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Estimation of the Interindividual Variability of Cytochrome 2D6 Activity from Urinary Metabolic Ratios in the Literature

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Summary: Cytochrome P450 2D6 (CYP2D6) is an enzyme with a large interindividual variability in its metabolic activity due to genetic polymorphisms. In the present study, both its intrinsic metabolic activity (CLint,CYP2D6,app) relative to extensive metabolizers (EM) and its variability were estimated by analyzing the urinary metabolic ratios (MR) based on the well-stirred model. Sparteine and debrisoquine were considered to be appropriate probes for our methodology, whereas dextromethorphan was not appropriate since the formation of its metabolite of interest is not described by the well-stirred model. From the analysis of MRs of sparteine and debrisoquine for Caucasian subjects in the literature, CLint,CYP2D6,app for intermediate metabolizers (IM) was estimated to be approximately 15% of that for EM. The coefficient of variability (CV) of CLint,CYP2D6,app was estimated to be approximately 60% for both EM and IM and 100% for the combined population of ultrarapid metabolizer, EM and IM [i.e., the non-poor metabolizer (non-PM) population]. Simulation of exposure in the non-PM population showed that the CV of exposure was 140% for dextromethorphan and 71% for metoprolol, which reflected the reported values of 110% and 53% for dextromethorphan and metoprolol, respectively. The present study should be useful for predicting the interindividual variability in exposure to investigational drugs that are metabolized by CYP2D6.

Keywords: interindividual variability; CYP2D6; urinary metabolic ratio; Monte Carlo simulation; drug development

Introduction

In the process of drug development, it is important to predict exposure to a drug candidate and its variability before a first-in-human trial in order to select the best drug candidate because interindividual variability in exposure leads to variability in pharmacologic effects, which can result in severe adverse effects or therapeutic failure. Our group is currently developing a methodology to predict the interindividual variability in drug exposure. In this methodology, the variability is estimated based on a Monte Carlo simulation using both physiological parameters (blood flow rate, organ size, etc.) and pharmacokinetic parameters (clearance, protein binding, etc.) as variables (Fig. 1). With this methodology, we succeeded in estimating the interindividual variability in exposure to drugs that were metabolized predominantly by CYP3A4.1) The key factor in this methodology is the estimation of variability in metabolic activity; Kato et al. estimated the coefficient of variability (CV) of CYP3A4 activity to be 33%.1)

Variability in metabolic activity is, in most cases, related to polymorphisms of the drug metabolizing enzymes. CYP2D6, an important enzyme that metabolizes about 25% of prescribed drugs,2) has been investigated intensively with respect to the influence of polymorphisms on enzyme activity.3) More than 80 polymorphisms have been described for CYP2D63) and the number of alleles is still increasing.4) Among the reported polymorphisms, some lead to increased activity by gene multiplication (*1×N, *2×N, *35×N), whereas others lead to a complete loss of enzyme activity as a result of chromosomal...
Fig. 1. A scheme for the prediction of interindividual variability in drug exposure from prior knowledge of the physiological and drug-related parameters

EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; MR, urinary metabolic ratio. Prior knowledge is listed in the scheme, but is not limited to those items listed.

Deletion (*5), splice site mutation (*4), or the insertion of a stop codon owing to the deletion of a single base pair (*3, *6). On the one hand, some polymorphisms lead to decreased function (*10, *17).5–7

The CYP2D6 genotype has been associated with interindividual variability in CYP2D6 activity in vivo.8,9 CYP2D6 activity in vivo has been evaluated on the basis of the urinary metabolic ratio (MR: unchanged drug in urine/metabolites in urine) of probe drugs. Sparteine, debrisoquine, metoprolol, and dextromethorphan were used as the probe drugs, and 2- and 5-dehydrosparteine, 4-hydroxydebrisoquine, α-hydroxymetoprolol, and dextrorphan were the metabolites of interest, respectively. Subjects who are defective in metabolizing these probe drugs exhibit a higher MR and are classified as poor metabolizers (PM). Subjects who can metabolize these probe drugs are subdivided into three phenotypes: ultra-rapid metabolizers (UM), extensive metabolizers (EM), and intermediate metabolizers (IM). UM exhibit extremely high enzyme activity, EM exhibit normal activity, and IM have decreased activity relative to EM. From a genetic viewpoint, PM correspond to those who are homozygotes of null-function alleles, whereas IM correspond to those who have two decreased-function alleles or a combination of one decreased-function allele and one null-function allele. EM correspond to those who have one or two functional alleles and UM correspond to those who have more than three functional alleles. It is been known that the composition of these phenotypes differs among ethnicities, and this is partly associated with differences in the prevalence of genetic polymorphisms in each ethnic group.10 However, most reports have focused on the proportion of PM without estimating the extent of the variability in CYP2D6 activity in each phenotype.

