Regular Article

Consideration of Reliable Concentrations for Prediction of Change in Enzyme Activity by Mechanism-based Inactivation Using Physiologically-based Pharmacokinetic Model Simulations

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Using physiologically-based pharmacokinetic model simulations with the assumption that elimination of inactivator is not altered by mechanism-based inactivation (MBI) of the target enzyme, we examined at what concentrations the influence of MBI could be accurately and simply predicted. The method utilizing maximum unbound systemic concentration as the inactivator concentration (method 1) tended to overestimate this influence, and accuracy expressed as the ratio of estimated and exact fold decrease in enzyme activity ranged from 0.80 to 8.41. In addition, when the volume of distribution was large or the absorption rate constant was small, method 1 provided relatively precise estimation, with the ratio of nearly 1. We propose use of two concentrations, the steady-state average unbound liver concentration and maximum limit of steady-state average unbound liver concentration, to predict the effects of MBI. The accuracy of prediction of MBI using these two concentrations ranged from 0.90 to 1.04 and 0.92 to 2.96, respectively, and was higher than that with method 1. These two concentrations can be obtained early in the drug development process, and estimated results can be expected to contribute to determination of the effects of MBI.

Keywords: mechanism-based inactivation; drug-drug interactions; physiologically-based pharmacokinetics; prediction; steady-state

Introduction

Cytochrome P450 (CYP) enzymes comprise a superfamily of heme-containing enzymes that catalyze the oxidative metabolism of a variety of substrates. Because drug-drug interactions have become significant medical problems,1–3 evaluation of the effects of drug-drug interactions on CYP enzymes has become more important early in the drug development process. Mechanisms of CYP enzyme inhibition include reversible inhibition and mechanism-based inactivation (MBI). The latter deserves more attention than the former, because it often results in serious side effects in clinical use. One of the most serious cases of toxicity associated with MBI occurred when 5-fluorouracil was used together with sorivudine.4 In this case, the target enzymes are not CYP enzymes but dihydropyrimidine dehydrogenase.

Several approaches have been used to predict the extent of effects of MBI on the pharmacokinetic characteristics of co-administered drugs in vivo. Mayhew et al. proposed a simple method to predict in vivo drug-drug interactions in terms of AUC change caused by MBI based on the following Equation5):
method, clinically relevant unbound plasma concentrations of 0.1 μM for fluoxetine, clarithromycin, and N-desmethyl diltiazem were used for \( I \) in Equation 1.

Obach et al. examined three different concentrations as inactivator concentrations in Equation 1, and successfully demonstrated that use of the maximum unbound systemic concentration as the inactivator concentration provided the most accurate predictions of clinical effects of MBI.⁹

Kanamitsu et al. simulated the effects of MBI caused by erythromycin using a physiologically-based pharmacokinetic (PBPK) model and demonstrated a good correlation with clinical results.⁷ The PBPK model is composed of three compartments, systemic blood, liver, and portal vein, and the change in enzyme activity by MBI is described using the concentration in the liver compartment. Although in theory PBPK model-based estimation is considered a precise method of predicting the effects of MBI, many pharmacokinetic parameters for humans must be determined with precision to perform simulations.

Early in the drug development process, PBPK model-based estimation of the effects of MBI is usually difficult to perform, since many pharmacokinetic parameters required for simulation are usually not available. On the other hand, the method of prediction using Equation 1 with maximum unbound systemic concentration as the inactivator concentration (method 1) can be applied. In this case, concentrations obtained in animal experiments can be used for inactivator concentrations, although concentrations in humans are not yet available. Although another study supports the usefulness of this method,⁹ it is uncertain whether method 1 is applicable to a wide variety of drugs.

In the present study, we examined the accuracy of method 1, and elucidated the factors affecting the accuracy of prediction of method 1 using PBPK model-based simulations. Furthermore, we propose use of alternative inactivator concentrations that can be applied to Equation 1 to predict the effects of MBI accurately and used early in the drug development process.

