table>
<thead>
<tr>
<th>CYP isoforms</th>
<th>0.1 μM</th>
<th>1 μM</th>
<th>10 μM</th>
<th>NF</th>
<th>SKF-525</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1A2</td>
<td>92.3</td>
<td>92.6</td>
<td>105.3</td>
<td>18.1</td>
<td>—</td>
</tr>
<tr>
<td>2C9-Arg</td>
<td>97.1</td>
<td>94.9</td>
<td>89.3</td>
<td>—</td>
<td>56.9</td>
</tr>
<tr>
<td>2C9-Cys</td>
<td>103.2</td>
<td>100.6</td>
<td>100.3</td>
<td>—</td>
<td>57.2</td>
</tr>
<tr>
<td>2C19</td>
<td>96.0</td>
<td>91.6</td>
<td>93.1</td>
<td>—</td>
<td>20.7</td>
</tr>
<tr>
<td>2D6-Val</td>
<td>96.8</td>
<td>90.2</td>
<td>74.1</td>
<td>—</td>
<td>2.5</td>
</tr>
<tr>
<td>3A4</td>
<td>90.5</td>
<td>55.8</td>
<td>28.2</td>
<td>—</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Control was examined without test article. α-Naphthoflavone (5 μM) was used as a reference control for CYP1A2. SKF-525 (300 μM) was used as a reference control for CYP2C9-Arg, 2C9-Cys, 2C19, 2D6-Val, 3A4.

Inhibitory Effects of Oxatomide on Various P450 Isoforms Expressed by Microsomes in Vitro

Inhibitory effects of oxatomide on the metabolism of model substrates for human P450 were examined using human-lymphoblastoid-derived microsomes expressing six P450 isoforms (CYP1A2, CYP2C9-Arg, CYP2C9-Cys, CYP2C19, CYP2D6-Val and CYP3A4). Oxatomide was incubated with the NADPH-generating coenzyme and P450 isoform-specific model substrates at 37 °C, and concentrations of metabolites were measured by analytical methods corresponding to each substrate. The ratio of the activity in the presence of the test substance to that in the absence of the test substance was calculated as the uninhibition rate (% of uninhibited activity), by which the inhibitory effects of oxatomide on the metabolism of various model substrates were compared.

As shown in Table 2, oxatomide did not affect the metabolism of 7-ethoxyresorufin, the model substrate for CYP1A2, while the uninhibition rate by the positive control, α-naphthoflavone, was 18.1%. Furthermore, oxatomide did not inhibit the metabolism of the model substrates for CYP2C9-Arg, CYP2C9-Cys and CYP2C19 (Table 2). In contrast, the
dine,\textsuperscript{16,17} In addition, the \textit{in vivo} metabolism of terfenadine is inhibited by itraconazole, a potent inhibitor for CYP3A4, which may lead to an increase in the blood concentration of the drug.\textsuperscript{34} On the other hand, it has been shown by Kishimoto \textit{et al.} that epinastine is metabolized by CYP3A4 and CYP2D6, and also slightly by CYP2B6, but it does not inhibit the metabolism of testosterone, the model substrate for CYP3A4, even at 100 \textmu M.\textsuperscript{33} 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 a possibility that oxatomide, as well as epinastine, does not affect the metabolism of other drugs, which differs from the actions of terfenadine and astemizole. To confirm this, we investigated whether oxatomide has inhibitory effects on the metabolism of model substrates specific to each P450 isozyme.

