Quantitative Prediction of *In Vivo* Drug-Drug Interactions from *In Vitro* Data Based on Physiological Pharmacokinetics: Use of Maximum Unbound Concentration of Inhibitor at the Inlet to the Liver

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Purpose. To assess the degree to which the maximum unbound concentration of inhibitor at the inlet to the liver ($I_{inlet,u,max}$), used in the prediction of drug-drug interactions, overestimates the unbound concentration in the liver.

Methods. The estimated value of $I_{inlet,u,max}$ was compared with the unbound concentrations in systemic blood, liver, and inlet to the liver, obtained in a simulation study based on a physiological flow model. As an example, a tolbutamide/sulfaphenazole interaction was predicted taking the plasma concentration profile of the inhibitor into consideration.

Results. The value of $I_{inlet,u,max}$ differed from the concentration in each compartment, depending on the intrinsic metabolic clearance in the liver, first-order absorption rate constant, non-hepatic clearance and liver-to-blood concentration ratio (Kp) of the inhibitor. The AUC of tolbutamide was predicted to increase 4-fold when co-administered with sulfaphenazole, which agreed well with in vivo observations and was comparable with the predictions based on a fixed value of $I_{inlet,u,max}$. The blood concentration of tolbutamide was predicted to increase when it was co-administered with as little as 1/100 of the clinical dose of sulfaphenazole.

Conclusions. Although $I_{inlet,u,max}$ overestimated the unbound concentration in the liver, the tolbutamide/sulfaphenazole interaction could be successfully predicted by using a fixed value of $I_{inlet,u,max}$, indicating that the unbound concentration of sulfaphenazole in the liver after its clinical dose is by far larger than the concentration to inhibit CYP2C9-mediated metabolism and that care should be taken when it is co-administered with drugs that are substrates of CYP2C9.

KEY WORDS: drug interaction; prediction; physiologically-based pharmacokinetics; tolbutamide, sulfaphenazole.

INTRODUCTION

The possible sites of drug-drug interactions which can change pharmacokinetic profiles include: (1) gastrointestinal absorption, (2) plasma and/or tissue protein binding, (3) carrier-mediated transport across plasma membranes (including hepatic or renal uptake and biliary or urinary secretion), and (4) metabolism. Drug-drug interactions caused by inhibition of metabolic enzymes are clinically the most common and are particularly important. We have proposed the following methodology to quantitatively predict such drug-drug interactions *in vivo* from *in vitro* data (1,2).

In clinical situations, the substrate concentration is usually much lower than Km, and the degree of inhibition (R = AUC(+ inhibitor)/AUC(control)) is expressed as R = 1 + Iu/Ki in the cases of competitive and non-competitive inhibition of the enzyme, where Iu represents the unbound concentration in the liver and Ki represents the inhibition constant of the inhibitor. In order to avoid a false negative prediction, due to underestimation of Iu, the plasma unbound concentration at the inlet to the liver, where the blood flow from the hepatic artery and portal vein meet, was considered to be the maximum value of Iu and was used for prediction. *In vivo* drug-drug interactions were quantitatively predicted by comparing the maximum value of the unbound concentration at the inlet to the liver ($I_{inlet,u,max}$) estimated using pharmacokinetic data and the value of Ki obtained *in vitro* (1,2). However, because the concentration of the inhibitor is actually changing with time, predictions using a fixed value of $I_{inlet,u,max}$ may result in substantial overestimation of the *in vivo* interactions. In the present work, simulation studies have been carried out in order to understand to what degree and under what conditions the $I_{inlet,u,max}$ mentioned above overestimates the unbound concentrations in the liver estimated from a physiological flow model.

Furthermore, as an example, a case of tolbutamide/sulfaphenazole interaction was quantitatively predicted by considering the concentration profile of sulfaphenazole. Sulfaphenazole is reported to increase the AUC of tolbutamide, an antidiabetic, up to 5-fold by inhibiting the CYP2C9-mediated metabolism of tolbutamide (3). Inhibition of plasma protein binding was also taken into consideration in the prediction model because it is also reported to be involved in this interaction (4).

METHODS

Evaluation of the Estimation Error of the Inhibitor Concentration

The pharmacokinetics of the inhibitor after oral administration was assumed to be characterized by the perfusion model shown in Fig. 1 and the pharmacokinetic parameters listed in Table 1. The mass-balance equations for the inhibitor can be expressed as follows:

$$V_{liver} \cdot (dI_{liver}/dt) = Qh \cdot I_{inlet} - Qh \cdot I_{liver}/Kp - fb \cdot CL_{int} \cdot I_{liver}/Kp \quad (1)$$

$$V_{inlet} \cdot (dI_{inlet}/dt) = Qh \cdot I_{sys} + v_{abs} - Qh \cdot I_{inlet} \quad (2)$$

$$v_{abs} = ka \cdot D \cdot Fa \cdot e^{-ka \cdot t} \quad (3)$$

$$V_{sys} \cdot (dI_{sys}/dt) = Qh \cdot I_{liver}/Kp - Qh \cdot I_{sys} - CL_{NH} \cdot I_{sys} \quad (4)$$

where V_{liver} and V_{inlet} represent the volume of liver and inlet to the liver (designated as "inlet"), respectively; V_{sys} represents the volume of distribution in the central compartment; I_{liver}, I_{inlet}, and I_{sys} represent the concentration in the liver, inlet, and