**Methods**

**Physiologically-based pharmacokinetic model (PBPK model):** The PBPK model in this study includes only the body and the liver in description of the pharmacokinetics of the inactivator (Fig. 1). The enzyme compartment is attached to liver to simulate the change in enzyme activity by MBI.⁸ Since many drugs are expected to exhibit good bioavailability, kinetics in the liver are assumed to be described by the well-stirred model. To simplify the model, elimination of the inactivator is assumed not to be altered by MBI. Material balance and change in enzyme activity are defined as follows:

![Fig. 1. Physiologically-based pharmacokinetic model to simulate concentrations in the body and liver, and changes in enzyme activity by mechanism-based inactivation](image)

- \( V_B \cdot \frac{dC_B}{dt} = Q_H \cdot C_H \cdot f_B + f_H \cdot C_H - f_B \cdot C_B \cdot \frac{CL_{int,B}}{Q_B} \)
- \( V_H \cdot \frac{dC_H}{dt} = Q_H \cdot C_H \cdot f_B + f_H \cdot C_H - f_B \cdot C_B \cdot \frac{CL_{int,H}}{Q_H} \)
- \( \frac{dE_{act}}{dt} = \frac{-k_{inact} \cdot E_{act} \cdot f_B \cdot C_H}{K_i} + k_{deg} \cdot (E_0 - E_{act}) \)

where \( V, C, Q, f, \) and \( CL_{act} \) represent the volume of distribution, concentration in tissue, blood flow rate, unbound fraction of the drug, and intrinsic clearance, respectively, with abbreviations \( B \) and \( H \) used for the body and liver compartments, respectively. \( E_{act}, E_0, k_{inact}, K_i, \) and \( k_{deg} \) represent the active enzyme content, initial enzyme content, inactivation rate constant of the enzyme, the apparent dissociation constant between the enzyme and inactivator, and the degradation rate constant (turnover rate constant) of enzyme, respectively. A drug is assumed to be absorbed in the liver compartment with a first-order absorption rate constant of \( k_a \), and the absorbed fraction is assumed to be 100%.

**Kinetic parameters for simulations:** Table 1 summarizes the pharmacokinetic parameters, physiological parameters, and MBI parameters used for two different cases of simulation.

Case 1: To cover typical drugs with different characteristics, all combinations (720 cases) of five different parameters including the volume of distribution in the body, intrinsic clearance in the body and liver, absorption rate constant, and dose were examined. Absorption rate constants and volumes of distribution reported in
the literature were analyzed, and results are expressed as the range of mean ± SD with the mean value in parenthesis.

Table 1. Pharmacokinetic parameters and mechanism-based inactivation parameters used in simulations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_d$ (L)</td>
<td>15, 45, 135, 405</td>
<td>135</td>
</tr>
<tr>
<td>$CL_{int, s}$ (mL/min)</td>
<td>0, 600, 1200</td>
<td>600</td>
</tr>
<tr>
<td>$f_b$</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_H$ (L)</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>$Q_H$ (mL/min)</td>
<td>1450</td>
<td></td>
</tr>
<tr>
<td>$CL_{int, H}$ (mL/min)</td>
<td>300(0.91), 900(0.76), 2700(0.52), 8100(0.26)</td>
<td>2700</td>
</tr>
<tr>
<td>$k_{degr}$</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Dose amount (mg)</td>
<td>1, 10, 100</td>
<td>3 (daily dose)</td>
</tr>
<tr>
<td>$k_i$ (min⁻¹)</td>
<td>0.002, 0.005, 0.0125, 0.0313, 0.0781</td>
<td>0.0125</td>
</tr>
<tr>
<td>Dosing interval (hr)</td>
<td>8</td>
<td>8, 24</td>
</tr>
<tr>
<td>Enzyme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{act}$ (min⁻¹)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>$K_i$ (g/g/mL)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>$k_{degr}$ (min⁻¹)</td>
<td>0.0003</td>
<td></td>
</tr>
</tbody>
</table>

