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Research Paper

Prediction of the Effects of Genetic Polymorphism on the Pharmacokinetics of CYP2C9 Substrates from *In Vitro* Data

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Purpose. The **2* and **3* alleles of CYP2C9, with decreased enzymatic activity, are highly polymorphic and contribute to inter-individual differences in pharmacotherapy of CYP2C9 substrates. Here, we sought for a simplified theoretical method to predict the pharmacokinetic changes with minimal *in vivo* data.
Methods. The changes in clearances of CYP2C9 substrates in subjects with these alleles were quantitatively estimated by parameters from literature data: intrinsic metabolic clearance and the enzyme expression level of mutated CYP2C9, contribution of CYP2C9 to the CYP-mediated clearance (f_{m2C9}), and the contribution of the dominant metabolic pathways to the total clearance (f_h). To validate the accuracy of our prediction, the changes were compared to reported *in vivo* values.
Results. Sufficient data were available for nine substrates: celecoxib, diclofenac, *S*-flurbiprofen, losartan, *S*-phenprocoumon, phenytoin, tolbutamide, torsemide, and *S*-warfarin. These predicted values, either using the intrinsic clearance specific to each substrate, or the averaged values (*2: 0.66, *3: 0.13, (ratio to *1)), correlated well with observed values (r^2=0.812, 0.786, respectively).
Conclusions. This theoretical method well estimated the quantitative changes in pharmacokinetics of CYP2C9 substrates in subjects with mutated alleles of CYP2C9. This can be applied to drug development even from the early clinical phases.

KEY WORDS: CYP2C9; drug development; prediction; *in vitro-in vivo* extrapolation; polymorphism.

INTRODUCTION

CYP2C9 is one of the major cytochrome P450 enzymes for phase I metabolism of several kinds of clinically used drugs, and the expression level of CYP2C9 in human liver is reported to be the second highest among CYP isoforms (1,2). Drugs that are substrates of CYP2C9 consist of more than 10–20% of all marketed drugs; the largest fraction next to CYP3A4 (3–5). CYP2C9 is one of the well-known polymorphic enzymes and the pharmacokinetics of CYP2C9 substrate drugs such as tolbutamide and phenytoin show multimodal distributions, suggesting the existence of extensive and poor metabolizers in the population (6,7). The major mutant alleles are **2* (Arg144Cys) and **3* (Ile359Leu), and previous reports have indicated that these alleles decrease the enzymatic activity (8–10). The frequencies of these mutations show racial differences. The frequencies of **1/*1*, **1/*2*, **1/*3*, **2/*2*, **2/*3*, and **3/*3* in Caucasians are 65.3%, 20.4%, 0.9%, 11.6%, 1.4%, and 0.4%, respectively, whereas the diplotypes of CYP2C9 in Asians are mainly composed of **1/*1* and **1/*3*, whose frequencies are 96.5% and 3.5%, respectively (11). These indicate that, especially in Caucasians, we must pay attention to the fact that subjects with **2* or **3* mutant alleles show increased systemic exposure to CYP2C9 substrate drugs and decreased exposure to active metabolites (e.g., E3174 (major active metabolite of losartan)), which sometimes modify subsequent pharmacodynamic and toxicological effects. For example, prolonged bleeding time and increased incidence of severe bleeding in warfarin therapy (12), higher possibility of low blood sugar levels during glipizide and tolbutamide therapy (13), and more frequent symptoms of overdose in phenytoin therapy (14) are reported in poor metabolizers. Therefore, the genetic polymorphisms in

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ABBREVIATIONS: ActR, enzymatic activity ratio; $Ae_{met,bile}$, amount of metabolites excreted into bile; $Ae_{met,urine}$, amount of metabolites excreted into urine; BA, bioavailability; $CL_{h,int}$, intrinsic hepatic clearance; CL_{int}, intrinsic clearance; CL_{oral}, oral clearance; CYP, cytochrome P450; ExpR, enzyme expression level ratio; Fa, fraction absorbed from the gastrointestinal tract; Fg, fraction escaped from intestinal metabolism; Fh, hepatic availability; f_h, contribution of specific metabolic pathway to total drug clearance; f_{m2C9}, the contribution of CYP2C9 in the total hepatic intrinsic clearance of wild-type subjects; f_{uB}, protein unbound fraction in blood; K_m, Michaelis constant; PD, pharmacodynamic; PK, pharmacokinetic; RAF, relative activity factor; Rb, blood-to-plasma concentration ratio; VKORC, vitamin K epoxide reductase complex; V_{max}, maximum velocity.

CYP2C9 and the racial difference of the allele frequency must be taken into account in drug development and postmarketing phases.

Several reports have indicated that the influence of CYP2C9 polymorphisms on the changes in clinical pharmacokinetics depends on the substrate (15). For example, the oral clearances of warfarin in **1/*1*, **2/*2*, and **3/*3* subjects varies considerably (39.6, 12.8, 3.7 L/h) (16), but those of losartan (64, 57, 39 L/h) (17) and diclofenac (20, 30, 23 L/h) (18) do not show large differences among subjects with mutant alleles. These kinds of substrate-specific effects of CYP2C9 mutations on the pharmacokinetics are attributed to differences in the contribution of CYP2C9 to the overall clearance of drugs and differences in the decrease in the intrinsic clearances by mutated enzymes. The quantitative contribution of CYP2C9 to overall metabolic clearance has been reported to be an important factor in extrapolating *in vitro* data to *in vivo* situations (19). Some studies have extrapolated *in vitro* data to *in vivo* situations, considering polymorphisms of metabolic enzymes and contribution of the CYP isozyme to the metabolic clearance (20). Furthermore, a successful *in vitro* to *in vivo* extrapolation of warfarin metabolism considering CYP2C9 polymorphisms has been reported (21,22). However, no report has yet been made regarding whether the effects of CYP2C9 polymorphisms on the pharmacokinetics of various CYP2C9 substrate drugs can be quantitatively predicted by *in vitro* experimental data using mutated CYP2C9 enzyme-expression systems.

In this report, we propose a method for predicting oral clearances of drugs in subjects with different CYP2C9 diplotypes based on the pharmacokinetic theory considering the following four parameters: the decrease in *in vitro* intrinsic clearances of mutated CYP2C9 enzymes, the changes in the expression levels of mutated enzymes, the contribution of CYP2C9 to CYP-mediated metabolism, and the contribution of specific metabolic pathways to overall oral clearance. We validated this method by acquiring these kinds of data for nine different CYP2C9 drug substrates from the literature. If we can obtain good results using data even from heterogeneous sources, we can think that everybody can make such good estimation by using some required information obtained by the same group.