The inhibitory effects of oxatomide on the metabolism of other drugs were examined using CYP1A2, CYP2C9-Arg, CYP2C9-Cys, CYP2C19, CYP2D6 and CYP3A4 expressed by lymphoblastoid-derived microsomes and the corresponding model substrates, 7-ethoxyresorufin, diclofenac, S(+)-mephenytoin, (\pm)-bufuralol and testosterone. Oxatomide did not affect the production of metabolites for 7-ethoxyresorufin, diclofenac or S(-)-mephenytoin, suggesting that the drug does not have inhibitory effects on the metabolic activity of CYP1A2, CYP2C9-Arg, CYP2C9-Cys or CYP2C19. In contrast, the amount of the metabolites of (\pm)-bufuralol and testosterone was decreased in the presence of oxatomide, indicating that the agent inhibited drug metabolism catalyzed by CYP2D6 and CYP3A4. Moreover, when it is assumed the reaction was competition antagonism from the result of Table 2, IC\textsubscript{50} was calculated as 21 \textmu M for CYP2D6 and 5.3 \textmu M for CYP3A4 using the least-squares method, and oxatomide showed more potent inhibition to CYP3A4. Furthermore, when the \textit{K}_i value was calculated using the following formula, CYP2D6 was 3.5 \textmu M and CYP3A4 was 1.8 \textmu M.

\begin{equation}
\frac{V}{V_{\text{max}}} \frac{S}{K_m+S} = \left(1 + \frac{IC_{50}}{K_i}\right)K_m+S
\end{equation}

\begin{equation}
V_{1/2} = \frac{V_{\text{max}} \cdot S}{IC_{50}} \left(1 + \frac{IC_{50}}{K_i}\right)K_m+S
\end{equation}

\begin{equation}
\frac{V_{1/2}}{V} = \frac{V_{\text{max}} \cdot S}{IC_{50}} \left(1 + \frac{IC_{50}}{K_i}\right)K_m+S
\end{equation}

When the relation of Eq. 3 is applied to various concentrations of a substrate, between the IC\textsubscript{50} and a preventive constant (\textit{K}_i), the following relation of Eq. 4 is realized.

\begin{equation}
IC_{50} = (n+1)\textit{K}_i
\end{equation}

\textit{The n} represents a substrate concentration indicating the number of times \textit{K}_m, the condition assumes an \textit{S}<<\textit{K}_m relation, IC\textsubscript{50} = \textit{K}_i could be considered, and when \textit{S} is 5 times \textit{K}_m, a relation of IC\textsubscript{50} = 6\textit{K}_i is drawn.

In this study, bufuralol, which is the probe substrate for CYP2D6, was used at 5 times general \textit{K}_m, and testosterone which is the probe substrate for CYP3A4 was used a 2 times general \textit{K}_m, as computed on the basis of the above assump-
tion. These results raise the possibility that oxatomide could be the substrate for CYP2D6 and CYP3A4, and that it simultaneously acts as an inhibitor for these enzymes responsible for the metabolism of the drug itself and inhibits CYP3A4 at a smaller concentration than that required for CYP2D6, in a manner similar to that of terfenadine and astemizole, but differently from epinastine.