When our methodology is expanded to predict the interindividual variability in exposure to drugs and drug candidates metabolized by CYP2D6, information on both the metabolic activity and the extent of its variability for each phenotype is required. In the present study, the relative CYP2D6 activity in IM to EM and the variability in IM and EM were estimated by analyzing the MRs of probe drugs. The prerequisite attributes of probe drugs for the present method were also clarified. The validity of the relative CYP2D6 activity and its variability estimated in the present study were assessed by comparing the simulated MR histograms of dextromethorphan and metoprolol with reported data, as well as comparing the CV of simulated exposure with reported values.

Methods

Phenotype definition: In the present study, a PM is a subject with an MR greater than 12.6,11 20,12 and 0.313 for debrisoquine, sparteine, and dextromethorphan, respectively. An IM is a subject with 1.2< MR<12.6 for debrisoquine, 1.2<MR<20 for sparteine, and 0.03<MR<0.3 for dextromethorphan.14–16 Whereas Dalen et al. identified intermediate subjects as
those whose debrisoquine MR was more than unity,14) we applied 1.2 as the threshold between EM and IM from a visual inspection of the histogram of the MR. An EM is a subject with an MR < 1.2 for debrisoquine, MR < 1.2 for sparteine, and MR < 0.03 for dextromethorphan. A sparteine MR of less than 0.15 was identified as UM.3) A debrisoquine MR of less than 0.03 was identified as UM.17)

Theory on the analysis of MR: MR is expressed as follows:

\[ MR = \frac{\text{amount of unchanged drug collected in urine}}{\text{amount of metabolite(s) collected in urine}} \]  

(1)

If linear pharmacokinetics is assumed and both the metabolite of interest and its subsequent metabolite(s) are quantified, MR is expressed as follows:

\[ MR = \frac{CLtot}{CLh} \left( 1 - \frac{CLh}{Q} \right) \cdot \frac{CLh}{CLr} \cdot R_{\text{m,r}} \]  

(2)

where \( CL_{\text{tot}} \), \( CL_h \), and \( CL_r \) represent the total, hepatic, and renal clearance of the unchanged drug, respectively. The parameters in equation 2 are model-independent. \( CL_{\text{tot}} \) is the summation of \( CL_h \) and \( CL_r \). \( CL_{\text{tot}} \) represents the organ clearance of the metabolic reaction of interest that is mediated predominantly by CYP2D6. If the metabolic reaction of interest exclusively contributes to the hepatic elimination of drugs, \( CL_h \) is equal to \( CL_{\text{tot}} \). \( Q \) represents the hepatic blood flow rate (22.8 mL/min/kg). \( R_{\text{m,r}} \) represents the urinary excretion fraction of the metabolite expressed as follows:

\[ R_{\text{m,r}} = \frac{CL_{\text{m,r}}}{CL_{\text{m,ox}}} \]  

(3)

where the subscript \( m \) represents the metabolite of interest. The subscripts \( r \) and \( tot \) represent the renal and total clearance, respectively. In the present study \( R_{\text{m,r}} \), was set as 1, unless specified otherwise, because there are no reports on \( R_{\text{m,r}} \) in humans. When \( R_{\text{m,r}} \) becomes smaller than 1 (0 < \( R_{\text{m,r}} \) < 1), the histogram shifts to the higher range while the distribution pattern of the histogram remains unchanged. When the well-stirred model is applied to \( CL_h \), MR is expressed as follows:18,19)

\[ MR = \frac{CL_r}{f_u \left( 1 + \frac{CL_r}{Q} \right) \cdot R_{\text{m,r}}} \]  