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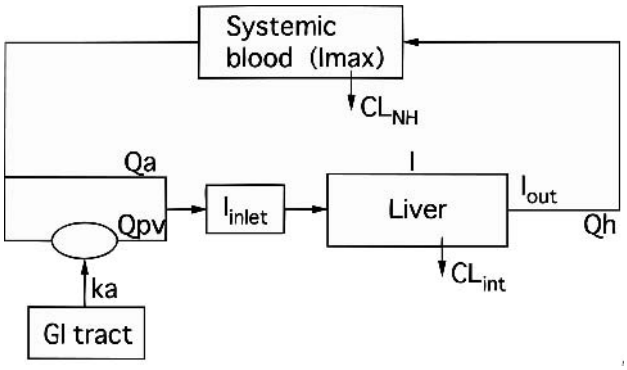

Fig. 1. Model for estimating the concentration of the inhibitor at the inlet to the liver after oral administration (I_{inlet}). I_{out}, I, and Imax represents the inhibitor concentration at the exit of the liver (hepatic vein side), the inhibitor concentration at the liver capillary, and maximum inhibitor concentration in the systemic circulation, respectively. Qa, Qpv and Qh (=Qa + Qpv) represents the blood flow at the hepatic artery, portal vein, and hepatic vein, respectively.

central compartment, respectively; Qh represents the blood flow rate; Kp represents the liver-to-blood concentration ratio; fb represents the unbound fraction in blood; CL_{int} represents the intrinsic metabolic clearance; CL_{NH} represents the non-hepatic clearance; v_{abs} represents the absorption velocity; ka represents the first-order absorption rate constant; and Fa represents the fraction absorbed from the gastrointestinal tract.

The following assumptions have been made in the above mass-balance equations:

(1) The inhibitor is orally administered.

(2) Distribution of the inhibitor in the liver rapidly reaches equilibrium and the unbound concentration in the hepatic vein is equal to that in the liver at equilibrium (Well-stirred model).

(3) Gastrointestinal absorption can be described by a first-order rate constant.

Using the parameters listed in Table 1, the above differential equations were numerically solved by STELLA II (High Performance Systems, Inc.) to simulate the concentration profiles of the inhibitor in the systemic blood, inlet and liver.

Values of ka, CL_{int} and CL_{NH} were changed in the simulation as follows: ka = 0.0003 − 0.1 min^{-1}, CL_{int} = 3 − 300 mL/min/kg, CL_{NH} = 0.6 − 600 mL/min/kg.

Table 1. Parameters for Simulation in Humans (70 kg)

Inhibitor	Physiological parameters
Dose = 1000 μg/kg	Qh = 30 mL/min/kg
Fa = 1	V_{liver} = 40 mL/kg
fb = 1	V_{sys} = 200 mL/kg
ka = 0.03 min^{-1}	V_{inlet} = 1 mL/kg
CL_{int} = 30 mL/min/kg	
CL_{NH} = 2 mL/min/kg	
Kp = 1	

Note: Qh: blood flow rate, V_{sys}: volume of distribution in the central compartment, V_{inlet}: volume of the inlet to the liver, V_{liver}: volume of liver, fb: unbound fraction in blood, ka: first-order absorption rate constant, CL_{int}: intrinsic metabolic clearance, CL_{NH}: non-hepatic clearance, Fa: fraction absorbed from the gastrointestinal tract, Kp: liver-to-blood concentration ratio.

The effect of the Kp value of the inhibitor was simulated by changing the Kp value from 0.2 to 30, assuming either a change in protein binding in the liver or active transport of the inhibitor into the liver. In the former case, the unbound concentration of the inhibitor in the liver is equal to that in the hepatic vein while, in the latter, the unbound concentration in the liver is obtained as that in the hepatic vein multiplied by Kp.

On the other hand, according to the model in Fig. 1, the maximum unbound concentration of the inhibitor at the inlet to the liver ($I_{inlet,u,max}$) was estimated by fb · (Imax+ka·D·Fa/Qh) as described elsewhere (1,2), in which the simulated maximum concentration in the systemic blood was used as Imax.