However, it has not been reported that oxatomide shows side effects due to concomitant administration with other drugs such as ketoconazole, as terfenadine and astemizole do. This might be because the clinical dose of oxatomide is generally much lower than the concentration at which the drug shows inhibition on CYP2D6 and CYP3A4 in vitro. For example, the maximal drug concentration ($C_{\text{max}}$) of oxatomide was 19.3 ng/ml ($\approx 45 \text{ nm}$) in single-dose administration (unpublished observation), and when 30 mg was given to adult asthma patients twice a day for 11 consecutive days, the $C_{\text{max}}$ immediately before and 4 h after administration was 20—30 ng/ml and $\approx 40 \text{ ng/ml}$, respectively, on day 6 and later. 35) On the other hand, Nicolas et al. examined the inhibitory effects of five antiallergic drugs with anti-histamine actions, and demonstrated that terfenadine and astemizole inhibited the demethylation of dextromethorphan, a model substrate for CYP2D6, at 18 and 26 $\mu \text{M}$ (IC$_{50}$) respectively. At the same time, they showed that terfenadine and astemizole inhibited the metabolism of testosterone, a model substrate for CYP3A4, at 23 and 21 $\mu \text{M}$ (IC$_{50}$), respectively. In the present study, the concentrations of oxatomide required for inhibiting the metabolism of the substrates for CYP2D6 and CYP3A4 were comparable to those of terfenadine and astemizole. Furthermore, there has been a report that the maximal plasma concentrations of terfenadine and astemizole are less than 3 and 2 nm, respectively, 18, 36) which are similar to that of oxatomide. Thus, it seems difficult to conclude that the difference in appearance of side effects by terfenadine and astemizole and by oxatomide can be attributed to a simple difference between the blood concentrations and the concentrations required for enzyme inhibition in vitro. However, no side effect by concomitant drugs, including cardiovascular ones, has been reported for oxatomide as described above. Another possibility is that the metabolism of oxatomide is not affected by other drugs. It has been reported that when terfenadine (60 mg) is administered with ketoconazole (200 mg), the time of maximal concentration ($T_{\text{max}}$) and $C_{\text{max}}$ 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. 57) It has also been shown that the blood concentration of terfenadine is increased from 1.3 ng/ml to less than 20 and 80 ng/ml when the ketoconazole concentration is increased from 0 to 1 and 5 $\mu \text{g/ml}$. 38) Moreover, the blood concentration of astemizole has been shown to increase from 2.73—3.63 to 15.85 ng/ml by combined administration with cimetidine in a single normal subject. 39) If oxatomide is affected by the agents inhibiting CYP3A4 and CYP2D6, like terfenadine and astemizole, the blood concentration of the drug might be increased, and side effects might occur. But there has been no report on such cardiovascular system side effect, suggesting that oxatomide might not be affected by other concomitant drugs. Moreover, oxatomide is mainly metabolized by the liver, similar to terfenadine and astemizole. As a result of rat study, it is known that the concentration in the inside of liver was 30 to 40 times as high as the concentration in plasma (unpublished). On the other hand, the inside of liver concentration of astemizole was 400 times as high as the concentration in plasma. 40) These distribution results might be considered the incidence causing different side effects among the two drugs. However Leeson et al. reported that the concentration of terfenadine in the liver was 5.6 times higher than its plasma level. 41) So the difference of distribution can’t explain the lack of cardiovascular system side effects with oxatomide.

A recent publication by Iwamoto et al. showed that not only a single administration of oxatomide, but also combined administration with itraconazole, did not elicit QT prolongation in dogs, suggesting that oxatomide does not possess any action on the cardiovascular system. 42) On the other hand, oxatomide has been reported to inhibit dopamine D1 and D2 receptors, and to induce catalepsy at high doses in mice. 43) In addition, oxatomide inhibits the uptake of dopamine, 44) which infrequently causes extrapyramidal disorder clinically. 25) Actually, healthy volunteers taking 30 mg of oxatomide, which then occupied the brain H1 receptor, suggests that oxatomide would pass the blood brain barrier, as shown by the PET studies reported by Yanai et al. 45) The plasma concentration of oxatomide wasn’t measured in this study, however, it is reported that 30 mg administered twice to adult asthmatic patients resulted in 20—30 ng/ml and 40 ng/ml plasma levels, respectively, on day 6 and later. 35) These results show that oxatomide could inhibit the dopamine receptor if the blood concentration increased more than 40 mg/ml. If the inhibitory effects of oxatomide on CYP3A4 and CYP2D6 interact with the drugs inducing catalepsy, the possibility of side effects should be carefully considered. Since the present study employed the in vitro expression systems derived from human lymphoblastoids as microsome preparations for P450, the obtained results might differ from those examined in the human liver. And the testosterone was used only for CYP3A4 in this study because the purpose of the examination was to clarify which isoform would metabolize oxatomide. Therefore, other substrates like nifedipine or midazolam might also be necessary to be examined. Detailed investigation using human liver homogenate will be performed in future study.

REFERENCES


