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Application of the PKCYP Test to Predict Caffeine Clearance Mediated by CYP1A2 in a Rat Acute Liver Injury Model

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Summary: We previously established a method for assessing in vivo drug-metabolizing capacity by pharmacokinetic estimation of the quantity of cytochrome P450 (CYP) in vivo (PKCYP test), in which an apparent liver-to-blood free concentration gradient in vivo (qg) is introduced (Matsunaga et al., Jpn. J. Hosp. Pharm., 26. 492–504 (2000)). This method was applied to estimate the amount of CYP2C11 in rats treated with carbon tetrachloride (CCl4-treated rats). In this study, we estimated the amount of CYP1A2 in CCl4-treated rats by using acetanilide and caffeine as a probe and a model drug, respectively.

In CCl4-treated rats, the total body clearance (CLtot) of acetanilide and caffeine was about one-fifth and one-eighth of that in control rats, respectively. In CCl4-treated rats, the amount of CYP1A2 was predicted as 0.60 ± 0.06 nmol/kg from the clearance of acetanilide mediated by CYP1A2. Moreover, the clearance of caffeine mediated by CYP1A2 in CCl4-treated rats was estimated as 0.47 ± 0.05 mL/min/kg by using the predicted amount of CYP1A2. The observed value was 0.44 ± 0.03 mL/min/kg, and the predicted value was within the 95% confidence interval of the observed value.

In conclusion, we have demonstrated that the PKCYP test can also be applied for estimating the amount of CYP1A2 in CCl4-treated rats.

Key words: PKCYP test; CYP1A2; acetanilide; caffeine; carbon tetrachloride-treated

Introduction

Recent technological advances in human gene analysis have made it possible to analyze genetic polymorphisms of drug-metabolizing enzymes and transporters in individual patients.1–9 Thus, attempts have been made to predict variations in drug pharmacokinetics among patients by analyzing the genetic polymorphisms of drug-metabolizing enzymes.10 However, drug-metabolizing capacity is also influenced by disease state. In order to achieve real-time evaluation of drug-metabolizing capacity of individual patients, specific probe drugs for cytochrome P450 (CYP) isoforms have been used.11 On the other hand, it has been reported that in vivo clearance can be predicted by multiplying the enzymatic activity in vitro by the enzyme level in liver.6,7

Several approaches have been used to predict in vivo drug metabolism, such as scaling of in vivo metabolic clearance from in vitro data obtained using human liver microsomes or a system of recombinant human CYP isoforms.8,9

We previously reported a new method for diagnosing drug-metabolizing capacity based on pharmacokinetic estimation of the quantity of CYP in vivo (the PKCYP test).10 An apparent liver-to-blood free concentration gradient in vivo (qg) was introduced in this PKCYP test. In rats whose CYP1A2 level has fluctuated, the amount of CYP1A2 could be predicted by the PKCYP test with acetanilide as a probe.11 Caffeine clearance mediated by CYP1A2 could also be predicted, based on the predicted amount of CYP1A2.10 The amount of CYP2C11 could also be predicted by employing the PKCYP test with tolbutamide as a probe in rats whose CYP2C11 level had been reduced by the administration of carbon tetrachloride (CCl4).12 In addition, we showed, using theophylline as a model drug, that the qg value in the PKCYP test is applicable to drugs metabolized by

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multiple CYP isoforms. Since several CYP isoforms are involved in in vivo drug metabolism, it is important to predict the amounts of multiple CYP isoforms in individual patients as the same time by applying the PKCYP test. Therefore, it is necessary that we confirm whether the PKCYP test can be used to predict the amount of multiple CYP isoforms as the same time in a rat model of disease.

It is well known that administration of CCl₄ induces acute liver injury in rats. It has been reported that the amount of CYP isoforms other than CYP2C11 is also reduced in rats treated with CCl₄ (CCl₄-treated rats). As mentioned above, we have already confirmed that the amount of CYP2C11 in CCl₄-treated rats could be predicted by the PKCYP test. In the present study, we examined whether the PKCYP test could also be used to predict the amount of CYP1A2 in CCl₄-treated rats using acenatline as a probe. Moreover, we also predicted the clearance of caffeine mediated by CYP1A2 using the predicted amount of CYP1A2 in CCl₄-treated rats, and compared the result with the observed value.