$V_d$ volume of the body; $CL_{int, s}$ body clearance; $f_b$ unbound fraction in blood; $V_H$ volume of the liver; $Q_H$ hepatic blood flow rate; $CL_{int, H}$ hepatic intrinsic clearance; $k_i$ absorption rate constant; $k_{act}$ inactivation rate constant of the enzyme; $K_i$ apparent dissociation constant between the enzyme and inactivator; $k_{degr}$ degradation rate constant (turnover rate constant) of enzyme

$value in parenthesis indicates hepatic availability

$same value as in left column

Table 2. Ranges of pharmacokinetic parameters reported in the literature

<table>
<thead>
<tr>
<th>n</th>
<th>$k_i$ (min⁻¹)</th>
<th>$V_H$ (L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>155</td>
<td>—</td>
<td>19–357(129)</td>
<td>Goodman &amp; Gilman⁷⁹</td>
</tr>
<tr>
<td>34</td>
<td>0.006–0.042(0.016)</td>
<td>29–600(131)</td>
<td>Kato et al.⁷¹</td>
</tr>
<tr>
<td>9</td>
<td>0.011–0.033(0.019)</td>
<td>30–549(83)</td>
<td>Grime et al.⁸⁰</td>
</tr>
</tbody>
</table>

Logarithmically transformed pharmacokinetic parameters in the literature were analyzed, and results are expressed as the range of mean ± SD to mean ± SD with the mean value in parenthesis.

the literature are summarized in Table 2. To cover the range of absorption rate constants in Table 2, the absorption rate constant was set as an initial value of 0.002, and increased with a geometric ratio of 2.5 up to a fifth parameter (0.0781 min⁻¹) in calculation mode of sensitivity analysis. Given a maximum absorption rate constant of 0.1 min⁻¹,¹⁰ which is equivalent to gastric emptying time, a maximum absorption rate constant of 0.0781 min⁻¹ was considered sufficient for simulations. The minimum distribution volume was set at 15 L, considering the range of values in Table 2 and volume of interstitial fluid in the body.¹¹ When the volume of distribution in the body was set as an initial value of 15 L with a geometric ratio of 3 in calculation mode of sensitivity analysis, the fourth value was 405 L. This value was within the range of maximum values in Table 2. Intrinsic clearance in the body was set at 0 to 1200 mL/min, based on renal blood flow. Hepatic clearance was set to cover a variety of drugs with various hepatic availabilities from 0.26 to 0.91. The physiological parameters including liver volume and liver blood flow were obtained from published data.¹¹ MBI parameters including $k_{act}$, $K_i$, and $k_{degr}$ were determined considering data in the literature.¹² Case 2: To examine dosing regimen, two different conditions, once-a-day and three-times-a-day with the same daily dose, were examined. In this case, intrinsic clearance in the body and liver, absorption rate constant, and distribution volume were each fixed to one value within the ranges indicated for Case 1.

Simulations: Simulations were performed using SimPBPK in-house software¹³ to construct a PBPK model to generate the above differential equations internally and perform numerical integration based on the Runge-Kutta-Gill method. The interval of calculation for numerical integration was set by comparing the results of simulation with different intervals of 0.05, 0.1, 0.2, 0.5, and 1 min for several PBPK parameter sets. Since relative error between the result using calculation interval of 0.05 min and that of other calculation interval was less than 0.2%, 0.6%, 1.7%, and 4.9%, respectively, the interval for numerical integration was set at 0.1 min. The effect of output interval on the calculated concentrations was similarly examined, and the interval for output was set at 1 min. The final time point for simulation was set at 240 hr in order to attain steady-state with all PBPK parameter sets.

Steady-state maximum unbound systemic concentration ($I_{u, ss, max, b}$): Steady-state maximum concentrations in the body compartment were determined from the time course of change in body concentration for each simulation result and multiplied by the unbound fraction in the body to obtain the unbound concentrations.