Quantitative Prediction of the Tolbutamide/Sulfaphenazole Interaction

Using a perfusion model, the differential equations for tolbutamide and sulfaphenazole can be expressed as follows:

For tolbutamide,

$$V_{liver} \cdot (dC_{liver}/dt) = Qh \cdot C_{inlet} - Qh \cdot C_{liver}/Kp - fb \cdot CL_{int} \cdot C_{liver}/Kp \quad (5)$$

(Inhibition of metabolism)

$$CL_{int} = CL_{int,1} + CL_{int,2} = \frac{Vmax}{Km,app + fb \cdot C_{liver}/Kp} + CL_{int,2} \quad (6)$$

$$Km,app = Km \cdot \left(1 + \frac{fb(I) \cdot C_{liver}(I)/Kp(I)}{Ki}\right) \quad (7)$$

(Inhibition of plasma protein binding)

$$fb = \frac{1}{R_B} \cdot \frac{1}{1 + \dfrac{n(Pt)}{Kd \cdot \left(1 + \dfrac{fb(I) \cdot C_{liver}(I)/Kp(I)}{Ki^*}\right) + Cu}} \quad (8)$$

$$Cu = \frac{-Kd - n(Pt) + C_{liver}/Kp/R_B + ((Kd + n(Pt) - C_{liver}/Kp/R_B)^2 - 4 \cdot Kd \cdot C_{liver}/Kp/R_B)^{1/2}}{2} \quad (9)$$

$$V_{inlet} \cdot (dC_{inlet}/dt) = Qh \cdot C_{sys} + v_{abs} - Qh \cdot C_{inlet} \quad (10)$$

$$v_{abs} = ka \cdot D \cdot Fa \cdot e^{-ka \cdot t} \quad (11)$$

$$V_{sys} \cdot (dC_{sys}/dt) = Qh \cdot C_{liver}/Kp - Qh \cdot C_{sys} - CL_{NH} \cdot C_{sys} \quad (12)$$

For sulfaphenazole,

$$V_{liver} \cdot (dI_{liver}/dt) = Qh \cdot I_{inlet} - Qh \cdot I_{liver}/Kp - fb \cdot CL_{int} \cdot I_{liver}/Kp \quad (13)$$

$$CL_{int} = Vmax/(Km + fb \cdot I_{liver}/Kp) \quad (14)$$

$$V_{inlet} \cdot (dI_{inlet}/dt) = Qh \cdot I_{sys} + v_{abs} - Qh \cdot I_{inlet} \quad (15)$$

$$v_{abs} = ka \cdot D \cdot Fa \cdot e^{-ka \cdot t} \quad (16)$$

$$V_{sys} \cdot (dI_{sys}/dt) = Qh \cdot I_{liver}/Kp - Qh \cdot I_{sys} - CL_{NH} \cdot I_{sys} \quad (17)$$

where V_{liver} and V_{inlet} represent the volume of liver and inlet to the liver, respectively; V_{sys} represents the volume of distribution in the central compartment; C_{liver} and I_{liver} represent the concentration in the liver; C_{inlet} and I_{inlet} represent the concentration at the inlet; C_{sys} and I_{sys} represent the concentration in the central compartment; Qh represents the blood flow rate; Kp represents the liver-to-blood concentration ratio; fb represents the unbound fraction in blood; CL_{int} represents the intrinsic metabolic clearance; CL_{NH} represents the non-hepatic clearance; $CL_{int,1}$ represents the CL_{int} for CYP2C9 metabolism; $CL_{int,2}$ represents the CL_{int} for non-CYP2C9 metabolism; Km represents the Michaelis-Menten constant; Vmax represents the maximum rate of metabolism; Ki represents the inhibition constant for metabolism; R_B represents the blood-to-plasma concentration ratio; n(Pt) represents the total number of binding sites; Kd represents the dissociation constant; Ki* represents the inhibition constant for protein binding; Cu represents the unbound concentration in plasma; v_{abs} represents the absorption velocity; ka represents the first-order absorption rate constant; and Fa represents the fraction absorbed from the gastrointestinal tract.

The following assumptions have been made in the above mass-balance equations for tolbutamide and sulfaphenazole:

(1) The hepatic elimination of both tolbutamide and sulfaphenazole can be described by the Michaelis-Menten equation.

(2) The distribution of both tolbutamide and sulfaphenazole in the liver rapidly reaches equilibrium and the unbound concentrations in the hepatic vein are equal to those in the liver at equilibrium (Well-stirred model).

(3) Only the unbound form in the liver is involved in the elimination.

(4) Gastrointestinal absorption can be described by a first-order rate constant.

(5) Inhibition of tolbutamide metabolism by sulfaphenazole is based on competitive inhibition of metabolism mediated by CYP2C9 and the contribution of CYP2C9 to tolbutamide metabolism in the liver under the linear condition is 80% in the absence of sulfaphenazole ($CL_{int,1}/(CL_{int,1} + CL_{int,2}) = 0.8$) (5).

(6) Tolbutamide binds to a single site on the plasma protein and this binding is competitively inhibited by sulfaphenazole.

Pharmacokinetic parameters of tolbutamide and sulfaphenazole were determined to describe well the blood concentration profiles after administration of each drug alone (Tables 2 and 3) (3,15). The parameters for inhibition of metabolism and plasma protein binding used in the simulation are shown in Table 4.

Using the parameters in Tables 2–4, the above equations were solved numerically by STELLA II to simulate the effect of sulfaphenazole co-administration on the concentration profile of oral tolbutamide (dose: 500 mg). Sulfaphenazole was assumed to be co-administered orally at doses ranging from 0.5 to 500 mg.