Methods

Materials: Chemicals were obtained from the following sources: acenatline, p-hydroxyacetanilide, caffeine, CCl₄, phenacetin, theobromine, and theophylline were from Wako Pure Chemicals (Osaka, Japan); 8-chlorotheophylline, 1,7-dimethyloxanthine, 1,2,3,7-trimethyluric acid, goat serum, and rabbit serum were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); [8-¹⁴C]caffeine was from American Radiolabeled Chemicals, Inc. (St. Louis, MO, U.S.A.); NADPH was from Oriental Yeast Co. (Tokyo, Japan); and anti-rat CYP1A1 (anti-rat CYP1A1 antibody) and anti-rat CYP1A2 (anti-rat CYP1A2 antibody) were from Daichi Pure Chemicals Co. (Tokyo, Japan). Anti-rat CYP1A antibody inhibited both CYP1A1 and CYP1A2 activity. All other reagents were of reagent grade or HPLC grade and were used without further purification.

Animals: Male Sprague-Dawley rats, 6 weeks of age, were purchased from Japan SLC, Inc. (Shizuoka, Japan). The rats were pretreated with CCl₄ (after an 8-hr fast, 2.5 mL/kg of 20% CCl₄, dissolved in olive oil, was administered i.p.) (CCl₄-treated rats). Control animals were used without pretreatment. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoritsu College of Pharmacy.

In vivo pharmacokinetic studies: The control and CCl₄-treated rats were given acenatline (20 mg/kg, dissolved in ethanol) or caffeine (20 mg/kg, dissolved in saline) by intravenous injection. Blood was collected from the jugular vein. Blood was centrifuged at 15000 × g (MX-150, Tomy Seiko Co., Ltd., Tokyo, Japan) for 10 min at 4°C to obtain serum. Serum was stored at −20°C until assayed.

Determination of serum protein binding of acenatline and caffeine: The serum protein binding of acenatline and caffeine was determined by ultrafiltration using a Centrifree device (Amicon, Inc., Beverly, MA, U.S.A.) as described previously.26,27

Quantitation of CYP1A2 in liver microsomes: Liver microsomes were prepared as previously described.28 The CYP1A2 level in liver microsomes was quantified by Western blotting, as described previously. Briefly, liver microsomes derived from the control (2–6 μg protein/lane) and CCl₄-treated (10–40 μg protein/lane) rats were separated by electrophoresis on a 10% SDS-polyacrylamide gel. Rat CYP1A2, purified as previously described,29 was also applied (0.01–0.04 pmol/lane) as a standard. The separated proteins were then transferred to a nitrocellulose membrane which was then incubated with antibodies. Anti-Syrian hamster CYP1A2, raised in rabbit as previously described,30 was used as the first antibody. The second antibody was against peroxidase-linked rabbit Ig, isolated from donkeys (Amersham, Aylesbury, UK). The antibody against Syrian hamster CYP1A2 was shown to cross-react with rat CYP1A2.31 To visualize peroxidase activity on the immunoblots, Western Blot Chemiluminescence Reagent (NEN, Boston, MA) was applied to the nitrocellulose membrane, which was then exposed to a photographic film (Hyper ECL, Amersham, Aylesbury, UK) for 30 sec. The CYP1A2 protein band identified on the film was quantified densitometrically with an electrophoresis documentation and analysis system (Eastman Kodak, Rochester, N.Y.), and the CYP1A2 protein content was determined from a standard curve generated using purified rat CYP1A2. In CCl₄-treated rats, no CYP1A2 protein bands could be detected.

Inhibitory effect of pretreatment CYP1A1 and CYP1A1 antibodies on metabolism of acenatline and caffeine in liver microsomes: The assay mixture for acenatline metabolism contained 0.25 mM acenatline, 0.4 mM NADPH, 0.67 mg/mL MgCl₂·6H₂O, and 25 mM Tris buffer (pH 8.0) in a final volume of 1.0 mL. CCl₄-treated rat liver microsomes (0.75 mg) were preincubated with 100 μL of anti-rat CYP1A1 or CYP1A1 antibody for 30 min at room temperature prior to the measurement of acenatline metabolite formation. Under these conditions, the CYP1A1 and CYP1A1 activity of CCl₄-treated rat microsomes, irrespective of their origin, was maximally inhibited. Control incubations contained an equivalent amount of preimmune goat (for CYP1A1) or rabbit (for CYP1A1) serum proteins. Then, the assay mixture without acenatline was added and preincubation was carried out in a shaking bath for 10 min at 37°C. The reaction was started by the addition of acenatline and incubation was carried out in a shaking bath for 10 min at 37°C. The reaction was halted by the
addition of 5 mL of ethyl acetate, and the acetalnilide metabolites in the samples were assayed.