(4)

where \( f_u \) represents the unbound fraction in blood and \( CL_{\text{int,CYP2D6,app}} \) represents the apparent intrinsic clearance of the metabolic reaction of interest that is mediated predominantly by CYP2D6. Here, \( CL_{\text{int,CYP2D6,app}} \) is considered to include the contribution of other enzyme(s), as is implied by the fact that even PM subjects who completely lack CYP2D6 activity can form the metabolite of interest. For instance, the metabolic reactions of debrisoquine 4-hydroxylation and sparteine dehydrogenation were reported to be catalyzed by CYP1A1 as well as CYP2D6.20) Therefore, \( CL_{\text{int,CYP2D6,app}} \) can be written as follows:

\[ CL_{\text{int,CYP2D6,app}} = CL_{\text{int,CYP2D6}} + CL_{\text{pseudo,CYP2D6}} \]  

(5)

where \( CL_{\text{pseudo,CYP2D6}} \) is the metabolic intrinsic clearance in a PM who completely lacks CYP2D6 activity. The log-transformed MR is expressed as follows:

\[ \log MR = \log CL - \log \left[ f_u \left( 1 + \frac{CL_r}{Q} \right) \cdot R_{\text{m,r}} \right] \]  

(6)

When the interindividual variability in \( CL_{\text{int,CYP2D6,app}} \) is larger than the other parameters, the variability of MR directly reflects the variability of CYP2D6 activity. In the present study, the variability of log MR was regarded as the variability of log-normalized CYP2D6 activity. However, since log MR is not a pure marker of enzyme activity, as shown in equation 6, the estimated variability in CYP2D6 activity is considered to be the largest estimate that could be applied.

Assuming that parameters other than \( CL_{\text{int,CYP2D6,app}} \) are not affected by the phenotype status, the difference of the averaged log-transformed MR between phenotypes represents the log-transformed activity ratio of CYP2D6 between phenotypes as follows:

\[ \log MR_1 - \log MR_2 = \log \frac{CL_{\text{int,CYP2D6,app}} \cdot 2}{CL_{\text{int,CYP2D6,app}} \cdot 1} \]  

(7)

Simulation of MR-activity relationship for probe drugs: In order to examine whether the probe drugs have the appropriate attributes for our methodology, the relationship between the MR and enzyme activity was investigated by simulating MRs based on the physiological and pharmacokinetic parameters for debrisoquine, sparteine, and dextromethorphan using equation 2 with different hepatic clearance models: the well-stirred model, the parallel tube model, and the dispersion model with a dispersion number of 0.17. The product \( f_u \cdot CL_{\text{int,CYP2D6,app}} \) varied from 0.1% of EM activity to 200% of EM activity, where 100% of EM activity corresponded to the enzyme activity that provided particular MR values in EM derived in the following section (MR: 0.437 for debrisoquine,17) 0.468 for sparteine,18) and 0.0033 for dextromethorphan21)). In this simulation, hepatic clearance through other metabolic route(s) was not considered. This was because the influence of these clearances on the simulation had been confirmed to be limited under the conditions where the contributions of these clearances to the metabolism of probe drugs were taken into consideration (≤ 10% for sparteine,22) ≤ 50% for debrisoquine,23) and ≤ 12% for dextromethorphan24,25).
Then, log MR was obtained based on equation 2 after CLh was estimated by using the well-stirred model, the parallel tube model, and the dispersion model. Meanwhile, the renal clearance was fixed (2.6 mL/min/kg for sparteine, 26 2 mL/min/kg for debrisoquine, 27) and 0.57 mL/min/kg for dextromethorphan.28 If the relationship between the MR and CYP2D6 activity is model-independent, the well-stirred model can be applied to analyze the histogram of MR based on equations 4 and 6. Otherwise, equations 4 and 6 are not applicable to the analysis of the MR histogram of the probe drug concerned.

**Analysis of the histogram of MR to estimate the variability of CYP2D6 activity:** The information on the histogram of MR for debrisoquine17,29 and for sparteine13,39 was collected from the published literature. The histograms were quantified by scanning the figures in the reports. Since the MR for individual subjects was not reported, the MR for every subject in a given class of histogram was represented by the class mean. The mean MR (MRmean) was obtained for each phenotype as follows:

$$\log \text{MR}_{\text{mean}} = \frac{\sum (N_i \cdot \log m_r)}{\sum N_i}$$  (8)

where m_r represents the class mean for each i-th class, and N_i represents the number of subjects in the i-th class. The standard deviation of MR was estimated as follows for each phenotype, assuming a normal distribution of log MR:

$$SD = \sqrt{\sum N_i \cdot (\log m_r)^2} - \left(\sum (N_i \cdot \log m_r)\right)^2 \over \sum N_i(\sum N_i - 1)$$  (9)

where SD represents the standard deviation of log-transformed CYP2D6 activity when based on equation 6. Because the literature generally provides information in the form of the algebraic mean and its standard deviation, the coefficient of variance of CYP2D6 activity was calculated by the following equation to maintain comparability:

$$CV^2 = e^{SD \times \ln (10)^2} - 1$$  (10)