MATERIALS AND METHODS

Data Collection from the Literature

All data were obtained from previously published papers searched on PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez) or cited from references provided by manufacturers (interview forms (Japan) or package inserts (USA)), or from the appendix of Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (23). Substrate drugs and their dominant metabolic pathways catalyzed by CYP2C9 were extracted from review articles (11,15). The kinetic parameters (K_m, V_{max}) of the enzymatic activities of mutated CYP2C9 for the metabolism of individual drugs were extracted from literature demonstrating the *in vitro* experiments using microsomes prepared from mutated CYP2C9-expressing Sf9 cells, yeast, COS-7 cells, or HepG2 cells. The expression levels of each variant CYP2C9 enzyme in human liver were estimated from the relative band density of CYP2C9 in Western blot analyses in the human liver microsomes with genotyping information as described previously (24–26). Observed human oral clearances of each substrate in subjects with CYP2C9 diplotypes were also cited from a number of publications.

To calculate f_{m2C9}, defined as the contribution of CYP2C9 to the total hepatic intrinsic clearance of wild-type subjects, the information about the inhibitable portion of metabolic clearance by anti-CYP2C9 functional neutralizing antibody or the specific CYP2C9 inhibitor such as sulfaphenazole in human liver microsomes or the contribution estimated by relative activity factor (RAF) method adjusted with CYP abundance (27) using expression systems of several CYP enzymes, were obtained from the literature. Renal and biliary elimination profiles of each substrate and its metabolites were also searched in order to calculate f_h, defined as the contribution of the specific metabolic pathway to the total clearance of drugs.

Strategy for the Prediction of the Effects of CYP2C9 Polymorphisms on the Pharmacokinetics of Drugs

Based on pharmacokinetic theory, we considered the changes in the following four kinds of clearances; CYP2C9-mediated intrinsic clearance per unit enzyme expression ($CL_{int,2C9}$), hepatic intrinsic metabolic clearance mediated by CYP2C9 ($CL_{h,int,2C9}$), CYP-mediated clearance, and oral clearance (CL_{oral}). Here, we predicted the decrease in oral clearances in subjects with variant diplotypes (**1/*2*, **1/*3*, **2/*2*, **2/*3*, **3/*3*) as the ratio to that of subjects with the wild-type diplotype (**1/*1*). In cases of the presence of multiple metabolic pathways mediated by CYP2C9, the parameters were estimated for each pathway. The basic strategy for the prediction of the effects of CYP2C9 polymorphisms on pharmacokinetics is shown below. The detailed calculation methods and their equations are shown in the Appendix.

1. The intrinsic metabolic clearance mediated by CYP2C9 per unit enzyme expression level ($CL_{int,2C9}$) for each allele was calculated from the reported kinetic parameters (K_m and V_{max}) for metabolic clearance of each substrate in microsomes expressing wild type or variant types of CYP2C9. The ratios of the $CL_{int,2C9}$ for *CYP2C9*2* and **3* alleles to that for wild-type allele (**1*) ($ActR_{2C9*2}$, $ActR_{2C9*3}$) were then calculated.
2. The ratio of the intrinsic hepatic clearance mediated by CYP2C9 ($CL_{h,int,2C9}$) in subjects with each diplotype to that in subjects with **1/*1* ($CL_{h,int,2C9}R$) was calculated by $ActR_{2C9}$ as well as the ratio of the expression level of each CYP2C9 variant in human liver microsomes to that of the wild type ($ExpR_{2C9}$), assuming that enzyme is produced evenly and independently from two alleles in single subject.
3. f_{m2C9}, the contribution of CYP2C9 to the total hepatic intrinsic clearance of wild-type subjects, and f_h, the contribution of the specific metabolic pathway to the total clearance of drugs, were calculated for each drug.
4. The ratio of oral clearance (CL_{oral}) in subjects with each diplotype of CYP2C9 to that in subjects with **1/*1* ($CL_{oral}R$) was predicted by using the parameters described above.

Calculation Methods

Calculation of ActR$_{2C9}$

$ActR_{2C9}$ values for *CYP2C9*2* and **3* were calculated by dividing the *in vitro* $CL_{int,2C9}$ (V_{max}/K_m) for *CYP2C9*2* or **3* by that for wild-type allele (**1*). When we could obtain $ActR_{2C9}$ values for the same metabolic pathway from different multiple sources, the average of the $ActR_{2C9}$ values obtained from each literature source was calculated. If multiple metabolites were produced from a single compound by CYP2C9, we calculated the $ActR_{2C9}$ value for each metabolic pathway separately.

Calculation of CL$_{h,int,2C9}$R

Based on the published data of CYP2C9 expression levels in subjects with each diplotype, i.e., **1/*2*, **1/*3*, **2/*2*, **2/*3*, and **3/*3* (24–26), the ratio of expression level of CYP2C9 in human liver samples from subjects with specific diplotype to that from subjects with wild-type (**1/*1*) ($ExpR_{diplotypeA}$) was calculated by the following equation:

$$ExpR_{diplotypeA} = \frac{ExpR_1 \times n_1 + ExpR_2 \times n_2 + ExpR_3 \times n_3}{n_1 + n_2 + n_3}, \quad (1)$$

where $ExpR_i$ and n_i represent the $ExpR_{diplotypeA}$ value and the number of individual batches of human liver samples in i^{th} literature, respectively. Assuming that CYP2C9 protein was expressed independently from two alleles belonging to one subject with equal contribution, the ratios of the expression level of CYP2C9*2 and *3 to that of wild type (*1) ($ExpR_{2C9*2}$, $ExpR_{2C9*3}$) were estimated by fitting the following equations (Eqs. 2, 3, 4, 5, and 6) simultaneously by using Microsoft Excel Solver tool (Microsoft, USA):

$$ExpR_{2C9*1/*2} = (1 + ExpR_{2C9*2})/2 \quad (2)$$

$$ExpR_{2C9*1/*3} = (1 + ExpR_{2C9*3})/2 \quad (3)$$

$$ExpR_{2C9*2/*2} = ExpR_{2C9*2} \quad (4)$$

$$ExpR_{2C9*2/*3} = (ExpR_{2C9*2} + ExpR_{2C9*3})/2 \quad (5)$$

$$ExpR_{2C9*3/*3} = ExpR_{2C9*3} \quad (6)$$

Then, the $CL_{h,int,2C9}R$ value was calculated using Eq. 11, as described in the Appendix.