The assay mixture for caffeine metabolism contained 100 μM [8-14C]caffeine, 0.67 mg/mL MgCl2·6H2O, and 25 mM Tris buffer (pH 8.0) in a final volume of 0.5 mL. The specific activity of [8-14C]caffeine was adjusted to 1924 Bq/nmol by dilution with unlabeled caffeine. CCl4-treated rat liver microsomes (0.25 mg) were preincubated with 50 μL of anti-rat CYP1A1 or CYP1A2 antibody for 30 min at room temperature prior to the measurement of caffeine metabolite formation. Under these conditions, the CYP1A1 or CYP1A2 activity of CCl4-treated rat liver microsomes, irrespective of their origin, was maximally inhibited. Control incubations contained an equivalent amount of preimmune goat (for CYP1A1) or rabbit (for CYP1A2) serum proteins. Then, the assay mixture without caffeine was added, and preincubation was carried out with shaking in a bath at 37°C for 10 min. The reaction was started by the addition of caffeine, and incubation was carried out with shaking in a bath at 37°C for 10 min. The reaction was divided by the addition of 500 μL of 20% perchloric acid, and the radioactivity due to each of the caffeine metabolites was measured.

The results were calculated as the percent of control activity. Since the inhibitory capacity of anti-rat CYP1A1 antibody towards CYP1A1 was 65%, the activities which were inhibited by anti-rat CYP1A1 antibody were corrected using this value. The contribution of each CYP isoform (fCYP) was calculated as the percent of control activity. When the percent of control activity was more than 100%, fCYP was taken as zero.

Assay of acetalnilide, caffeine, and their metabolites: Acetalnilide and caffeine in serum and acetalnilide metabolites in the samples for inhibition studies of in vitro acetalnilide metabolism were assayed by HPLC, as described previously.10,11 The radioactivity of caffeine metabolites in the samples for inhibition studies of in vitro caffeine metabolism were measured after separation by HPLC, as described previously.11

Prediction of the liver CYP1A2 content in CCl4-treated rats: The total body clearance (CLint) was used to predict the liver CYP1A2 content in CCl4-treated rats. The intrinsic metabolic clearance (CLint) can be expressed by CLint, the unbound fraction of drug in serum (fS), and the hepatic blood flow rate (Qt), as follows:

\[ CL_{int} = \frac{Q_t \cdot CL_{int}}{f_s \cdot CL_{int} - 1} \]

Then, the in vivo amount of specific CYP (A-CYPint) in CCl4-treated rats was predicted according to Eq. A7 as follows:

\[ A-CYP_{int} = \frac{f_{CYP} \cdot Q_t \cdot CL_{int}}{Q_t - CL_{int} \cdot f_S} \cdot \frac{K_{int}}{V_{int,CYP}} \]

Data analysis: Serum concentration versus time data were analyzed by model-independent moment analysis. The last determined serum concentration was extrapolated to infinity using the terminal slope of the last two points of the log serum concentration-time curve. The volume of distribution at the steady-state (Vss) and the CLint, were estimated as described by Yamaoka et al. Data were analyzed by using Student’s t test for comparison of unpaired means of two sets of data. Analysis of variance was used to compare more than two sets of data. The number of determinations (n) is noted in the table. A p value of 0.05 or less was taken as the criterion of a significant difference between sets of data.

Results

Comparison of the pharmacokinetics of acetalnilide and caffeine in control and CCl4-treated rats: The serum concentration versus time profiles of acetalnilide and caffeine in control and CCl4-treated rats are shown in Figs. 1 and 2. Since the acetaminol and caffeine profiles suggest linear pharmacokinetics, the CLint was determined under linear conditions. The results of linear model-independent moment analysis are given in Tables 1 and 2. The CLint of acetaminol and caffeine in CCl4-treated rats was approximately one-fifth and one-
The physiological data for the acetanilide and caffeine-treated rats used in this study are also summarized in Tables 1 and 2. There were no differences in body weight between control and CCL-treated rats (Tables 1 and 2). There were also no differences in the \( k_{p} \) or the \( V_{CYP} \) for acetanilide and caffeine between control and CCL-treated rats (Tables 1 and 2).