Steady-state average unbound liver concentration: Steady-state average unbound liver concentration after oral administration ($I_{u, ss, ave, H}$) was defined as

$$I_{u, ss, ave, H} = \frac{f_b \cdot F_{hit} \cdot Dose}{\tau \cdot (CL_{H} + CL_{NH})} \left(1 + \frac{CL_{NH}}{Q_H}\right)$$

where $F_{hit}$, Dose, $\tau$, $CL_H$, and $CL_{NH}$ represent hepatic availability, dose, dosing interval, hepatic clearance, and extra-hepatic clearance, respectively. In the present PBPK model, $CL_{NH}$ was equal to $f_s \cdot CL_{int, b}$.

The maximum limit of steady-state average unbound liver concentration after oral administration ($I_{u, ss, limit, H}$) was obtained by eliminating $CL_{NH}$ in Equation 2 as follows:

$$I_{u, ss, limit, H} = \frac{Dose}{\tau \cdot CL_{int, H}}$$

The concentrations defined by Equations 2 and 3 were calculated using each set of pharmacokinetic parameters in Table 1.

Assessment of accuracy of prediction of MBI: The accuracy of prediction of MBI using the three con-
centrations indicated above was evaluated by comparing predicted change in enzyme activity with actual change.

Predicted fold decrease in enzyme activity at steady-state by MBI (RI) was calculated using Equation 1 and the three inactivator concentrations, \( I_{u, \min, 0}, I_{u, \max, 0} \) and \( I_{u, \text{sc, limit}} \).

Exact fold decrease in enzyme activity at steady-state by MBI (RE) was calculated using initial enzyme concentration \( (E_0) \) and steady-state minimum enzyme concentration \( (E_{\text{ss}, 0}) \), which were determined from the time course of change in the enzyme compartment for each simulation result, as follows:

\[
RE = \frac{E_0}{E_{\text{ss}, 0}} 
\]

(4)

The accuracy of RI values was determined by calculating the ratio of RI to RE, and the effects of change in pharmacokinetic parameters on the ratio of RI to RE were examined.

**Results**

Accuracy of prediction of MBI using the steady-state maximum unbound systemic concentration: As shown in Figure 2, the relationship between the actual inactivation potential RI and estimated inactivation potential RI calculated by method 1 for 720 virtual drugs in Case 1 in Table 1 was found to vary significantly. The accuracy of prediction of MBI for method 1 was expressed as the ratio of RI to RE and summarized in Table 3. The ratio of RI to RE varied from 0.72 to 8.41. The effects of each pharmacokinetic parameter on the relationships between RI and RE are shown in Figure 3. The correlation between RI and RE improved with increase in the volume of distribution in the body. Neither change in intrinsic clearance of the body nor that in the liver appeared to influence the correlation between RI and RE. Change in intrinsic clearance of the body had slight effect on RE values, while that in liver shifted RE values significantly. With decrease in the absorption rate constant, the correlation between RE and RI improved. The relationships between accuracy of prediction of MBI expressed as the ratio of RI to RE and two pharmacokinetic parameters, the volume of distribution in the body and the absorption rate constant, are summarized in Table 4. With decrease in the volume of distribution and increase in the absorption rate constant, the ratio of RI to RE grew larger and the range of the ratio of RI to RE became broader.

Accuracy of prediction of MBI using the steady-state average unbound liver concentration: The relationships between RE and RI calculated using the steady-state average and the maximum limit of steady-state average unbound liver concentrations for 720 virtual drugs in Case 1 in Table 1 are shown in Figure 4, and the ratios of RI to RE are summarized in Table 3.

The correlation between RE and RI calculated using the steady-state average unbound liver concentrations was high, while that between RI and RE calculated using the maximum limit of steady-state average unbound liver concentrations was moderate. The range of the ratio of RI to RE was 0.71 to 1.46 with use of the former concentrations and 0.72 to 2.96 with use of the latter.

Effects of dosing regimen on accuracy of prediction of MBI: Simulations were performed with two different dosing regimens, once-a-day and three-times-a-day with the same daily dose (Case 2). Enzyme activity and concentration profiles in the body are shown in Figure 5. The decrease in enzyme activities at steady-state was similar for once-a-day and three-times-a-day administration, and RE values were 2.1 and 2.0, respectively. RI values calculated using the steady-state maximum unbound systemic concentrations were 5.3 and
Fig. 3. Effect of each pharmacokinetic parameter on the relationship between actual fold decrease in enzyme activity (RE) and estimated fold decrease in enzyme activity (RI) calculated using steady-state maximum unbound systemic concentration for all virtual drugs in Case 1 of Table 1. The line represents 1:1 correlation.