Calculation of f_{m2C9} and f_h

The f_{m2C9} value was calculated based on the literature information described above. If multiple concentrations of substrate and CYP2C9-specific inhibitor (anti-CYP2C9 functional neutralizing antibody and sulfaphenazole) were used in one experiment, the data obtained at the highest concentration of the inhibitor and lowest concentration of substrate were selected for further calculation. When we could obtain f_{m2C9} values from different multiple sources, the average of the f_{m2C9} values obtained from each literature source was calculated. The f_h value, defined as the contribution of the specific metabolic pathway to the total clearance of drugs, was calculated as the fraction of dose excreted into bile and urine as a specific metabolite after intravenous administration. If the pharmacokinetic data were only available for oral administration, when the hepatic clearance was much smaller than hepatic blood flow, assuming that the amount of the metabolites collected both in urine and bile is not formed in gut wall during the first-pass entrance of drugs into systemic circulation because CYP2C9 is not thought to be abundantly expressed in the small intestine, f_h was calculated using the following equation:

$$f_h = \frac{CL_{h,met}}{CL_{h,met} + CL_{others}} = \frac{Ae_{met,bile} + Ae_{met,urine}}{dose} \times \frac{1}{BA}, \quad (7)$$

where $CL_{h,met}$, CL_{others}, $Ae_{met,bile}$, $Ae_{met,urine}$ and BA, represent the hepatic clearance for the specific metabolic pathway, the residual total clearance other than $CL_{h,met}$, amount of the metabolite of interest which is excreted into bile, feces, and or urine, and bioavailability.

Calculation of CL$_{oral}$R

Two sets of prediction were conducted with different $ActR_{2C9}$ values. In the first method, considering that $ActR_{2C9}$ values are different for each metabolic pathway, we used a substrate-specific $ActR_{2C9}$ for the further calculation. In another method, assuming that $ActR_{2C9}$ values are basically the same regardless of the substrates, we used averaged $ActR_{2C9}$ value for all metabolic pathways.

$CL_{oral}R$ value of each diplotype, defined as the ratio of oral clearance (CL_{oral}) in subjects with each diplotype of CYP2C9 to that in subjects with **1/*1*, was predicted by incorporating the parameters described above into Eq. 18 in the Appendix, after confirming that they exhibit low intrinsic clearance ($Q_h >> f_{uB} \cdot CL_{h,int}$). The observed $CL_{oral}R$ value was calculated from the following equation:

$$CL_{oral}R = \frac{\sum_i CL_{oral}R_i \times n_i}{\sum_i n_i}, \quad (8)$$

where $CL_{oral}R_i$ and n_i represent the $CL_{oral}R$ value and the number of subjects who participated in the clinical study in the i^{th} literature, respectively. Then, the observed $CL_{oral}R$ was compared with predicted value to check the predictability of this method. When the total number of subjects with a specific haplotype was two or less, the data were not used for comparison.

RESULTS

Selection of the CYP2C9 Substrate Drugs for the Prediction

In this study, we selected CYP2C9 drug substrates [and their metabolic pathways] whose pharmacokinetic informa-

Table I. Summary of *in vitro* CYP2C9 genotype-specific kinetic parameters (ratio to *1) from reports using recombinant microsomes

	CYP2C9*2					CYP2C9*3				
	K_m	V_{max}	V_{max}/K_m	N^a	Ref	K_m	V_{max}	V_{max}/K_m	N^a	Ref
Celecoxib [hydroxylation]	0.94 [0.73, 1.16]	0.73 [0.46, 1.00]	0.76 [0.66, 0.86]	2	(24,25)	1.62 [1.09, 2.16]	0.34 [0.10, 0.57]	0.21 [0.09, 0.32]	2	(24,25)
Diclofenac [4′-hydroxylation]	1.07 [0.76, 1.39]	0.67 [0.44, 0.90]	0.62 [0.59, 0.65]	2	(26,49)	4.54 [2.13]	0.93 [0.35]	0.23 [0.11]	6	(26,49–53)
S-flurbiprofen [4′-hydroxylation]	1.14 [1.57, 0.71]	0.49 [0.53, 0.46]	0.51 [0.36, 0.65]	2	(26,54)	4.06 [2.45]	0.29 [0.14]	0.09 [0.05]	3	(26,54,55)
Losartan [E3174 formation]	0.71	0.46	0.65	1	(49)	1.27	0.18	0.14	1	(49)
S-phenprocoumon [4′-hydroxylation]	1.05	0.74	0.69	1	(56)	–	–	–	–	–
S-phenprocoumon [6-hydroxylation]	1.09	0.77	0.71	1	(56)	–	–	–	–	–
S-phenprocoumon [7-hydroxylation]	1.11	0.79	0.71	1	(56)	–	–	–	–	–
Phenytoin [4′-hydroxylation]	1.00	0.71	0.71	1	(57)	3.00 [2.00, 4.00]	0.14 [0.08, 0.21]	0.04 [0.04, 0.05]	2	(53,57)
Tolbutamide [methylhydroxylation]	0.64 [0.68, 0.60]	0.57 [0.60, 0.54]	0.90 [0.88, 0.92]	2	(9,57)	4.72 [4.59]	0.62 [0.51]	0.14 [0.10]	4	(9,51,53,58)
Torsemide [methylhydroxylation]	0.60	0.54	0.92	1	(59)	2.13	0.33	0.15	1	(59)
S-warfarin [7-hydroxylation]	0.91 [0.37]	0.42 [0.31]	0.42 [0.26]	3	(26,57,60)	4.45 [1.79]	0.43 [0.40]	0.11 [0.09]	8	(9,26,50,53,58,60,61,62)
S-warfarin [6-hydroxylation]			0.27	1	(60)	4.56	0.18	0.04	1	(61)
Mean	0.93	0.63	0.66			3.37	0.38	0.13		
SD	0.20	0.13	0.18			1.39	0.25	0.07		
CV (%)	21	21	28			41	66	51		
Min	0.60	0.42	0.27			1.27	0.14	0.04		
Max	1.14	0.79	0.92			4.72	0.93	0.23		

If $N=2$, data are presented as mean [reported values]; if $N \leq 3$, mean [SD]

[a] N number of reports

tion for the prediction of oral clearance in subjects with each diplotype could be sufficiently obtained from the literature. These were celecoxib [hydroxylation], diclofenac [4′-hydroxylation], *S*-flurbiprofen [4′-hydroxylation], losartan [E3174 formation], *S*-phenprocoumon [4′-hydroxylation, 6-hydroxylation, 7-hydroxylation], phenytoin [4′-hydroxylation], tolbutamide [methylhydroxylation], torsemide [methylhydroxylation], and *S*-warfarin [7-hydroxylation, 6-hydroxylation]. In spite of the multiple metabolic pathways reported for losartan, the required kinetic data could be obtained only in the E3174 (active metabolite of losartan) formation pathway. Therefore, the formation clearance of E3174, defined as urinary excretion of E3174 divided by losartan AUC, was compared instead of the clearance of losartan. Similarly, *S*-phenprocoumon and *S*-warfarin are metabolized *via* several pathways, but as the kinetic data for each pathway were reported, the clearances of parent drugs were compared with the predicted values.

