Which Concentration of the Inhibitor Should Be Used to Predict In Vivo Drug Interactions From In Vitro Data?

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ABSTRACT When the metabolism of a drug is competitively or noncompetitively inhibited by another drug, the degree of in vivo interaction can be evaluated from the $[I]_u / K_i$ ratio, where $[I]_u$ is the unbound concentration around the enzyme and K_i is the inhibition constant of the inhibitor. In the present study, we evaluated the metabolic inhibition potential of drugs known to be inhibitors or substrates of cytochrome P450 by estimating their $[I]_u / K_i$ ratio using literature data.

The maximum concentration of the inhibitor in the circulating blood ($[I]_{\max}$), its maximum unbound concentration in the circulating blood ($[I]_{\max,u}$), and its

maximum unbound concentration at the inlet to the liver ($[I]_{in,\max,u}$) were used as $[I]_u$, and the results were compared with each other. In order to calculate the $[I]_u / K_i$ ratios, the pharmacokinetic parameters of each drug were obtained from the literature, together with their reported K_i values determined in in vitro studies using human liver microsomes.

For most of the drugs with a calculated $[I]_{in,\max,u} / K_i$ ratio less than 0.25, which applied to about half of the drugs investigated, no in vivo interactions had been reported or "no interaction" was reported in clinical studies. In contrast, the $[I]_{\max,u} / K_i$ and $[I]_{\max} / K_i$ ratio was calculated to be less than 0.25 for about 90% and 65% of the drugs, respectively, and more than a 1.25-fold increase was reported in the area under the concentration-time curve of the co-administered drug for about 30% of such drugs. These findings indicate that the possibility of underestimation of in vivo interactions (possibility of false-negative prediction) is greater when $[I]_{\max,u}$ or $[I]_{\max}$ values are used compared with using $[I]_{in,\max,u}$ values.

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INTRODUCTION As fractions of human tissues such as human liver microsomes and human hepatocytes have become more easily available for in vitro studies, attempts have been made to quantitatively predict in vivo drug metabolism or drug interactions in humans from in vitro data.¹⁻³ In the case of a competitive or noncompetitive inhibition of drug metabolism, the degree of in vivo interaction can be evaluated from the $[I]_u / K_i$ ratio, where $[I]_u$ is the unbound concentration around the enzyme and K_i is the in vitro inhibition constant of the inhibitor. In clinical situations, the substrate concentration is usually much lower than K_m and the maximum degree of interaction ($R = \text{area under the curve [AUC] (+ inhibitor) / AUC (control)}$) is expressed as $R = 1 + [I]_u / K_i$, assuming that the substrate is eliminated from the body only by the inhibited pathway.¹ Although K_i values can be determined by kinetic analyses of in vitro data using human liver microsomes or recombinant enzymes, it is usually impossible to directly measure $[I]_u$ in humans.

In the case of drugs that are transported into the liver by passive diffusion, $[I]_u$ may be assumed to be equal to the unbound concentration in the liver sinusoid at steady-state. In order to avoid a false-negative prediction due to underestimation of $[I]_u$, we have proposed the use of the maximum unbound concentration at the inlet to the liver, where the blood flow from the hepatic artery and portal vein meet ($[I]_{in,max,u}$), as the maximum value of $[I]_u$.^{1,4} We have succeeded in semiquantitative prediction of several cases of drug interactions using this $[I]_{in,max,u}$ value as $[I]_u$.^{1,5} This method was thus proposed to be useful for predicting the maximal degree of inhibition.^{1,4}

In the present study, the metabolic inhibition potential of a number of drugs known to be inhibitors or substrates of cytochrome P450 (CYP) has been evaluated by estimating their $[I]_u / K_i$ ratio using literature data. The maximum concentration in the circulating blood ($[I]_{max}$), the maximum unbound concentration in the circulating blood ($[I]_{max,u}$), and $[I]_{in,max,u}$ have been used as $[I]_u$ and the results have been compared with each other.

MATERIALS AND METHODS

Using the values of K_i and pharmacokinetic data reported in the literature, the $[I]_u / K_i$ ratios were calculated for a number of drugs known to be inhibitors or substrates of human CYP enzymes. Inhibitors and substrates of various human CYP isozymes were selected mainly from the reviews or textbooks.^{3,6,7}

K_i values evaluated using human liver microsomes (or recombinant human CYP isozymes) were collected together with the inhibition type and the CYP isozyme involved. To avoid the underestimation of in vivo interactions, the smallest K_i value was used in the analysis if more than 2 values of K_i were reported for a drug. If no information of K_i was available, K_m (Michaelis constant) was used instead of K_i . Calculation was not conducted if the inhibition type was other than competitive or noncompetitive inhibition.