Table 4. Relationships between range of ratios of RI to RE and volume of distribution and absorption rate constant for virtual drugs in Case 1, with RE values smaller than 10

<table>
<thead>
<tr>
<th>$k_a$ (min$^{-1}$)</th>
<th>0.002</th>
<th>0.005</th>
<th>0.0125</th>
<th>0.03125</th>
<th>0.0781</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_a$ (L)</td>
<td>15</td>
<td>0.96–1.30</td>
<td>1.17–1.81</td>
<td>1.60–3.04</td>
<td>2.46–5.20</td>
</tr>
<tr>
<td>45</td>
<td>0.89–1.20</td>
<td>1.07–1.53</td>
<td>1.39–2.28</td>
<td>1.80–3.45</td>
<td>2.20–5.00</td>
</tr>
<tr>
<td>135</td>
<td>0.83–1.09</td>
<td>0.90–1.23</td>
<td>1.04–1.53</td>
<td>1.17–1.98</td>
<td>1.29–2.54</td>
</tr>
<tr>
<td>405</td>
<td>0.80–1.02</td>
<td>0.82–1.06</td>
<td>0.86–1.14</td>
<td>0.91–1.27</td>
<td>0.96–1.46</td>
</tr>
</tbody>
</table>

2.6, respectively, RI values calculated using the steady-state average unbound liver concentrations were 2.1 for both conditions.

Discussion

MBI has been thought to result in serious and complex problems, and many approaches have been used to estimate its effects. Among them, method 1 is considered suitable for predicting the effect of MBI in screening of drug candidates in pharmaceutical companies early in the drug development process. Determination of the characteristics of method 1 is thus important.

The purposes of this study were to evaluate the relationship between change in enzyme activity by MBI and $I_{u, u, max, B}$, and propose an alternative method for prediction of the effects of MBI using $I_{u, u, ave, H}$ and $I_{u, u, limit, H}$. Elucidation of these relationships must include evaluation of the relationships between these concentrations and pharmacokinetic parameters. Since these three concentrations are similarly affected by change in each organ volume, as by change in each organ clearance, a general multi-organ PBPK model was not considered required for evaluation of the effects of these pharmacokinetic parameters. Moreover, since pre-systemic exposure occurs after oral administration and MBI takes place in the liver, we constructed a simple PBPK model including body (systemic) and liver compartments.

When the inactivator concentration is high compared to $K_i$ in Equation 1, RI becomes constant. The accuracy
of estimation of $RI$ by method 1 is thus high with high inactivator concentrations. In Figure 3, this applies within the region where $RE$ values are larger than 30. Because a large $RE$ value is not itself a proper characteristic of a drug, the accuracy of prediction of MBI should be discussed for the results obtained with low $RE$ values. The accuracy of method 1 expressed as the ratio of $RI$ to $RE$ varied widely from 0.80 to 8.41 in the case of smaller $RE$ values less than 10. It is thus essential to determine when method 1 provides accurate estimates of MBI.

If mean values with variance of each parameter and co-variance between parameters are available, Monte Carlo simulation may be the proper method. Unfortunately, the number of pharmacokinetic parameter sets for commercial drugs is still insufficient, and we therefore performed simulations using sensitivity analysis mode with each pharmacokinetic parameter altered in stepwise fashion so as to cover the ranges of representative drugs on the market. It should therefore be noted that the incidence of existence of virtual drugs is not the same as those for marketed drugs. For $CL_{int,b}$, we used values of 0, 600, and 1200 mL/min, which were considered to be within the range of possible values. However, the number of drugs which have $CL_{int,b}$ values larger than 1200 mL/min is small. As shown in Figure 3, change in $CL_{int,b}$ value had slight effects on the maximum value of the range of prediction, and the likelihood of underestimation probably increases as with increase in $CL_{int,b}$. Therefore, the possibility of underestimation of MBI by method 1 is considered small. When the estimated fold decrease in enzyme activity determined by method 1 is close to 1, the drug is unlikely to cause MBI. Method 1 is thus likely to avoid false-negative findings. In this study, we assumed that elimination of an inactivator is not altered by MBI of the target enzyme. However, when the target enzyme of MBI is involved in the main route of elimination of the inactivator, the fold decrease in enzyme activity becomes larger. In such cases the extent of overestimation by method 1 should thus be smaller.