Calculation of $ActR_{2C9}$ Values for *2 and *3 Alleles

The ratio of the kinetic parameters (K_m and V_{max}) of *CYP2C9*2* and **3* variants to that of wild-type (**1*) ($ActR_{2C9*2}$, $ActR_{2C9*3}$) in wild-type and variant forms of CYP2C9-expressing microsomes retrieved from the literature is shown in Table I. CL_{int}, but not K_m and V_{max} for $ActR_{2C9*2}$ in warfarin 6-hydroxylation in **2* variants, was reported. K_m values of the **2* variant were changed only slightly compared with those of the wild type, but V_{max} values of the **2* variant were lower than those of the wild type. The intrinsic clearance of the **2* variant was decreased to 66% that of the wild type. On the other hand, the **3* variant had significantly increased K_m values and decreased V_{max} values, which resulted in the drastic decrease of intrinsic clearance compared with that of the wild type. The **3* data for *S*-phenprocoumon were excluded from further calculation because of the abnormal value for 4′-hydroxylation, whose CL_{int} was calculated to be higher than that of the wild type.

Estimation of the Relative Expression Level of CYP2C9 ($ExpR_{2C9}$) in each Haplotype

Literature information about the expression levels of CYP2C9 protein was retrieved from three publications (24–26) (Table II). Based on the relative expression levels of CYP2C9 in human liver microsomes from subjects with each diplotype, $ExpR_{2C9*2}$ and $ExpR_{2C9*3}$ were estimated to be 0.68 and 0.48, suggesting that these mutations might decrease the expression level as well as intrinsic metabolic clearance, and that the influence of the **3* allele was more prominent than that of the **2* allele on both expression level and intrinsic metabolic clearance. The predicted values for each diplotype calculated using $ExpR_{2C9*2}$ and $ExpR_{2C9*3}$ values were similar to the reported ones.

Estimation of f_{m2C9} and f_h Values for Each Metabolic Reaction

The f_{m2C9} value for each metabolic reaction was calculated from literature data (Table III). Among multiple data sources, the most popular method for estimating the f_{m2C9} values was to observe the inhibitory effect of sulfaphenazole, which is well known to be a selective inhibitor for CYP2C9 on the clearance for each metabolic reaction compared to the other methods such as inhibition of CYP2C9-mediated metabolism by functional neutralizing antibody or the RAF method adjusted with CYP abundance.

The f_h value for each metabolic reaction was calculated from literature data (Table IV). For losartan, whose oral E3174 formation clearance was predicted, f_h was fixed as 1, because f_h was considered to be the contribution of this metabolic pathway to the E3174 formation clearance of the drug, not to the total clearance of the drug. Data based on intravenous administration were only available for torsemide. Data on bioavailability were calculated from references for diclofenac and phenprocoumon, and cited from the literature (23) for diclofenac, flurbiprofen, tolbutamide, phenytoin, and *S*-warfarin. For celecoxib, bioavailability (Fa × Fg × Fh) was kinetically estimated to be 0.72, as $Fa \times Fg \approx 1$ (as merely 2.6% of the drug excreted unchanged in feces when orally administered (28)), and $Fh \approx 0.72$ (calculated using PK parameters (23), on the assumption that Rb=1). If the bioavailability or f_h exceeded 1.00, the values were fixed as 1 for further calculation.

Correlation Between Predicted and Observed $CL_{oral}R$ Values of CYP2C9 Substrate

Except for diclofenac and celecoxib (partly), the observed $CL_{oral}R$ value for each variant diplotype was lower than that for wild type, which is consistent with the decrease in $ActR_{2C9}$ and $ExpR_{2C9}$ of **2* and **3* variants compared with those of the wild-type (**1*) (Table V). No observed $CL_{oral}R$ values of celecoxib (**2/*3*) and flurbiprofen (**2/*2*, **2/*3*, **3/*3*) were available. Because the total number of subjects who participated in all of the reported clinical studies was two or less for the data on celecoxib (**2/*2*), losartan (**3/*3*), and phenytoin (**3/*3*), these data were excluded from our analyses.

Table II. Summary of CYP2C9 genotype-specific protein levels in human liver microsomes (presented as ExpR [number of subjects])

Ref	*1/*1		*1/*2		*1/*3		*2/*2		*2/*3		*3/*3	
(24)	1.00	[14]	0.96	[6]	0.67	[8]	0.57	[4]	0.81	[2]	0.43	[1]
(25)	1.00	[8]	0.80	[2]	0.61	[2]	–	–	–	–	–	–
(26)	1.00	[2]	0.52	[2]	0.47	[3]	–	–	–	–	–	–
Mean[a]	1.00		0.84		0.61		0.57		0.81		0.43	
Predicted expression level[b]	1.00		0.84		0.74		0.68		0.58		0.48	

[a] Mean was weighed by number of subjects
[b] Predicted by least squares method using Microsoft Excel Solver tool (Microsoft, USA); see the "Materials and Methods" section for details

Table III. Contribution of CYP2C9 to CYP-mediated metabolic pathway (f_{m2C9}) of various CYP2C9 substrates

Drug [reaction]	f_{m2C9} (average)	f_{m2C9}	Method[a]	Ref
Celecoxib [hydroxylation]	0.82	0.77	a	(63)
		0.88	b	
		0.86	c	
Diclofenac [4′-hydroxylation]	0.95	0.90	a	(64)
		0.95	b	
		0.96	a	(26)
		0.98	b	
S-flurbiprofen [4′-hydroxylation]	0.96	0.95	a	(26)
		0.98	b	
Losartan [E3174 formation]	0.84	0.89	a	(65)
		0.78	b	
S-phenprocoumon [4′-hydroxylation]	0.46	0.31	b	(66)
		0.71	c	
		0.41	a	(67)
S-phenprocoumon [6-hydroxylation]	0.64	0.52	b	(66)
		0.76	c	
S-phenprocoumon [7-hydroxylation]	0.66	0.56	b	(66)
		0.65	c	
		0.72	a	(67)
Phenytoin [4′-hydroxylation]	0.83	0.86	a	(68)
		0.80	c	
Tolbutamide [methylhydroxylation]	0.98	0.96	a	(69)
		0.99	c	
Torsemide [methylhydroxylation]	0.97	0.97	a	(70)
S-warfarin [7-hydroxylation]	0.94	0.96	a	(26)
		0.94	b	
		0.95	a	(71)
		0.92	b	
S-warfarin [6-hydroxylation]	0.70	0.80	a	(72)
		0.60	a	(73)

[a] Method: (a) inhibition by CYP2C9-specific inhibitor (sulfaphenazole), (b) inhibition by CYP2C9 functional neutralizing antibody, (c) RAF method adjusted with CYP abundance. In the presence of multiple values in one experiment, the value of the highest inhibitor/antibody concentration or of the lowest substrate concentration was used.