**Estimation of the contribution of CYP1A2 to the liver microsomal metabolic activity for acetanilide and caffeine in CCL-treated rats:** The contributions of CYP1A2 to liver microsomal acetanilide and caffeine metabolism in CCL-treated rats were estimated using anti-rat CYP1A1 and CYP1A2 antibodies. The effects of these antibodies on liver microsomal metabolism of acetanilide and caffeine in CCL-treated rats are shown in Table 3. Control activity towards acetanilide in the presence of preimmune serum in CCL-treated rats was 53 ± 21 pmol/min/mg. Control activity towards caffeine in the presence of preimmune serum in CCL-treated rats was 7.8 ± 3.5 pmol/min/mg. As shown in Table 3, there was no significant difference in the contributions of CYP1A2 to liver microsomal acetanilide and caffeine metabolism between control and CCL-treated rats.

**Prediction of liver CYP1A2 content in CCL-treated rats:** The A-CYP1A2 in CCL-treated rats was predicted as 0.60 ± 0.06 nmol/kg using the parameters listed in Tables 1 and 3. Reported values of \( q_{C} \) (1.90), \( K_{m,\text{CYP}} \) (1.05 x 10^7 M), and \( V_{\text{max, CYP}} \) (1.23 x 10^6 mol/min/nmol CYP1A2) were also used in this prediction. Since the standard deviation of \( f_{CYP} \) was the largest among the parameters used in the prediction, we only introduced the standard deviation of \( f_{CYP} \) to equations when making any predictions. The observed value of the A-CYP1A2 in CCL-treated rats could not be obtained by Western blotting, because it was below the detection limit of the assay method.

**Prediction of caffeine clearance mediated by CYP1A2 in CCL-treated rats:** Since the \( f_{CYP} \) for CYP1A2 for

### Table 1. Pharmacokinetic and biochemical parameters of acetanilide in control and CCL-treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CCL-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-CYP &lt;sub&gt;1A2&lt;/sub&gt; (pmol/mg of microsomal protein)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-/-</td>
</tr>
<tr>
<td>(nmol/kg)</td>
<td>4.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-/-</td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>6±6</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>186±5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>174±5</td>
</tr>
<tr>
<td>( CL_{in} ) (mL/min/kg)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.64±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>( f_{CYP} )</td>
<td>0.63±0.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.69±0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microsome content (g/kg)</td>
<td>1.09±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.97±0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>( Q_{in} ) (mL/min/kg)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>59.7±4.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>52.4±5.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>( V_{CYP} ) (mL/kg)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>700±95&lt;sup&gt;e&lt;/sup&gt;</td>
<td>818±136&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± S.D. (n = 3–4).<br>
<sup>b</sup>In vivo amount of CYP1A2.<br>
<sup>c</sup>Total body clearance. <br>
<sup>d</sup>Unbound fraction of drug in serum.<br>
<sup>e</sup>Hepatic blood flow rate.<br>
<sup>f</sup>Volume of distribution at steady-state.<br>
<sup>g</sup>Ref. 11. <br>
<sup>h</sup>Not detectable. <br>
<sup>i</sup>p<0.05.

### Table 2. Pharmacokinetic parameters of caffeine in control and CCL-treated rats<sup>h</sup>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control&lt;sup&gt;i&lt;/sup&gt;</th>
<th>CCL-treated&lt;sup&gt;i&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>199±10</td>
<td>173±8</td>
</tr>
<tr>
<td>( CL_{in} ) (mL/min/kg)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>3.26±0.09&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.43±0.01&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>( f_{+} )</td>
<td>0.85±0.03&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.85±0.04&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>( V_{CYP} ) (mL/kg)&lt;sup&gt;k&lt;/sup&gt;</td>
<td>723±45</td>
<td>729±35</td>
</tr>
</tbody>
</table>

<sup>h</sup>Values are means ± S.D. (n = 3–4).<br>
<sup>i</sup>Total body clearance.<br>
<sup>j</sup>Unbound fraction of drug in serum.<br>
<sup>k</sup>Volume of distribution at the steady-state.<br>
<sup>l</sup>Ref. 11. <br>
<sup>m</sup>p<0.05.