Because change in dosing regimen affects the concentration used in method 1 and that defined by Equation 2 differently, we performed simulations using two dosing regimens (Fig. 5). Since the systemic concentration for once-a-day administration is about three times that for three-times-a-day administration, method 1 provided two different $RI$ values, and the $RI$ value for once-a-day administration was especially different from the $RE$ values. Since the concentrations defined by Equation 2 were identical for the two different dosing conditions, they yielded the same $RI$ values. Since the increase in differ-
ence between the maximum systemic concentration and average liver concentration due to the decrease in number of daily doses is the cause of overestimation of MBI effect, it appears that method 1 should be applied with care in cases with small numbers of daily doses, and that further characterization of the features of method 1 is needed.

The accuracy of prediction using the concentrations define by Equation 2 was very high, with a ratio of RI to RE that ranged from 0.90 to 1.04, in the case of RE values less than 10 (Table 3). This suggests that it is not necessary to perform PBPK model based simulations for estimation of the change in steady-state enzyme activity due to MBI. The accuracy of prediction using the concentrations defined by Equation 3 ranged from 0.92 to 2.96, and thus appears to exceed 2 in some cases. This situation occurred with drugs with the common pharmacokinetic characteristics of small hepatic clearance and large body clearance. Equation 3 should thus be applied with care in cases in which extra-hepatic clearance is predominant. Sekiguchi et al. succeeded in predicting the clinical effects of MBI of some drugs with use of Equation 1 and steady-state average unbound systemic concentration.20) This concentration was considered equivalent to the concentration defined by Equation 3 under their assumptions. Equation 3 thus appears useful.

Early in the drug development process, hepatic clearance can be estimated using the results of metabolic stability screening tests and MBI parameters, such as $K_I$ and $k_{\text{inact}}$, can be obtained through the enzyme inhibition screening test. Although the clinical dose is not available, we can use the estimated clinical dose obtained by converting the effective animal dose based on body surface area. With these parameters, we can estimate the effect of MBI using Equation 3 and Equation 1. When extra-hepatic clearance is negligible, the concentration defined by Equation 3 is equivalent to a steady-state average unbound systemic concentration, which is calculated by dividing the unbound systemic AUC in an animal pharmacological model by dosing interval. This can be substituted for the concentration defined by Equation 3 to predict the effect of MBI using Equation 1, when pharmacological activity appears to depend on AUC. Since, as noted above, there may be cases in which the accuracy of prediction using Equation 3 decreases, characteristics such as physicochemical properties and pharmacokinetic profiles in animals which may suggest the existence of extra-hepatic elimination must be considered. As the non-clinical stage advances, estimation of non-hepatic clearance and protein binding tests will be carried out, and estimation using Equation 2 and Equation 1 can be performed for precise estimation of MBI. This approach thus appears to be useful early in the drug development process.

In conclusion, we have shown that the accuracy of method 1 mainly depends on the volume of distribution, the absorption rate constants, and dosing interval. When the volume of distribution is large, the absorption rate constants are small, or dosing interval is short, method 1 can predict the effect of MBI relatively accurately. We propose use of the steady-state average unbound liver concentration and maximum limit of steady-state average unbound liver concentration as inactivator concentrations. Prediction of MBI using these two concentrations is more accurate than the result obtained with method 1. The strategy of predicting MBI using these two concentrations in stepwise fashion according to stage of development should improve determination of the effects of MBI early in the drug development process.

References