Table IV. Contribution of the metabolic pathway to overall metabolism (f_h) of various CYP2C9 substrates

Drug [reaction]	f_h[a]	Metabolite renal excretion [of dose]	Metabolite biliary excretion [of dose]	BA	Ref
Celecoxib [hydroxylation]	1[b]	0.20	0.55	0.72	(23,28)
Diclofenac [4′-hydroxylation]	0.49	0.17 (po)	0.12 (po)	0.59	(23,74,75, Voltaren Interview Form (Novartis, 2005))
S-flurbiprofen [4′-hydroxylation]	0.65	0.47 (po)	–	0.92	(23,76)
Losartan [E3141 formation]	1[c]	–	–	–	
S-phenprocoumon [4′-hydroxylation]	0.12	0.12 (po)[d]			
S-phenprocoumon [6-hydroxylation]	0.14	0.14 (po)[d]		1[b]	(77,78)
S-phenprocoumon [7-hydroxylation]	0.42	0.42 (po)[d]			
Phenytoin	1[b]	0.98 (po)	0.01 (po)	0.9	(23,79,80)
		0.95 (po)[d]			
Tolbutamide	0.94	0.80 (po)[d]		0.85	(23,81)
Torsemide	0.75	0.75 (iv)	[0]	–	(82)
S-warfarin [7-hydroxylation]	0.81	0.75 (po)	[0]	0.93	(23,83)
S-warfarin [6-hydroxylation]	0.17	0.16 (po)	[0]		

(po) data from oral administration, *(iv)* data from intravenous administration, *[0]* approximately zero, *BA* bioavailability

[a] f_h was calculated as the total excreted amount of biliary and urinary metabolites of the pathway after intravenous administration (ratio to dose). If the data were for oral administration, the value was divided by the bioavailability (BA)

[b] Values fixed to 1 because of calculated values >1

[c] For losartan, whose E3174 formation clearance was evaluated, f_h was fixed to 1, as f_h was considered to be the contribution of this metabolic pathway to the E3174 formation clearance of the drug, not to the total clearance of the drug

[d] Metabolite renal and biliary excretion [of doses]

Table V. Summary of $CL_{oral}R^a$ value of each diplotype from literature data (presented as $CL_{oral}R$ [number of subjects])

	*1/*1		*1/*2		*1/*3		*2/*2		*2/*3		*3/*3		ref
Celecoxib	1	[10]	1.17	[5]	0.65	[4]	0.84	[2]	–	–	1.09	[1]	(84)
	1	[4]	–	–	0.66	[4]	–	–	–	–	0.30	[3]	(85)
	1	[12]	1.01	[2]	0.40	[2]	–	–	–	–	0.23	[1]	(25)
Mean[b]	1	[26]	1.12	[7]	0.60	[10]	0.84	[2]	–	–	0.45	[5]	
Diclofenac	1	[10]	1.08	[6]	1.30	[4]	0.58	[2]	–	–	1.39	[1]	(84)
	1	[3]	1.42	[4]	1.11	[4]	1.47	[3]	2.02	[3]	1.14	[3]	(18)
	1	[6]	–	–	1.36	[6]	–	–	–	–	–	–	(86)
	1	[6]	0.62	[3]	0.68	[5]	1.13	[1]	0.52	[4]	1.36	[1]	(87)
Mean[b]	1	[25]	1.08	[13]	1.11	[19]	1.12	[6]	1.16	[7]	1.23	[5]	
S-flurbiprofen	1	[5]	0.73	[5]	0.56	[5]	–	–	–	–	–	–	(88)
Losartan [E3174 formation]	1	[5]	0.50	[5]	0.72	[5]	0.85	[1]	–	–	–	–	(36)
	1	[6]	0.56	[3]	0.57	[5]	0.62	[3]	0.23	[4]	0.01	[1]	(17)
Mean[b]	1	[11]	0.52	[8]	0.64	[10]	0.68	[4]	0.23	[4]	0.01	[1]	
S-phenprocoumon	1	[7]	0.78	[4]	0.82	[5]	0.69	[3]	0.49	[4]	0.63	[3]	(89)
Phenytoin	1	[68]	0.75	[13]	0.74	[16]	0.63	[3]	–	–	0.70	[1]	(90)
	1	[18]	0.67	[7]	0.68	[4]	0.37	[1]	0.37	[1]	–	–	(91)
	1	[37]	0.60	[9]	0.70	[9]	0.69	[3]	0.42	[2]	–	–	(92)
	1	[151]	–	–	0.690	[18]	–	–	–	–	–	–	(93)
Mean[b]	1	[274]	0.70	[29]	0.71	[47]	0.62	[7]	0.40	[3]	0.70	[1]	
Tolbutamide	1	[15]	0.91	[7]	0.71	[3]	0.67	[1]	–	–	–	–	(94)
	1	[6]	0.89	[4]	0.58	[4]	0.77	[3]	0.46	[3]	0.15	[3]	(95)
	1	[5]	0.71	[5]	0.52	[5]	–	–	–	–	–	–	(96)
	1	[12]	–	–	0.75	[6]	–	–	–	–	–	–	(97)
Mean[b]	1	[38]	0.84	[16]	0.64	[18]	0.75	[4]	0.46	[3]	0.15	[3]	
Torsemide	1	[12]	0.98	[9]	0.54	[9]	0.59	[1]	0.44	[3]	0.22	[2]	(98)
	1	[80]	–	–	–	–	0.96	[15]	–	–	0.33	[2]	(99)
Mean[b]	1	[92]	0.98	[9]	0.54	[9]	0.94	[16]	0.44	[3]	0.33	[4]	
S-warfarin	1	[118]	0.66	[32]	0.58	[27]	0.55	[2]	0.31	[6]	0.12	[3]	(100)
	1	[74]	0.73	[30]	0.50	[15]	–	–	–	–	–	–	(101)
	1	[54]	0.58	[15]	0.52	[16]	0.32	[2]	0.23	[4]	0.09	[2]	(16)
	1	[42]	–	–	0.34	[4]	–	–	–	–	0.10	[1]	(62)
Mean[b]	1	[288]	0.67	[77]	0.53	[62]	0.44	[4]	0.28	[10]	0.11	[6]	

[a] The ratio to oral clearance (CL_{oral}) in subjects with each diplotype of CYP2C9 to that in subjects with *1/*1
[b] Mean were calculated as weighed values for study size

Based on the calculated parameters estimated from the literature indicated above, two kinds of prediction of $CL_{oral}R$ values were performed (see the "Materials and Methods" section). In the first method, we used substrate-specific $ActR_{2C9}$ values for the prediction of $CL_{oral}R$ (Table VI A and Fig. 1A). In another method, we used averaged $ActR_{2C9}$ values for all metabolic pathways (Table VI B and Fig. 1B). Overall, the predicted $CL_{oral}R$ values of several CYP2C9 substrates in subjects with each diplotype were similar to the observed $CL_{oral}R$ values, suggesting that this method of estimation can successfully predict the effect of CYP2C9 polymorphisms on the pharmacokinetics of several drug substrates (Figs. 1A, B). Comparing the results from the two prediction methods, the averaged absolute difference between observed and predicted $CL_{oral}R$ values relative to observed values for each data point were similar (0.16±0.17 *vs.* 0.19±0.18), yet scarcely better in the prediction utilizing substrate-specific $ActR_{2C9}$.