Furthermore, the simulation was also carried out by fixing the concentration of sulfaphenazole at the maximum unbound concentration at the inlet ($I_{inlet,u,max}$), which was estimated by fb· (Imax + ka· D· Fa/Qh). Using the maximum concentration in the systemic blood obtained by the simulation as Imax, the values of $I_{inlet,u,max}$ were calculated to be 13.1, 1.31, 0.131 and 0.0131 μg/mL after sulfaphenazole doses of 500, 50, 5 and 0.5 mg, respectively.

RESULTS

Evaluation of the Estimation Error of the Inhibitor Concentration

Effect of CL_{int}

Figure 2 shows the simulated profiles of the unbound concentration of the inhibitor in the systemic blood, inlet and liver. The estimated maximum unbound concentration at the inlet ($I_{inlet,u,max}$), calculated as fb· (Imax + ka· D· Fa/Qh), is also shown. Although the value of $I_{inlet,u,max}$ was higher than the maximum unbound concentration at the inlet obtained by the simulation, the difference was not very great. The error tended to be large when the CL_{int} value was low, and was 1.6 times when CL_{int} = 3 or 30 mL/min/kg and 1.3 times when CL_{int} = 300 mL/min/kg. Furthermore, $I_{inlet,u,max}$ also overestimated the unbound concentration in the liver which actually participated in the inhibition of metabolism. This error tended to be large when CL_{int} value was high, and the error at the maximum liver concentration was 1.5 times when CL_{int} = 3 mL/min/kg, 2.6 times when CL_{int} = 30 mL/min/kg and 11 times when CL_{int} = 300 mL/min/kg.

Effect of ka, CL_{int} and CL_{NH}

Figure 3 shows the results of simulation studies changing the values of ka, CL_{int} and CL_{NH}.

When the ka value was changed, $I_{inlet,u,max}$ overestimated the maximum unbound concentration at the inlet obtained by the simulation, but both concentrations were closer when ka value became smaller (<0.001 min^{-1}). The error tended to increase with an increase in ka value, reaching a maximum of 1.6 times when ka = 0.1 min^{-1}. Furthermore, $I_{inlet,u,max}$ also overestimated the maximum unbound concentration in the liver. This error also tended to increase with an increase in the ka value, reaching a maximum of 2.9 times when ka = 0.1 min^{-1}.

When the CL_{int} value was changed, as described above, $I_{inlet,u,max}$ overestimated the maximum unbound concentration at the inlet obtained by the simulation and the error tended to increase as the CL_{int} value fell, the maximum error being 1.6 times. Furthermore, $I_{inlet,u,max}$ also overestimated the maximum unbound concentration in the liver and the error increased as the CL_{int} value increased, the maximum error being 11 times.

When the CL_{NH} value was changed, $I_{inlet,u,max}$ overestimated the maximum unbound concentration at the inlet obtained by the simulation and the error increased as the CL_{NH} value fell, the maximum error being 1.6 times. Furthermore, $I_{inlet,u,max}$ also overestimated the maximum unbound concentration in the liver and the error tended to increase as the CL_{NH} value fell, the maximum error being 2.6 times.

Effect of Kp

The Kp value was changed from 0.2 to 30 assuming a change in protein binding in the liver (Fig. 4 left) or active transport into the liver (Fig. 4 right).

Table 2. Pharmacokinetic Parameters of Tolbutamide

			Ref.
Fa	0.93	BA = Fa (because of low clearance)	(6)
fb	0.13	calculated from R_B = 0.71 (rabbit), fu[a] = 0.093	(7),(8)
Kp	0.184	in rabbits	(7)
V_{liver}	2800 mL		
V_{inlet}	70 mL		
Qh	1610 mL/min		(9)–(11)
CL_{int}	109 mL/min	calculated from CL_{oral} = 14.2 mL/min = fb × CL_{int}	(3)
$CL_{int,1}$	87.2 mL/min	CL_{int} × 0.8	(5)
$CL_{int,2}$	21.8 mL/min	CL_{int} × 0.2	(5)
Km	32.4 μg/mL		(12)
V_{max}	2820 μg/min	calculated from Km × $CL_{int,1}$	
ka	0.00866/min	calculated from Tmax = 4 hr, kel = 0.0952/hr[−1b]	(3)
V_{sys}	6600 mL	calculated using eq. (5)–(6) and (10)–(12) to fit the reported blood concentration (3).	
CL_{NH}	0 mL/min		(13)

[a] Fu represents the unbound fraction in plasma.
[b] Calculated from Tmax = *ln*(ka/kel)/(ka−kel).

Table 3. Pharmacokinetic Parameters of Sulfaphenazole

			Ref.
Fa	0.85		(14)
fb	0.32		(14)
Kp	1	assumed	
V_{liver}	2800 mL		
V_{inlet}	70 mL		
Qh	1610 mL/min		(9)–(11)
ka	0.0303/min	calculated from Tmax = 2 hr, kel = 0.0533/hr[−1a]	(15)
CL_{int}	30 mL/min	calculated using eq. (13)–(17) to fit the reported blood concentration (15).	
V_{sys}	4800 mL	calculated using eq. (13)–(17) to fit the reported blood concentration (15).	
CL_{NH}	0 mL/min		(15)

[a] Calculated from Tmax = *In*(ka/kel)/(ka−kel).