**Simulation of the histograms of MR for dextromethorphan and metoprolol:** In order to assess the validity of the estimated CV of CYP2D6, the MRs for dextromethorphan and metoprolol were simulated by means of a Monte Carlo simulation, and then the simulated histograms of MR and drug exposure were compared to those reported in the literature. Physiological and pharmacokinetic parameter sets were generated for 200 subjects as listed in Table 4 assuming log-normal distributions for each of the parameters. Each parameter was distributed independently. Interrelationships between parameters were not taken into consideration. Drugs were assumed to be metabolized by CYP2D6 to provide the MR (CLint,CYP2D6,app) and anything other than the route of interest for the MR (CLint,other). The free fraction of drugs in plasma was used in the calculation due to its availability, so that the partition coefficient of drugs to red blood cells (R_b) should be introduced to estimate f_v. Since the quotient R_b/R_m,r is proportional to MR and is assumed to be constant between subjects, R_b/R_m,r just shifts the histogram of MR to the higher or lower range without affecting the distribution pattern of the simulated histogram. In the present study, values of 1 and 5 were applied to R_b/R_m,r for dextromethorphan and metoprolol, respectively, to give an appropriate MR in PM. Typical values of the metabolic activity-related parameters used in the simulation were estimated as follows. As the first step, typical values of CLint,other and CLint,pseudoCYP2D6 in PM were obtained arithmetically based on equation 2 and by using a dispersion model to give both the PM’s dose-normalized exposure, AUC/D (0.00406 min/mL for dextromethorphan, 0.00353 min/mL for metoprolol)28,31 and the PM’s MR (0.48 for dextromethorphan, 1.95 for metoprolol).32 All these target values were derived from the literature. Second, the obtained typical value of CLint,other was used for the other phenotypic groups, assuming that CLint,other was independent of the CYP2D6 phenotype. The typical value of CLint,CYP2D6,app in EM was then estimated to give the target AUC/D (0.000160 min/mL for dextromethorphan and 0.000833 min/mL for metoprolol) from the literature.31,33 Third, the typical value of CLint,CYP2D6,app in IM was assumed to be 15% of that in EM, an estimate from the present study. Then CLint,CYP2D6,app and CLint,other were generated for 200 subjects around the typical values with a variability (CV) of 60% for CLint,CYP2D6,app and an approximate estimate obtained in the present study, and with a CV of 33% for CLint,other, an estimate for CYP3A4 as a reference. The contribution of CYP2D6 to the metabolism was assessed by the following equation:

$$\text{Contribution of CYP2D6} = \frac{\text{CL}_{\text{int,CYP2D6,app}}}{\text{CL}_{\text{int,CYP2D6,app}} + \text{CL}_{\text{int,other}}}$$  (11)

The dispersion model with a dispersion number of 0.17 was applied in calculating AUC/D. A hypothetical population was assumed in which the proportion of each phenotype was EM:IM:PM = 80:15:5. As further analysis to confirm the effect of CV of CLint,CYP2D6,app on the distribution pattern of the histogram of MR, the histogram for metoprolol was simulated for different CVs of CYP 2D6 (30%, 90%, and 120%) while fixing the other parameters. All calculations, including the generation of parameters, were done using Microsoft Excel 2003.

**Results**

**Simulation of the MR-activity relationship for probe drugs:** The relationship between the MR and CYP2D6 activity was examined by using three pharmacokinetic models with varying relative enzyme activity ranging from 0.1% of EM activity to 200% of EM activity.
Fig. 2. Relationship between the urinary metabolic ratio (MR) and CYP2D6 activity for debrisoquine (a), sparteine (b), and dextromethorphan (c).