### Table 3. The contribution of CYP1A2 to the liver microsomal metabolic activity for acetanilide and caffeine in control and CCL-treated rats<sup>n</sup>

<table>
<thead>
<tr>
<th>% of control activity</th>
<th>CYP1A1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CYP1A2&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>108±10</td>
<td>42±4</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>49±5</td>
<td>0.51±0.05</td>
</tr>
<tr>
<td>Caffeine Control</td>
<td>117±6</td>
<td>18±6</td>
</tr>
<tr>
<td>Caffeine CCL-treated</td>
<td>155±1</td>
<td>0.86±0.05</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± S.D. (n = 3).<br>
<sup>b</sup>C<sub>A</sub>=[(100-B)-(100-A)]/100 (A < 100), C<sub>B</sub>=[(100-B)/100] (A > 100).<br>
<sup>c</sup>Ref. 11.
metabolism of caffeine in CCL-treated rats was not unity, we tried to predict the clearance of caffeine mediated by CYP1A2 (CL_{CYP1A2}) by applying the qg value. The CL_{CYP1A2} in CCL-treated rats can be predicted by using a CYP expression, which was predicted from the acetanilide clearance mediated by CYP1A2 according to Eq. A8. The CL_{CYP1A2} in CCL-treated rats was predicted as 0.47 ± 0.05 mL/min/kg (Table 4) using the parameters listed in Tables 2 and 3. Reported values of the qg (0.59), K_{m,ac} (200 μM), and V_{max,ac} (267 nmol/min/nmol CYP1A2) were also used in this prediction. The predicted CL_{CYP1A2} in CCL-treated rats was within the 95% confidence interval of the observed value (Table 4).

**Discussion**

In the present study, we examined the application of the PKCYP test to CYP1A2 in CCL-treated rats using acetanilide and caffeine as a probe and a model drug, respectively.

Firstly, we examined the pharmacokinetics of acetanilide and caffeine in CCL-treated rats. The CL_{ac} values of acetanilide and caffeine in CCL-treated rats were about one-fifth and one-eighth of that in control rats, respectively (Table 2). The decrease of the CL_{ac} of caffeine in CCL-treated rats is in agreement with a previous report. The CL_{ac} values of acetanilide and caffeine in CCL-treated rats were linearly related to dose.

Secondly, we estimated the contribution of CYP1A2 to liver microsomal acetanilide and caffeine clearance in CCL-treated rats. We have reported that some CYP isoforms other than CYP1A2 contribute to the liver microsomal acetanilide and caffeine clearance in control rats and some rat models of disease. In CCL-treated rats, the f_{CYP} values for CYP1A2 with respect to liver microsomal acetanilide and caffeine were 0.51 ± 0.05 and 0.86 ± 0.05, respectively (Table 3). Therefore, some CYP isoforms other than CYP1A2 also contributed to the liver microsomal acetanilide and caffeine clearance in CCL-treated rats. Moreover, the f_{CYP} for CYP1A2 with respect to liver microsomal acetanilide and caffeine metabolism in CCL-treated rats was almost the same as that in control rats. As shown in Table 3, when anti-rat CYP1A1 antibody was added to assay mixture, the percent of control activity for acetanilide and caffeine in control and CCL-treated rats was larger than 100%. The reason for this has not been reported. It may be due to the effect of non specific binding between preimmune serum and acetanilide or caffeine. Therefore, we considered there was no contribution of CYP1A1 to liver microsomal metabolic activity for acetanilide and caffeine in control and CCL-treated rats.

Thirdly, we predicted the amount of CYP1A2 in CCL-treated rats. The predicted A-CYP_{ac} corresponding to CYP1A2 from the clearance of acetanilide, according to Eq. (2) in CCL-treated rats, was about one-seventh of that in control rats. In CCL-treated rats, because of the detection limit of the assay method, the observed value of A-CYP_{ac} could not be obtained by Western blotting. Consequently, it was impossible to confirm whether the predicted and observed A-CYP_{ac} values agreed or not. Therefore, we tried estimating the clearance of caffeine mediated by CYP1A2 in CCL-treated rats using the predicted A-CYP_{ac}. The predicted CL_{CYP1A2} according to Eq. A8 in CCL-treated rats was within the 95% confidence interval of the observed value (Table 4). Since the CL_{CYP1A2} of caffeine in CCL-treated rats could be predicted by using the predicted A-CYP_{ac}, the value of the predicted A-CYP_{ac} seemed to be appropriate. As well as our previous reports, by using the qg values for acetanilide and caffeine, which were estimated in control rats, the amount of CYP1A2 and the CL_{CYP1A2} of caffeine in CCL-treated rats could be estimated. Therefore, results show that the qg values are not changed by fluctuation of the amount of CYP.

In conclusion, we have been able to show that the amount of CYP1A2 in CCL-treated rats can be predicted by using the PKCYP test as well as the amount of CYP2C11. If probe drugs for each CYP isoform were administered as a cocktail, the amount of each CYP isoform could be predicted at the same time. In future, it should be possible to estimate the amount of each CYP isoform for individual patients, as an indicator of hepatic metabolism capacity, by using the PKCYP test. Moreover, using these values, it will also be possible to design drug dosage regimens for individual patients.