When the change in Kp value was due to a change in protein binding in the liver, $I_{inlet,u,max}$ overestimated the maximum unbound concentration at the inlet obtained by the simulation and the error remained almost constant over the range 1.5–1.7 times, independent of the Kp value. Furthermore, $I_{inlet,u,max}$ also overestimated the maximum unbound concentration in the liver and the error increased slightly as the Kp value fell, the maximum error being 4.3 times.

When the change in Kp value was due to active transport into the liver, $I_{inlet,u,max}$ overestimated the maximum unbound concentration at the inlet obtained by the simulation and the error increased as the Kp value fell, the maximum error being 1.6 times. In addition, $I_{inlet,u,max}$ also overestimated the maximum unbound concentration in the liver and the error increased as the Kp value fell, the maximum error being 7.5 times.

Table 4. Parameters for Inhibition of Metabolism and Protein Binding

			Ref.
Inhibition of metabolism			
Ki	0.0314 μg/mL		(12)
Inhibition of protein binding			
Kd	81 μg/mL	in rats	(4)
R_B	0.71	in rabbits	(4)
Ki*	102 μg/mL	in rats	(4)
n(Pt)	800 μg/mL	calculated using eq. (5)–(12) and fb = 0.13.	

Quantitative Prediction of the Tolbutamide/Sulfaphenazole Interaction

Figure 5 shows the simulated time-profiles of Iu/Ki following oral administration of sulfaphenazole. Using the simulated unbound concentration at the inlet to the liver as Iu, the value of Iu/Ki remained as high as about 10 even at 50 hr (ca. 7 $t_{1/2}$ of tolbutamide) after administration of 500 mg sulfaphenazole (Fig. 5(A)), while the corresponding value after 0.5 mg administration declined to about 0.01 (Fig. 5(D)). Similar profiles were obtained when the unbound concentration in the systemic blood or that in the liver was used as Iu (data not shown).

The simulated and the reported concentration profiles of tolbutamide after co-administration with sulfaphenazole (dose: 500 mg) are shown in Figs. 6(B) and 6(A), respectively. A 5.3-fold increase in both the AUC after oral administration (AUC_{po}) and the elimination half-life ($t_{1/2}$) of tolbutamide has been

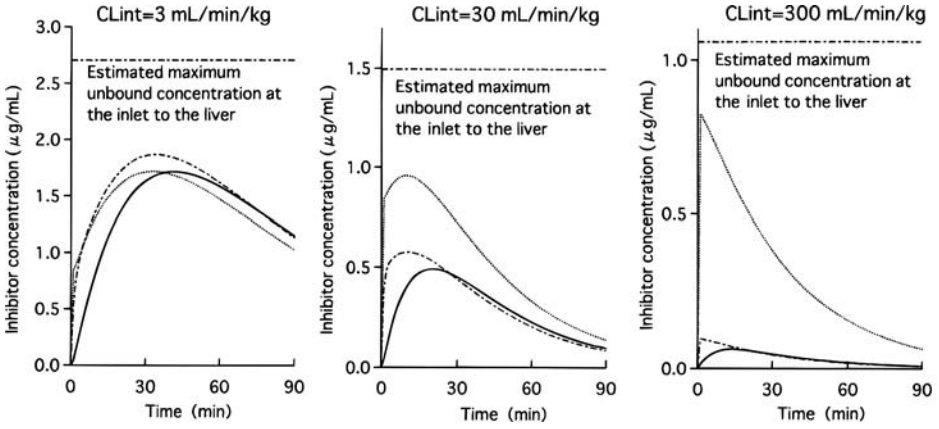

Fig. 2. Simulation of the concentration profiles of an inhibitor based on a physiological flow model (Fig. 1). ——: unbound concentration in the systemic blood, -----: unbound concentration at the inlet to the liver, – – –: unbound concentration in the liver. The basic parameters used in the simulation are listed in Table 1.

observed on co-administration of sulfaphenazole (Fig. 6(A)) (3). In the simulation study, the AUC_{po} and $t_{1/2}$ of tolbutamide was predicted to increase 4.1 and 4.4 times, respectively, using the fixed value of maximum unbound concentration of sulfaphenazole at the inlet ($I_{inlet,u,max}$). Considering the concentration profile of sulfaphenazole, the predicted increase was 4.2 and 4.6 times, respectively, which was comparable with the in vivo observations. Thus, the estimated concentration profile of tolbutamide, fixing the concentration of sulfaphenazole at $I_{inlet,u,max}$, was almost same as that taking into account the concentration profile of sulfaphenazole.

When the dose of sulfaphenazole was changed, the estimated concentration of tolbutamide fixing the concentration of sulfaphenazole at $I_{inlet,u,max}$ slightly overestimated the value considering the concentration profile of sulfaphenazole (Fig. 6(C)–(E)). When the dose of sulfaphenazole was low, a clear difference was observed after 15 hr post-administration, when the blood concentration of sulfaphenazole had declined. Even when the dose of sulfaphenazole was reduced by a factor of 100 to 5 mg, the AUC_{po} of tolbutamide was predicted to increase 2.6 times using a fixed concentration of sulfaphenazole and 1.7 times considering the concentration profile of sulfaphenazole.