MR was calculated by the well-stirred model (solid lines), the dispersion model (broken lines), and the parallel tube model (dotted lines) with varying intrinsic CYP2D6 activity ranging from 0.1% to 200% of the EM activity. 100% of EM activity corresponds to the activity that provides the average MR for the EM (MR: 0.437 for debrisoquine, 0.468 for sparteine, and 0.0033 for dextromethorphan).

Estimation of Variability in CYP2D6 Activity

No difference was observed among the models for both debrisoquine and sparteine, suggesting that the metabolic reactions of interest followed intrinsic clearance-limited kinetics. In contrast, the relationship between the MR and CYP2D6 activity for dextromethorphan was model-dependent, where the MR values calculated by both the parallel tube model and the dispersion model were more sensitive to the change in enzyme activity at around 100% of EM activity than those calculated by the well-stirred model. On the other hand, the relationships were almost parallel in the range below 10% of EM activity. Based on these results, the well-stirred model can be applied to the analysis of MR histograms of debrisoquine and sparteine to investigate the variability of CYP2D6 activity. In contrast, because it is not appropriate to apply a well-stirred model to extensive metabolizers, dextromethorphan would not serve as a good probe for the variability analysis in our methodology.

The relative metabolic activities ($CL_{int,CYP2D6,app}$, unit: % of EM) corresponding to the antimode and threshold between IM and EM were calculated from equation 2 with different clearance models (Table 1). These results suggest that PM subjects have less than 4% of EM activity in metabolizing probe drugs. The thresholds of IM used in the present study were 1.2, 1.2, and 0.03 for debrisoquine, sparteine, and dextromethorphan, respectively. The metabolic activities corresponding to these MRs were slightly different depending on the model applied; they were approximately 40% of EM for debrisoquine and sparteine and approximately 28% of EM for dextromethorphan for the parallel tube model.

Analysis of the histogram of MR to estimate the variability of CYP2D6 activity: The average MR for the EM population was 0.437 for debrisoquine, 0.468 for sparteine, and 0.0033 for dextromethorphan (Table 1).

The relative CYP2D6 activities in IM and PM were estimated from the MR histograms of sparteine and debrisoquine using equation 7 (Table 2). CYP2D6 activity was estimated to be around 15% of EM activity in the IM population and below 1.0% of EM activity in the PM population.

The estimated interindividual variability in CYP2D6 activity was from 45% to 69% for EM and from 61% to 64% for IM (Table 3). The CV for the overall population was estimated to be from 96% to 120%. The CV for PM was also estimated, although it should be noted that this is caused by variability in $CL_{int,pseudoCYP2D6}$.

Simulation of the histogram of MR for dextromethorphan and metoprolol: Typical values of $CL_{int,other}$, $CL_{int,CYP2D6,app}$, and $CL_{int,pseudoCYP2D6}$ that gave the target values of AUC/D and MR were obtained (Table 4). According to the results, the contribution of CYP2D6 in EM was 95% for dextromethorphan and 82% for metoprolol. The pseudo-CYP2D6 activity in PM was 0.3% of EM for dextromethorphan and 1% of EM for metoprolol. The product $f_r \cdot CL_{int,CYP2D6,app}$ for dextromethorphan was 2.9 mL/min/mL liver in EM, which was approximately 2.4-fold higher than the typical value of Q (1.22 mL/min/mL liver), while the product $f_r \cdot CL_{int,CYP2D6,app}$ in PM was 0.009 mL/min/mL liver. Those for metoprolol were 0.64 and 0.0062 mL/min/mL liver in EM and PM, respectively.
Table 1. Relative metabolic activities (CL\textsubscript{int, CYP2D6-app}) corresponding to the antimode and threshold between EM and IM calculated using an analytical model for MR with different clearance models

<table>
<thead>
<tr>
<th>Probe drugs</th>
<th>MR</th>
<th>Log MR</th>
<th>Description</th>
<th>CL\textsubscript{int, CYP2D6-app} (% of EM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WS</td>
</tr>
<tr>
<td>Sparteine</td>
<td>0.468</td>
<td>-0.33</td>
<td>Average MR for the EM population</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.08</td>
<td>Threshold between EM and IM</td>
<td>39.1</td>
</tr>
<tr>
<td>Debrisoquine</td>
<td>0.437</td>
<td>-0.36</td>
<td>Average MR for the EM population</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.08</td>
<td>Threshold between EM and IM</td>
<td>36.5</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>0.0033</td>
<td>-2.48</td>
<td>Average MR for the EM population</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>-1.52</td>
<td>Threshold between EM and IM</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>-0.52</td>
<td>Antimode between IM and PM</td>
<td>1.1</td>
</tr>
</tbody>
</table>