**Appendix**

**Theoretical basis for determination of qg** We used a well-stirred model to describe the hepatic metabolism of acetanilide and caffeine. As described by Houston,

$$\text{CL}_{\text{CYP1A2}} = \text{CL}_{\text{CYP1A2}}^{\text{ac}} \cdot \text{qg}$$

where \text{CL}_{\text{CYP1A2}} is purely a measure of enzyme activity towards a drug and is not influenced by other physiological factors associated with liver clearance, such as hepatic blood flow or drug binding within the blood matrix.

The rate of metabolism in vivo (v) can be expressed as
follows:

\[ v = CL_{int} \cdot C_L \]

(A1)

where \( C_L \) is the free concentration (not bound to macromolecules) of drug within the hepatic vein.

Let us assume that the drug is metabolized by a single CYP isoform. From a biochemical viewpoint, under linear conditions, when the drug concentration at the enzyme site (\( C_E \)) is less than 20% of the Michaelis constant (\( K_m \)), the \( CL_{int} \) can be considered in terms of the enzyme parameters of the Michaelis-Menten relationship shown in Eq. A2.

\[ v = \frac{V_{max,\text{in vivo}} \cdot A - \text{CYP}_{\text{in vivo}} \cdot C_L}{K_{m,\text{in vivo}}} \]

(A2)

Where \( A - \text{CYP}_{\text{in vivo}} \) is the \textit{in vivo} amount of specific CYP, \( K_{m,\text{in vivo}} \) is the \textit{in vitro} Michaelis constant for the drug-enzyme interaction and \( V_{max,\text{in vivo}} \) is the \textit{in vitro} maximal rate of metabolism.

From this, a relationship between \( CL_{int} \) and \( A - \text{CYP}_{\text{in vivo}} \) can be derived as follows:

\[ CL_{int} = \frac{V_{max,\text{in vivo}} \cdot A - \text{CYP}_{\text{in vivo}} \cdot C_L}{K_{m,\text{in vivo}}} \]

(A3)

Generally, the free concentration of drug within the hepatic vein is assumed to be \( C_L \). However, if there is a concentration gradient \textit{in vivo} (\( q_g \)) between \( C_E \) and \( C_L \), this equation can be rearranged to determine the \( A - \text{CYP}_{\text{in vivo}} \).

\[ A - \text{CYP}_{\text{in vivo}} = \frac{CL_{int} \cdot q_g}{V_{max,\text{in vivo}}} \cdot \frac{K_{m,\text{in vivo}}}{C_L} \]

(A4)

Although the above equation allows estimation of the individual \( A - \text{CYP}_{\text{in vivo}} \) in patients, a preliminary evaluation of \( q_g \) is necessary. The \( q_g \) value may be defined as follows, using a standard procedure:

\[ q_g = \frac{CL_{int}}{A - \text{CYP}_{\text{in vivo}}} \cdot \frac{K_{m,\text{in vivo}}}{V_{max,\text{in vivo}}} \]

Since the \( q_g \) value cannot be determined practically \textit{in vivo}, the measured values of \( CL_{int} \), \( K_{m,\text{in vivo}} \), \( V_{max,\text{in vivo}} \), and \( A - \text{CYP}_{\text{in vivo}} \) may be used to define it.

Moreover, when the drug metabolized by multiple CYP isoforms, the \( CL_{int} \) can be expressed as the sum of the \( CL_{int} \) of each CYP isoform. Therefore, the \( q_g \), \( A - \text{CYP}_{\text{in vivo}} \) and \( CL_{int} \) of each CYP isoform can be expressed as follows:

\[ q_g = \frac{f_{\text{CYP}} \cdot CL_{int}}{A - \text{CYP}_{\text{in vivo}}} \cdot \frac{K_{m,\text{in vivo}}}{V_{max,\text{in vivo}}} \]

(A6)

\[ A - \text{CYP}_{\text{in vivo}} = \frac{f_{\text{CYP}} \cdot CL_{int}}{q_g} \cdot \frac{K_{m,\text{in vivo}}}{V_{max,\text{in vivo}}} \]

(A7)

\[ f_{\text{CYP}} \cdot CL_{int} = \frac{V_{max,\text{in vivo}} \cdot A - \text{CYP}_{\text{in vivo}} \cdot q_g}{K_{m,\text{in vivo}}} \]

(A8)

Where \( f_{\text{CYP}} \) is the contribution of each CYP isoform.

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References