DISCUSSION

As multiple drug therapy is now widely used in clinical practice, many drug-drug interactions involving metabolic inhibition are being reported when two or more drugs are administered concomitantly. It is helpful to know about changes in pharmacokinetic parameters such as hepatic clearance due to drug-drug interactions during multiple drug therapy, because these parameters are directly related to drug effects and adverse drug reactions. The use of human hepatic preparations has become possible in recent years and so the ability to predict *in vivo* drug interactions from *in vitro* studies using human preparations is certainly proving to be very useful.

Iu/Ki has been proposed as an important factor to predict the degree of *in vivo* drug interactions based on competitive or noncompetitive inhibition of drug metabolism (1,2,16,17). Although Iu should be the unbound concentration of inhibitor

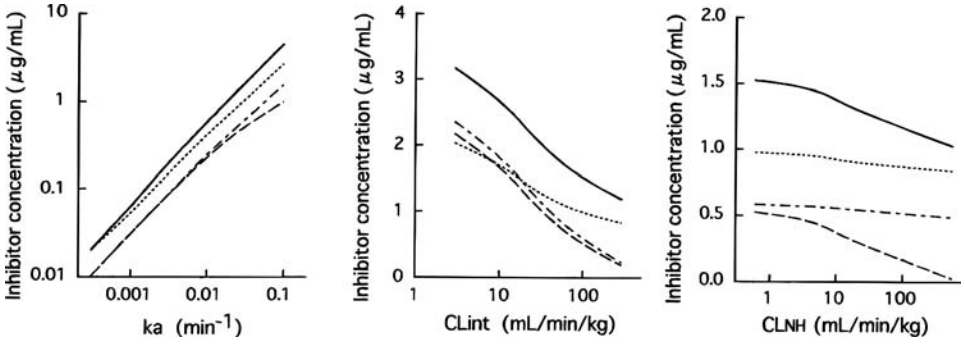

Fig. 3. Effects of the values of ka, CL_{int} and CL_{NH} on the estimated concentrations of an inhibitor. ——: estimated maximum unbound concentration at the inlet to the liver ($I_{inlet,u,max}$), -----: maximum unbound concentration at the inlet to the liver obtained by the simulation, – – –: maximum unbound concentration in the liver obtained by the simulation, – – –: maximum unbound concentration in the systemic blood obtained by the simulation. The basic parameters used in the simulation are listed in Table 1.

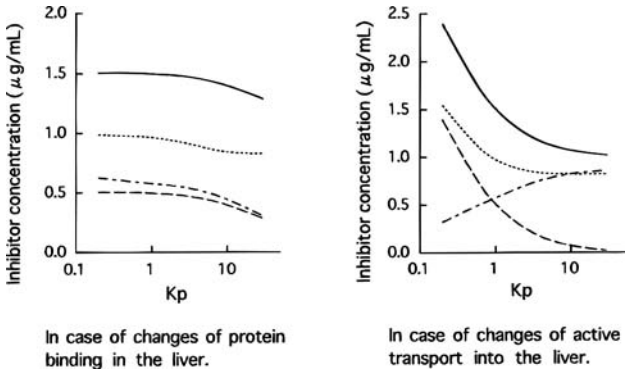

Fig. 4. Effects of the values of Kp on the estimated concentrations of an inhibitor. ——: estimated maximum unbound concentration at the inlet to the liver ($I_{inlet,u,max}$), -----: maximum unbound concentration at the inlet to the liver obtained by the simulation, –·–·-: maximum unbound concentration in the liver obtained by the simulation, –––: maximum unbound concentration in the systemic blood obtained by the simulation. The basic parameters used in the simulation are listed in Table 1.

around the metabolic enzyme in the liver, it is impossible to directly measure this *in vivo*. However, many drugs are transported into the liver by passive diffusion, allowing one to assume that the unbound concentration in the liver equals that in the liver capillary at steady-state. This means that estimating the unbound concentration of the inhibitor in the liver capillary may be enough for some drugs. In the case of drugs which are actively transported into the liver, the unbound concentration in the liver can be estimated by multiplying that in the liver capillary by the concentration ratio due to the active transport. In order to avoid a false negative prediction due to underestimation of Iu, the plasma unbound concentration at the inlet to the liver, where the blood flow from the hepatic artery and portal vein meet, was taken as the maximum value of Iu.