WS, well-stirred model; Disp, dispersion model; P-tube, parallel tube model; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer

Table 2. CYP2D6 activity (CL\textsubscript{int, CYP2D6-app}) in IM and PM estimated from an analysis of the histograms of MR in the literature

<table>
<thead>
<tr>
<th>Probe drug</th>
<th>Reference no.</th>
<th>Ethnicity</th>
<th>Mean log MR</th>
<th>CL\textsubscript{int, CYP2D6-app} (% of EM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EM</td>
<td>IM</td>
</tr>
<tr>
<td>Sparteine</td>
<td>Ref. 3 (n = 316)</td>
<td>European</td>
<td>-0.33</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Ref. 30 (n = 195)</td>
<td>German</td>
<td>-0.42</td>
<td>0.45</td>
</tr>
<tr>
<td>Debrisoquine</td>
<td>Ref. 17 (n = 1011)</td>
<td>Swedish</td>
<td>-0.36</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Ref. 29 (n = 758)</td>
<td>Swedish</td>
<td>-0.31</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Table 3. Variability of apparent CYP2D6 activity (CL\textsubscript{int, CYP2D6-app}) estimated from an analysis of histograms of MR in the literature

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Sub-phenotype</th>
<th>Cl\textsubscript{int, CYP2D6-app} (% of EM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EM</td>
</tr>
<tr>
<td>EM</td>
<td>UM</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>EM</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>IM</td>
<td>64%</td>
</tr>
<tr>
<td>UM + EM + IM</td>
<td>96%</td>
<td>102%</td>
</tr>
<tr>
<td>PM</td>
<td>UM</td>
<td>60%</td>
</tr>
</tbody>
</table>

UM, ultra-rapid metabolizers
aNot calculated because of low observation number (n = 1)

With these parameters and the variability listed in Table 4, the exposure (AUC/D) and the MR for dextromethorphan and metoprolol were predicted for 200 subjects. For dextromethorphan, the CV of AUC/D was 140% in the non-PM population and 42% in PM. For metoprolol, the CV of AUC/D was 71% in the non-PM population and 24% in PM, which reflect the actual observations of 53% in the non-PM population and 14% in PM. The distribution patterns of the simulated histograms of MR for dextromethorphan and metoprolol were similar to those reported (Fig. 3). The effect of the CV of CL\textsubscript{int, CYP2D6-app} on the distribution pattern of the simulated histogram of MR was examined by varying the CV of CL\textsubscript{int, CYP2D6-app} while fixing the other parameters for metoprolol (Fig. 4). When a CV of 30% was applied, IM and PM could be discriminated; however, the distribution was somewhat limited compared to that of the actual report (Fig. 4). On the other hand, when a CV of 90% was applied, it was hard to discriminate distinctly between IM and PM in the histogram obtained. When a CV of 120% was applied, the distribution of log-transformed MR became broader, ranging from -2.5 to 2.7, and the IM population was apparently confused with the EM population.

Discussion

The methodology that we have developed is based on a
Table 4. Physiological and pharmacokinetic parameters used in the simulation of the histograms of MR. The means of the metabolic intrinsic clearances were estimated as described in Materials and Methods

<table>
<thead>
<tr>
<th>Physiological parameters</th>
<th>Mean</th>
<th>CV(%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qh (mL/min/mL liver)</td>
<td>1.22</td>
<td>13.8</td>
<td>Ref. 1</td>
</tr>
<tr>
<td>Liver weight (mL/kg)</td>
<td>19.5</td>
<td>10.9</td>
<td>Ref. 1</td>
</tr>
<tr>
<td>Body weight (kg/person)</td>
<td>70</td>
<td>9.7</td>
<td>Ref. 1</td>
</tr>
</tbody>
</table>