DISCUSSION

In this study, we performed a prediction of the effects of genetic polymorphisms of CYP2C9 on the oral clearances of nine different CYP2C9 substrate drugs in subjects with each diplotype by considering multiple factors, such as the decrease in *in vitro* intrinsic clearances per unit protein expression and protein expression levels of CYP2C9 variants, the contribution of CYP2C9 to the CYP-mediated metabolism, and the contribution of the specific metabolic pathway to the overall oral clearances. We observed clear correlation between the predicted ratio of oral clearance in subjects with

Table VI. Predicted $CL_{oral}R$ values utilizing substrate-specific $ActR_{2C9}$ (A) and averaged $ActR_{2C9}$ (B)

	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
A						
Celecoxib	1	0.80	0.63	0.60	0.43	0.26
Diclofenac	1	0.87	0.79	0.73	0.66	0.59
S-flurbiprofen	1	0.80	0.70	0.59	0.50	0.40
Losartan[a]	1	0.77	0.61	0.54	0.38	0.22
S-phenprocoumon[b]	1	0.89	–	0.82	–	–
Phenytoin	1	0.79	0.59	0.57	0.38	0.19
Tolbutamide	1	0.82	0.57	0.65	0.39	0.14
Torsemide	1	0.87	0.67	0.73	0.53	0.33
S-warfarin	1	0.68	0.58	0.36	0.26	0.16
B						
Celecoxib	1	0.77	0.61	0.55	0.39	0.23
Diclofenac	1	0.87	0.78	0.74	0.65	0.56
S-flurbiprofen	1	0.83	0.71	0.66	0.53	0.41
Losartan[a]	1	0.77	0.61	0.54	0.38	0.22
S-phenprocoumon[b]	1	0.88	–	0.58	–	–
Phenytoin	1	0.77	0.61	0.54	0.38	0.22
Tolbutamide	1	0.75	0.57	0.50	0.32	0.14
Torsemide	1	0.80	0.66	0.60	0.46	0.32
S-warfarin	1	0.76	0.59	0.52	0.34	0.17

[a] Oral E3174 formation clearance was predicted
[b] Prediction with *S*-phenprocoumon in *3 alleles was not calculated (see the "Results" section for details)

each diplotype of CYP2C9 to that in subjects with *1/*1 ($CL_{oral}R$) and observed values.

There have been reports on successful prediction of the oral clearance in subjects with each CYP2C9 diplotype of warfarin (21) and tolbutamide (29), which are mostly metabolized by CYP2C9. The changes in enzymatic activities and expression levels of CYP2C9 variants (*2* and *3*) were incorporated in their prediction, but this method was not extended to other CYP2C9 substrates. Recently, in some reports, when estimating the impact of the change in the metabolic activity of specific enzyme caused by genetic polymorphisms or drug interaction on the pharmacokinetics of substrate drugs, the contribution of specific CYP isozyme to the overall clearance of drugs was considered (30,31), but this method has not been systematically applied to the prediction of the effect of genetic polymorphisms of CYP2C9 on the clinical pharmacokinetics of various drugs. Therefore, we proposed a general theoretical method for the prediction of the effects of CYP2C9 polymorphisms on the pharmacokinetics of several kinds of substrate drugs from *in vitro* data by incorporating not only the changes in enzymatic activities and expression levels, but also the contribution of CYP2C9 to the overall clearance. To consider the latter, we introduced two additional parameters, f_{m2C9} and f_h, in order to separate the metabolic clearance mediated by CYP2C9, from the rest of the total clearance. Of the two parameters, f_{m2C9} can be obtained from *in vitro* experiments using human liver microsomes and specific inhibitors, and f_h can be estimated from *in vivo* pharmacokinetic data including conjugation, renal clearances, and bioavailability. These two parameters play important roles in making precise predictions for general compounds because not all substrate drugs undergo metabolism solely by a single enzyme.

In vitro literature data indicated decrease in enzymatic activity for each substrate as well as expression level of CYP2C9*2 and *3 variants (Tables I and II). Some reports have indicated the substrate-specific decrease (8–10), whereas others mentioned that the decrease in metabolic activity may be independent of substrates (32,33). A large variance in $ActR_{2C9*2}$ (0.27–0.92) and $ActR_{2C9*3}$ (0.04–0.23) for each substrate was observed (Table II). However, by comparing the correlation between predicted and observed $CL_{oral}R$ values with or without considering the substrate-specific $ActR_{2C9}$, the precision of the prediction in both cases (Figs. 1A, B) was almost similar, but scarcely better in Fig. 1A. Thus, judging from the current knowledge, we may suppose that the use of averaged $ActR_{2C9}$ values for all CYP2C9 substrates is sufficient for the practical estimation, whereas the use of substrate-specific $ActR_{2C9}$ values would yield a better prediction.

Among test compounds in this study, *S*-phenprocoumon and *S*-warfarin are reported to be metabolized mainly by CYP2C9, but multiple metabolic pathways are involved in their metabolism and the literature indicated that the contribution of CYP2C9 to each metabolic reaction is different (Table III). Drugs with multiple metabolic pathways are likely to be preferred as drug candidates because the change in the function of single protein by and large does not affect the overall clearance. For a better prediction, it is also important to collect kinetic parameters for every major metabolic reaction. Furthermore, the accuracy of the prediction should improve with more data, which were restricted due to availability of observed values.

Several reports on discrepancy between CYP2C9 genotypes and the metabolic ratio of losartan (ratio of excreted amount of E3174 to that of unchanged losartan) have been published (34–36). It may be due to be multiple metabolic pathways for losartan and the lack of information on the contribution of CYP2C9 to overall clearance of E3174. The accuracy of the formation clearance of E3174 in this study was rather insufficient, probably because the calculation was

dependent only on E3174 urinary excretion, and not on the total E3174 elimination.

CL_{oral} values for drug substrates in subjects with CYP2C9 **2* or **3* alleles are generally lower than for wild type with a few exceptions, such as diclofenac (18). In this study, there were large discrepancies between the predicted and observed $CL_{oral}R$ values of celecoxib (**1/*2*) and diclofenac, whose observed values were higher than 1. Because *in vitro* $CL_{int,2C9}$ values were decreased in all substrates, we could not explain the reason theoretically. Large discrepancies among the reported values (Table V) may attribute to this. Further studies will be needed to elucidate this matter.