In the present study, the maximum unbound concentration of the inhibitor at the inlet ($I_{inlet,u,max}$) obtained as fb·(Imax + ka·D·Fa/Qh) was compared with the unbound concentration in the systemic blood, inlet and liver obtained by a simulation study based on a physiological flow model. The parameter, $I_{inlet,u,max}$, differed from the concentrations in each compartment depending on the pharmacokinetic parameters of the inhibitor such as intrinsic metabolic clearance in the liver, first-order absorption rate constant, non-hepatic clearance and Kp value (in case of changes in both protein binding in the liver and active transport into the liver). Over the range studied, the error of $I_{inlet,u,max}$ was within 1.7 times of the maximum unbound concentration at the inlet and 11 times of that in the liver. The value of $I_{inlet,u,max}$ never underestimated the unbound concentration in the liver for inhibitors with any pharmacokinetic parameters. As far as avoiding a false negative prediction is concerned, this finding suggests the validity of the prediction method of drug interactions based on the maximum value of Iu estimated by fb·(Imax + ka·D·Fa/Qh).

However, drug concentration in the body usually changes in a time-dependent manner. Taking the interaction between tolbutamide and sulfaphenazole as an example, the degree of overestimation of *in vivo* interactions by the above method was investigated by comparing the concentration profiles of

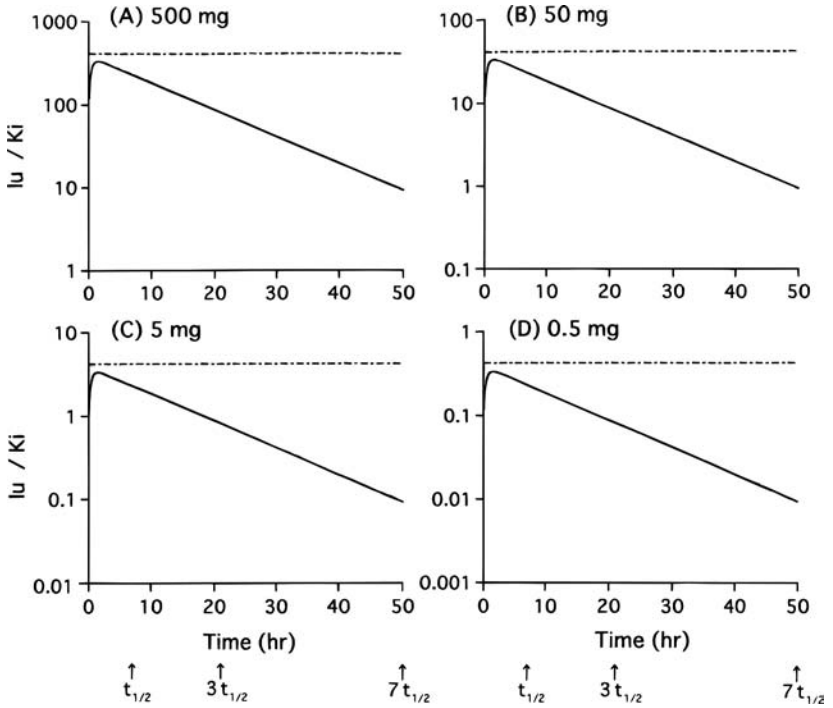

Fig. 5. Simulated profiles of Iu/Ki following oral administration of sulfaphenazole at a dose of (A) 500 mg, (B) 50 mg, (C) 5 mg, and (D) 0.5 mg. ——: using the simulated unbound concentration at the inlet to the liver as Iu, –·–·-: using a fixed value of $I_{inlet,u,max}$ as Iu. Arrows indicate $t_{1/2}$, 3 $t_{1/2}$, and 7 $t_{1/2}$ of tolbutamide administered alone.