**Dextromethorphan**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>CV(%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fraction (%)</td>
<td>75</td>
<td>2.8</td>
<td>In-house data</td>
</tr>
<tr>
<td>(\text{CL}_r) (mL/min/kg)</td>
<td>0.57</td>
<td>50</td>
<td>Ref. 28</td>
</tr>
<tr>
<td>(\text{CL}_{\text{int, CYP2D6, app}}) (EM) (mL/min/mL liver)</td>
<td>3.9</td>
<td>60</td>
<td>Estimated</td>
</tr>
<tr>
<td>(\text{CL}_{\text{int, pseudoCYP2D6}}) (PM) (mL/min/mL liver)</td>
<td>0.012</td>
<td>60</td>
<td>Estimated</td>
</tr>
<tr>
<td>(\text{CL}_{\text{int, other}}) (mL/min/mL liver)</td>
<td>0.2</td>
<td>33</td>
<td>Estimated</td>
</tr>
</tbody>
</table>

**Metoprolol**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>CV(%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fraction (%)</td>
<td>80</td>
<td>2.8</td>
<td>Ref. 41</td>
</tr>
<tr>
<td>(\text{CL}_r) (mL/min/kg)</td>
<td>1.1</td>
<td>50</td>
<td>Ref. 31</td>
</tr>
<tr>
<td>(\text{CL}_{\text{int, CYP2D6, app}}) (EM) (mL/min/mL liver)</td>
<td>0.8</td>
<td>60</td>
<td>Estimated</td>
</tr>
<tr>
<td>(\text{CL}_{\text{int, pseudoCYP2D6}}) (PM) (mL/min/mL liver)</td>
<td>0.0078</td>
<td>60</td>
<td>Estimated</td>
</tr>
<tr>
<td>(\text{CL}_{\text{int, other}}) (mL/min/mL liver)</td>
<td>0.18</td>
<td>33</td>
<td>Estimated</td>
</tr>
</tbody>
</table>

Fig. 3. Predicted (a, c) and reported (b, d) urinary metabolic ratios of dextromethorphan (a, b) and metoprolol (c, d). A parameter set for 200 subjects was generated based on the conditions listed in Table 4. (Ref. 21 and Ref. 32), DEM, dextromethorphan; DOR, dextrorphan.

Monte Carlo simulation in which the information on the metabolic activity and the extent of its variability for each phenotype is required (Fig. 1). To obtain this essential information, we used the urinary MR data of probe drugs. The MRs of debrisoquine, sparteine, and dextromethorphan are well-established markers of CYP2D6 activity and have been used to investigate the relative size of the PM group in populations of various ethnicities. However, the validity of using the MRs of these probe drugs as an index for variability in CYP2D6 activity...
Fig. 4. Simulated histograms of MR for metoprolol with various variabilities of CYP2D6 activity with other parameters fixed. The parameters used are listed in Table 4. The coefficients of variance are set at 30% (a), 60% (b), 90% (c), and 120% (d).

Takashi Ito, et al. has not yet been confirmed. Therefore, we started by examining the pharmacokinetic attributes of probe drugs and then estimated the variability in CYP2D6 activity.

Because our methodology is based on the analysis of urinary MR data of probe drugs using equation 6, which is valid only if the well-stirred model is applicable, it was important to clarify the pharmacokinetic attributes of the probe drugs. The three probe drugs were examined with respect to the relationship between MR and $CL_{int, CYP2D6, app}$, demonstrating that the relationship in the case of dextromethorphan was model-dependent, while those for the other two probes were not (Fig. 2). This kind of model dependency implies that dextrorphan formation is not an example of intrinsic clearance-limited kinetics and that the dispersion model or the parallel tube model could be appropriate in this case, in contrast to the case for the other two probes. This is supported by the fact that the absolute difference in log MR between EM and IM is approximately 0.8 for sparteine and debrisoquine (Table 2), while that for dextromethorphan is 1.54 (−2.48 for EM, and −0.94 for IM) when the histogram of the MR data of dextromethorphan reported by Funck-Brentano et al. is analyzed.21 These observations are demonstrated by the curvilinear relationship of the MR of dextromethorphan as represented by the dispersion model and the parallel tube model (Fig. 2). Therefore, dextromethorphan is not appropriate for our methodology, whereas debrisoquine and sparteine were considered to be appropriate probes for our methodology.