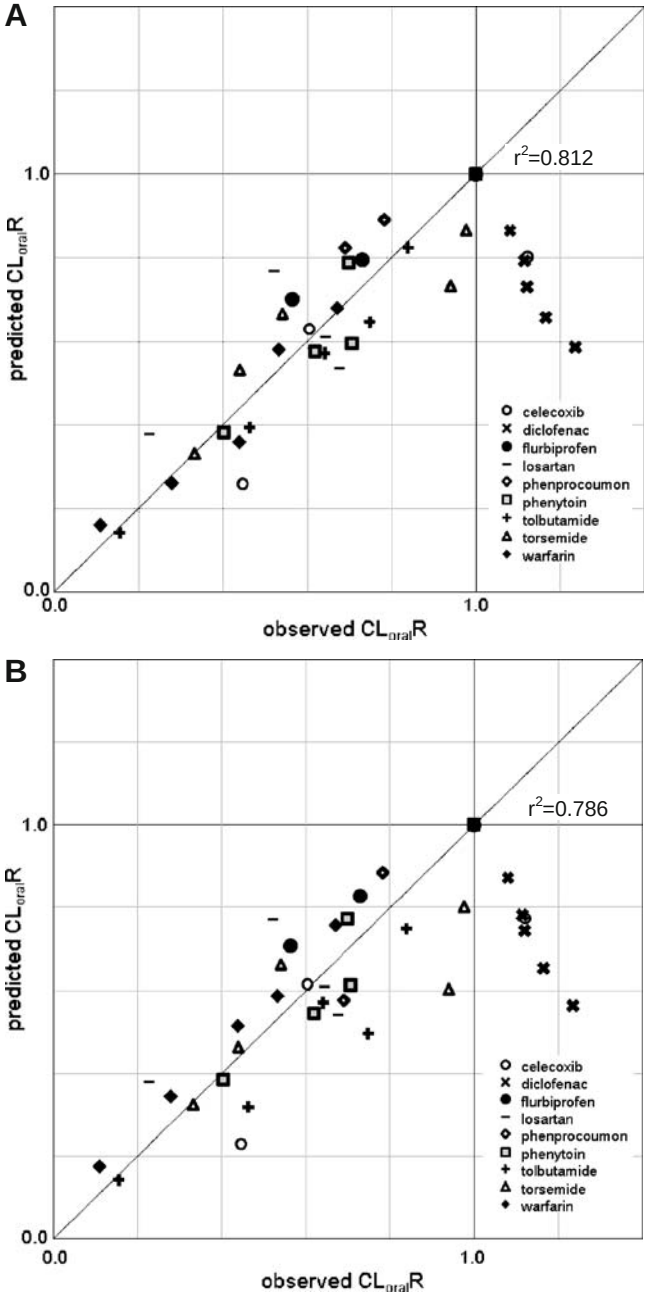

Fig. 1. Comparison between predicted and reported oral clearances of nine substrates in CYP2C9 **2* and **3* variants (ratio to *1/*1; $CL_{oral}R$) using substrate-specific $ActR_{2C9}$ values (Panel **A**) or averaged $ActR_{2C9}$ values (Panel **B**). Oral E3174 formation clearance, not total oral clearance, was evaluated for losartan. Data for celecoxib (*2/*2, *2/*3) and flurbiprofen (*2/*2, *2/*3, *3/*3), losartan (*3/*3), *S*-phenprocoumon (*1/*3, *2/*3, *3/*3), and phenytoin (*3/*3) were not eligible to be plotted (see the "Results" section for details).

In this study, we defined f_h as the contribution of the specific metabolic pathway to the total clearance of drugs including conjugation, biliary excretion, and renal clearance in an unchanged form. Diclofenac showed a lower f_h compared to other drugs (Table IV) because of the significant amount of diclofenac was considered to be directly glucuronidated. Previous reports have indicated that the contribution of CYP2C9 to the overall clearance of diclofenac was approximately 0.3 due to direct glucuronidation (19,37,38). CYP-mediated metabolism is oberved in *in vitro* experiments using human liver microsomes, but not conjugation reactions such as glucuronidation. Therefore, the f_h value was overestimated, and thus predicted CL_{oral} values should be underestimated. Careful considerations of non-CYP metabolic clearance pathways such as excretion in an unchanged form and phase II conjugation reaction are inevitable.

It is useful to determine pharmacokinetic profiles in the early phases of drug development since the pharmacokinetic property is one of the important determinants for good therapeutic candidates. To achieve this kind of prediction, f_{m2C9} and f_h values are required. The isozymes involved in metabolism of drugs and their relative contribution to the CYP-mediated metabolic clearance (f_m) can be evaluated in early phases by recombinant enzyme-expressing and human liver microsomes. However, the involvement of non-CYP metabolic reactions, such as direct conjugation, and biliary and/or renal clearance in an unchanged form is not easily determined. One of the possible strategies is to use cryopreserved human hepatocytes. The function of uptake transporter and conjugation enzymes is preserved in some batches of human hepatocytes. Regarding the transporter-mediated clearance, if the uptake clearance is a rate-determining process of the overall hepatic clearance, the extrapolation of *in vitro* results of uptake assays using human hepatocytes and kidney slices into *in vivo* hepatic and renal clearance may be possible (39,40). Sandwich-cultured hepatocytes may also be useful for predicting biliary clearance, though few examples have been published using human hepatocytes (41,42). In the early phase of drug development, it would also be useful if pharmacokinetic properties of drugs, such as routes of drug elimination, involvement of transporters, and non-CYP metabolism, could be predicted by their physicochemical character predicted from chemical structure. The Biopharmaceutics Drug Disposition Classification System (BDDCS) is a good example of a method for rough prediction of pharmacokinetic properties (43). Recently, various *in silico* tools to predict the pharmacokinetics of drugs in any situation are available and with the aid of *in vitro* experiments and *in silico* quantitative prediction of the pharmacokinetic properties of drugs from their chemical structure, the effects of genetic polymorphisms, and furthermore, interindividual and interracial differences in pharmacokinetics can be easily estimated using the method reported here (44,45). However, few examples of the successful prediction have been reported to date, and more validation results of the prediction methods and softwares should be accumulated.

Some recent reports have indicated that several factors must be considered simultaneously to predict the pharmacokinetics and pharmacodynamics of CYP2C9 substrate drugs. Though torsemide has been reported to be mainly metabolized by CYP2C9, it was reported recently that genetic polymorphisms of both uptake transporters, OATP1B1 and CYP2C9, affected its clearance (46). Based on pharmacokinetic theory, it is possible that uptake clearance is solely determined by the overall hepatic clearance even if drugs are extensively metabolized (47). Recently, the genetic polymorphisms of VKORC1, along with polymorphisms of CYP2C9, were reported to greatly influence the pharmacodynamics of warfarin (48). Differences in pharmacodynamics of warfarin between CYP2C9 variants have been predicted by integrating changes in pharmacokinetics into pharmacodynamic modeling (21). In 2007 the US Food and Drug Administration (FDA) innovated an individualized warfarin dosing based on CYP2C9 and VKORC1 genotypes. Together with PD analyses, our theoretical prediction of the changes in the pharmacokinetics of drugs in subjects with CYP2C9-variant alleles could be integrated to predict the pharmacological and toxicological diversity in a specific population in drug development or postmarketing phase.