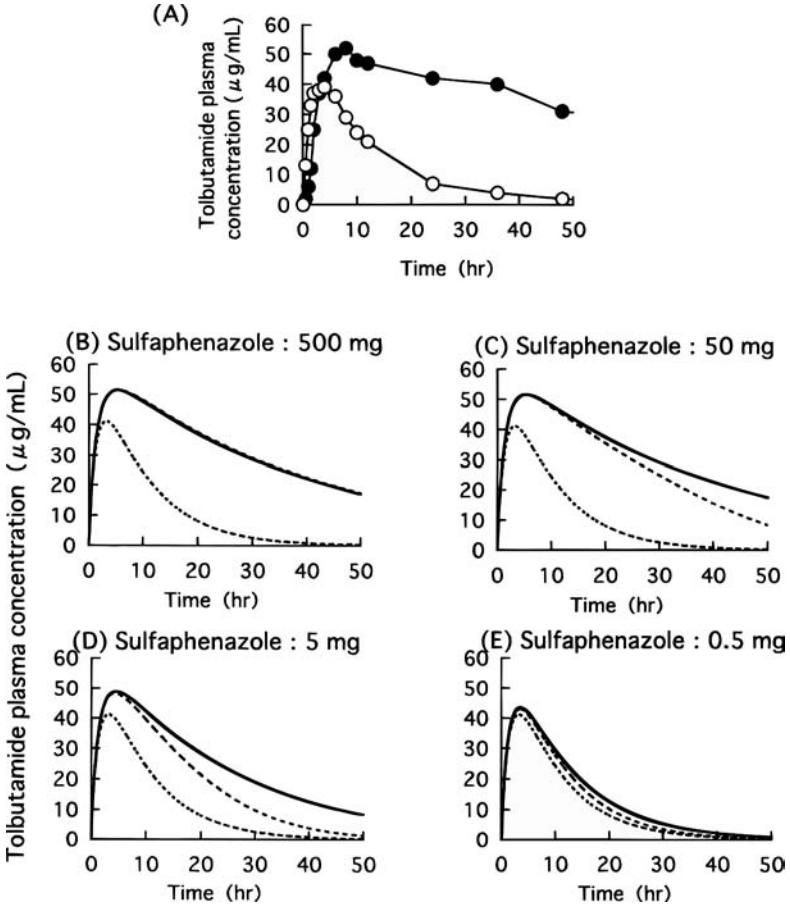

Fig. 6. Prediction of the interaction between tolbutamide and sulfaphenazole. Panel (A) represents the plasma concentration profiles of tolbutamide in humans reported by Veronese *et al.* (3). Open circles: control, closed circles: co-administration of sulfaphenazole (500 mg, b.i.d.). Panels (B), (C), (D), and (E) represents the simulated effect of co-administration of sulfaphenazole at a dose of 500, 50, 5, and 0.5 mg, respectively, on the plasma concentration profile of tolbutamide. -----: control, −−−−: co-administration of sulfaphenazole, ——: simulation using a fixed concentration of sulfaphenazole ($I_{inlet,u,max}$).

tolbutamide simulated using a fixed maximum Iu value of sulfaphenazole and those simulated by taking into consideration the concentration profile of sulfaphenazole.

In clinical situations, it is known that interactions between tolbutamide and sulfa-agents cause serious side-effects such as hypoglycaemic shock (18). Veronese *et al.* (3) reported about 5-fold increase from 587 ng·hr/mL to 3100 ng·hr/mL in AUC_{po} and from 7.3 hr to 38.8 hr in the $t_{1/2}$ of tolbutamide at a dose of 500 mg in humans following co-administration of 500 mg sulfaphenazole (Fig. 6(A)). The main metabolic pathway of tolbutamide in vitro is CYP2C9-mediated hydroxylation, and the metabolite undergoes sequential metabolism forming a carboxylate *in vivo* (6,19). Because sulfaphenazole is a specific inhibitor of CYP2C9, it is reasonable that metabolic inhibition should be involved in the increase in the AUC_{po} of tolbutamide. The Ki of sulfaphenazole for tolbutamide methyl hydroxylation in human liver microsomes *in vitro* is reported to be 0.0314 μg/mL (12). The $I_{inlet,u,max}$ of sulfaphenazole, estimated by fb·(Imax + ka·D·Fa/Qh), is 13.1 μg/mL and $I_{inlet,u,max}$/Ki is calculated to be 417. Sulfaphenazole also inhibits the plasma protein binding of tolbutamide, resulting in about a 3-fold increase in fb (18). However, the inhibition of metabolism is considered to be almost complete in terms of the product of fb and CL_{int} as the extent of inhibition of CL_{int} is much greater than that of plasma protein binding. The contribution of the CYP2C9-related metabolic pathway of tolbutamide is about 80% of the total elimination (5). Therefore, complete inhibition of this metabolic pathway leads to an 80% reduction in CL_{int}, and the AUC_{po} is predicted to be 5 times larger than the control value, which is consistent with the observed increase (2).

In the present simulation study, the concentration profile of tolbutamide predicted by fixing the sulfaphenazole concentration at the maximum value was comparable with that predicted by considering the concentration profile of sulfaphenazole. Both results showed a marked increase in tolbutamide concentration compared with the control value when no sulfaphenazole was co-administered, and this agreed well with the *in vivo* observations (Fig. 6(A), (B)). A reason for the successful prediction of tolbutamide concentrations, even by fixing the sulfaphenazole concentration at the overestimated maximum

value, is the following: at a sulfaphenazole dose of 500 mg, the value of Iu/Ki is high enough to completely inhibit the CYP2C9-mediated metabolism, whether the sulfaphenazole concentration is fixed or the concentration profile is considered (Fig. 5(A)). That is, the Iu/Ki values are approximately 230, 80, and 9 at 7 hr ($t_{1/2}$ of tolbutamide), 21 hr (3 $t_{1/2}$) and 50 hr (7 $t_{1/2}$), respectively, at this dose of sulfaphenazole. In such a situation, the concentration profile of tolbutamide depends on the clearance which is not related to CYP2C9, and it seems to be accurately estimated in the present prediction study.

When the dose of sulfaphenazole was decreased, the prediction fixing the sulfaphenazole concentration at the maximum value overestimated the interaction (Fig. 6(C)–(E)). In other words, no difference was observed between the results of both predictions in the case of a high value of Iu/Ki, while the prediction fixing the concentration at the maximum value resulted in overestimation in the case of a low value of Iu/Ki (Fig. 5). Even if the dose of sulfaphenazole was reduced to 5 mg, which corresponds to 1/100 of the therapeutic dose, a 2-fold increase in AUC_{po} of tolbutamide was predicted, indicating that sulfaphenazole is a very strong inhibitor of CYP2C9. Some sulfa-agents are still in clinical use and so care should be taken when co-administering them with substrates of CYP2C9 such as tolbutamide, phenytoin, and warfarin.

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