According to analysis of the MR histograms of debrisoquine and sparteine, the average CYP2D6 activities were approximately 15% of EM for the IM population and below 1% of EM for the PM population (Table 2), which was within the range estimated by the simulation analysis (Table 1). These estimated activities are in agreement with the report in which the EM subgroup had an almost 5-fold higher median clearance (410 mL/min) than the IM subgroup (87 mL/min) in the case of sparteine.30

In terms of the variability, the CV values for EM and IM were approximately 60% (Table 3), which was somewhat larger than the variability estimated for CYP3A4 (33%).1 However, these reference values were based on the cytochrome P450 contents using microsomes prepared from each individual. There were no clear descriptions of the CYP2D6 phenotype of subjects in these studies, implying that the subjects were a non-PM (UM + EM + IM) population. The CV for the non-PM population estimated in the present study was approximately 100% (Table 3), which reflects the variability in enzyme activity. The MR data used in the present study consisted of summary
histograms from the literature; overlap of the distribution for subpopulations was not taken into consideration. A more detailed and precise analysis could be possible if the distribution of individuals’ MR data was modeled statistically.\textsuperscript{21,38}

In the simulation of the AUC/D and MR histograms of dextromethorphan and metoprolol, the metabolic activity-related parameters were estimated. The relationship between Q and the product $\frac{L}{V} \cdot \text{CL}_{\text{int,CYP2D6,app}}$ suggested that dextorphan formation followed blood flow-limited kinetics in EM, while that in PM followed intrinsic clearance-limited kinetics. It was suggested that $\alpha$-hydroxylation of metoprolol followed intrinsic clearance-limited kinetics in both EM and PM. The estimated $\text{CL}_{\text{int,prod,CYP2D6}}$ in PM was below 1% of that in EM for both drugs, which was within the range shown in Table 1. The contribution of CYP2D6 to the metabolism was in good agreement with the reported values of $>90\%$ for dextromethorphan\textsuperscript{39} and $83\%$ for metoprolol.\textsuperscript{40} All these results suggest that equation 2 would be useful for estimating the pharmacokinetic parameters of a drug from its MR histogram.

When we simulate the exposure and variability of a given CYP2D6 substrate, we have to consider the effect of genetic polymorphism of CYP2D6, especially for the IM population, because genetic polymorphism may have an impact on both the relative activity and its variability in the IM population. It was reported that the effect of mutation on the metabolic activity was substrate-dependent. For example, the metabolic clearance of nortriptyline 10-hydroxylation and codeine O-demethylation by CYP2D6\textsuperscript{1} was 1.32% and 27.9% of CYP2D6.1, respectively.\textsuperscript{7} This suggests that the relative activity in IM should be determined depending on the substrate of interest. On the one hand, the IM population is a heterogeneous population with various combinations of CYP2D6 alleles, suggesting that the variability could differ between ethnicities depending on the prevalence of different polymorphisms. In our methodology, neither the substrate dependency of the effect of polymorphism on enzyme activity nor the ethnic difference in genetic polymorphisms was taken into account in order to construct a general framework for the simulation of exposure to CYP2D6 substrates. Instead, the IM population was dealt with as an apparent phenotype population. The applicability of the present study might be limited to Caucasians and particular substrates. However, considering that it is rare for the relative activity of every combination of alleles to be obtained for a given CYP2D6 substrate before a clinical trial, the relative activity (15% of EM) and variability (60% CV) could be applied as typical values as a first step. These could be adjusted depending on the substrate and ethnicity of interest if sufficient prior information becomes available.

Although we should be aware of the limitations of the present methodology, it is possible to predict the interindividual variability of exposure for a drug metabolized by CYP2D6 with our methodology (Fig. 1). There is also the possibility of simulating exposure to a drug in a given ethnicity by changing the composition of the EM, IM, and PM populations in order to predict ethnic differences. With information on the variability of the catalytic activity for other CYP isoforms and the magnitude of their contributions, exposure to a drug that is cleared by multiple CYP isoforms could also be simulated. This kind of virtual clinical simulation would be valuable in drug development. In conclusion, a methodology for the estimation of interindividual variability was proposed. The urinary MR of a probe drug whose metabolic route exhibits intrinsic clearance-limited kinetics can be used as an index for the variability of metabolic activity in this methodology. CYP2D6 activity in the IM population was estimated to be approximately 15% of EM activity, and CY2D variability was approximately 60% in both the EM and IM populations and 100% in the non-PM population (UM + EM + IM). These results could be useful for prediction of the interindividual variability of exposure to investigational drugs that are metabolized by CYP2D6.

References