CONCLUSION

We have proposed a theoretical method to quantitatively predict the changes of CL_{oral} in subjects with CYP2C9*2 and *3 alleles, considering the changes in enzymatic activities and expression levels as well as the contribution of CYP2C9 to the overall clearance with two parameters: f_{m2C9} and f_h. This method could be applied to predict interracial diversity of the pharmacokinetics of substrate drugs in their development and in clinical use. Of course, this concept can be generally applied to the prediction of the effects of other polymorphic metabolic enzymes on the pharmacokinetics of drugs.

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APPENDIX

In this study, we predicted the decrease in oral clearances (CL_{oral}) of the variant CYP2C9 diplotype (*1/*2, *1/*3, *2/*2, *2/*3, *3/*3) as a ratio to that of the wild type (*1/*1). In the presence of multiple metabolic pathways, the parameters were estimated for each pathway. The algorithm is shown below.

First, the changes in CYP2C9 mediated hepatic intrinsic clearance of the mutant diplotypes ($CL_{h,int,2C9'}$) were expressed as the ratio to homozygous wild-type ($CL_{h,int,2C9\text{-}wt}$). The ratio was calculated using Eqs. 9, 10, and 11, with the CYP2C9 enzymatic activity (intrinsic clearance) ratio ($ActR_{2C9}$), and the enzyme expression level ratio ($ExpR_{2C9}$) of the mutant allele to wild type, on the assumption that the two alleles were to be expressed evenly and independently, and the substrate drugs were to influx into hepatocytes passively and instantly:

$$ActR_{2C9-i} = \frac{CL_{int,2C9-i}}{CL_{int,2C9-wt}} \tag{9}$$

$$ExpR_{2C9-i} = \frac{Exp_{2C9-i}}{Exp_{2C9-wt}} \tag{10}$$

$$\frac{CL_{h,int,2C9'}}{CL_{h,int,2C9-wt}} = (ActR_{2C9-1} \times ExpR_{2C9-1} + ActR_{2C9-2} \times ExpR_{2C9-2}) \times \frac{1}{2} \tag{11}$$

Here, $CL_{int,2C9\text{-}i}$, $CL_{int,2C9\text{-}wt}$, $Exp_{2C9\text{-}i}$, $Exp_{2C9\text{-}wt}$ are the CYP2C9 intrinsic clearance (per unit enzyme level) of the i^{th} allele (i=1 or 2), the CYP2C9 mediated intrinsic clearance (per unit enzyme level) of wild type, the expression level of the CYP2C9 enzyme in the ith allele (i=1 or 2), and the expression level of the CYP2C9 enzyme in wild type, respectively.

Next, the following two parameters were defined in order to predict the changes in oral clearance from the changes in CYP2C9 mediated intrinsic hepatic clearance.

(1) f_{m2C9}: the contribution of CYP2C9 in the total hepatic intrinsic clearance of wild-type subjects:

$$f_{m2C9} = \frac{CL_{h,int,2C9-wt}}{CL_{h,int,2C9-wt} + CL_{h,int,others}} \tag{12}$$

Assuming solely passive transport, $CL_{h,int,others}$ indicates only the CYP-mediated hepatic intrinsic clearance other than CYP2C9, whereas non-CYP clearance (conjugation, biliary excretion of the parent drug) is excluded.

(2) f_h: the contribution of the metabolic pathway to the total clearance of the drug:

$$f_h = \frac{CL_h}{CL_h + CL_{others}} \tag{13}$$

Here, CL_h and CL_{others} are the hepatic clearance of the specific metabolic pathway and the residual total clearance, respectively. CL_{others} includes not only extrahepatic clearance, but also direct conjugation and biliary excretion of the parent drug.

$CL_{h,int,others}$ and CL_{others} are calculated with f_{m2C9} and f_h as follows:

$$CL_{h,int,others} = CL_{h,int,2C9-wt} \times \left(\frac{1}{f_{m2C9}} - 1\right) \tag{14}$$

$$CL_{others} = CL_h \times \left(\frac{1}{f_h} - 1\right) \tag{15}$$

When a drug is administered orally, the first-pass effect in the intestine and liver must be considered. The

ratio of CL_{oral} in the variants to wild type can be calculated using Eq. 16:

$$\frac{CL'_{oral}}{CL_{oral}} = \frac{CL'_h + CL_{others}}{CL_h + CL_{others}} \times \frac{Fa \times Fg \times Fh}{Fa \times Fg' \times Fh'} = \left(f_h \times \frac{CL'_h}{CL_h} + (1 - f_h)\right) \times \frac{Fg \times Fh}{Fg' \times Fh'} \quad (16)$$

Here, Fa, Fg, and Fh are the fraction absorbed through the gastrointestinal tract (Fa), the intestinal availability (Fg), and the hepatic availability (Fh), whereas Fg′ and Fh′ are the Fg and Fh in CYP2C9 variants.

If the substrate exhibits high intrinsic clearance ($Q_h << f_{uB} \cdot CL_{h,int}$, Q_h: hepatic blood flow, f_{uB}: fraction unbound in blood) even in CYP2C9 variants, Eq. 16 would be approximated to Eq. 17, because $CL_h \approx Q_h$ and $Fh \approx Q_h/(f_{uB} \cdot CL_{h,int})$:

$$\frac{CL'_{oral}}{CL_{oral}} = \frac{CL'_{h,int,2C9-wt}}{CL_{h,int,2C9-wt}} \times \frac{Fg}{Fg'} = \left(f_m \times \frac{CL'_{h,int,2C9-wt}}{CL_{h,int,2C9-wt}} + (1 - f_m)\right) \times \frac{Fg}{Fg'} \quad (17)$$

On the other hand, if the substrate exhibits low intrinsic clearance ($Q_h >> f_{uB} \cdot CL_{h,int}$), Eq. 16 would be approximated to Eq. 18, because $CL_h \approx f_{uB} \cdot CL_{h,int}$ and $Fh \approx 1$:

$$\frac{CL'_{oral}}{CL_{oral}} = \left(f_h \times \frac{CL'_h}{CL_h} + (1 - f_h)\right) \times \frac{Fg \times Fh}{Fg' \times Fh'} = \left(f_h \times f_m \times \frac{CL'_{h,int,2C9-wt}}{CL_{h,int,2C9-wt}} + (1 - f_h \times f_m)\right) \times \frac{Fg}{Fg'} \quad (18)$$

Because CYP3A4 is the major isozyme contributing to gastrointestinal drug metabolism, Fg is often considered negligible when studying other isozymes. Thus, we considered the Fg′/Fg ratio as 1.

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