According to the perfusion model shown in **Figure 1**, the maximum concentration of inhibitor at the inlet to the liver ($[I]_{in,max}$) can be calculated as follows¹⁻⁴:

$$[I]_{in,max} = [I]_{max} + k_a \times \text{Dose} \times F_a / Q_h \quad (1)$$

where k_a is the absorption rate constant, F_a is the fraction absorbed from gut to the portal vein, and Q_h is the hepatic blood flow rate. Using the unbound fraction in the blood (f_u), $[I]_{in,max,u}$ can be obtained as follows:

$$[I]_{in,max,u} = f_u \times [I]_{in,max} \quad (2)$$

Figure 1. Model for estimating the concentration of inhibitor at the inlet to the liver after oral administration ($[I]_{in}$). $[I]_{out}$, $[I]$, and $[I]_{max}$ represent the inhibitor concentration at the exit of the liver (hepatic vein side), the inhibitor concentration at the liver sinusoid, and maximum inhibitor concentration in the systemic circulation, respectively. Q_a , Q_{pv} , and Q_h ($= Q_a + Q_{pv}$) represent the blood flow at the hepatic artery, portal vein, and hepatic vein, respectively.

The value of $[I]_{max}$ after a single oral administration of a therapeutic dose was estimated from reported plasma concentrations assuming a linear pharmacokinetics. To avoid underestimation of interactions, the maximum value of $[I]_{max}$ or f_u was used if the parameter was reported with a range. The values of k_a , F_a , Q_h , and R_B (blood-to-plasma concentration ratio) were assumed to

be 0.1 min^{-1} (a first-order rate constant reported for the gastric emptying⁸), 1, 1610 mL/min, and 1, respectively.

In the case of drugs that inhibit more than 2 CYP isozymes, the $[I]_u / K_i$ ratios were calculated for each CYP isozyme. When any metabolites of the drug were also reported to inhibit the enzyme, the $[I]_{in,u} / K_i$ ratio was calculated for both the parent drug and the metabolite(s), and the largest value was used in the analysis.

Reports of in vivo interactions in clinical studies involving the investigated drugs were also examined, and the degree of the interaction was expressed as percentage increase in the area under the concentration-time curve (AUC) of a co-administered substrate of the corresponding CYP isozyme.

RESULTS AND DISCUSSION

Table 1 shows the calculated values of $[I]_{in,max,u} / K_i$, $[I]_{max,u} / K_i$, and $[I]_{max} / K_i$ ratios and the parameters used in the calculation. The calculated values of the absorption term (the second term in Equation 1) were found to be much larger than the $[I]_{max}$ for most of the drugs. Although this may result from using the maximum possible values of 0.1 min^{-1} and 1 for k_a and F_a , respectively, which may vary among the drugs, this finding suggests that the absorption term should not be ignored to avoid the underestimation of drug interactions.

In **Table 2**, the calculated $[I]_{in,max,u} / K_i$ ratios were classified according to the CYP isozyme involved. Figures in this table indicate the number of the inhibitor-CYP isozyme combinations. CYP3A4 and CYP2D6 were found to be inhibited by the largest number of drugs, followed by CYP1A2 and. No clear difference was observed in the distribution of $[I]_{in,max,u} / K_i$ ratios among the CYP isozymes involved.

Table 2. Classification of $[I]_{in,max,u} / K_i$ ratios based on CYP isozymes

$[I]_{in,max,u} / K_i$	CYP isozyme								Total
	1A1	1A2	2A6	2C9	2C19	2D6	2E1	3A4	
0 to 0.1	1	3		4	4	10	1	20	43
0.1 to 0.25		4	1		4	3	1	5	18
0.25 to 0.5		2		1		1		1	5
0.5 to 1.0				3		9		4	16
1.0 to 2.0		3	1	1		3		3	11
2.0 to 3.0						2		1	3
3.0 to 5.0				2		2	1	1	6
5.0 to 10.0									
10.0 or more		1		1		2		5	9
Total	1	13	2	12	8	32	3	40	111

Table 3 shows the relationship between the calculated $[I]_{in,max,u} / K_i$ ratio and the degree of in vivo interaction, indicated as the increase in the AUC of the co-administered substrates. Nine of the investigated drugs were reported not to cause any interactions with the substrates of the corresponding CYP isozymes in vivo. Reports of in vivo interactions were not found for more than half of the drugs investigated, but the $[I]_{in,max,u} / K_i$ ratios of most of the drugs in this group were calculated to be less than 0.25. As shown in **Table 3**, the $[I]_{in,max,u} / K_i$ ratio was calculated to be less than 0.25 for about half of the drugs investigated and no in vivo drug-drug interactions have been reported or "no interaction" was reported in clinical studies for about 80% of such drugs. In contrast, the $[I]_{in,max,u} / K_i$ ratio was calculated to be greater than 2.0 for about 16% of the drugs investigated, and about 80% of such drugs were reported to cause in vivo drug-drug interactions in which more than a 2-fold increase was observed in the AUC of the co-administered substrate of the corresponding CYP isozyme. Moreover, the $[I]_{in,max,u} / K_i$ ratio was calculated to be greater than 2.0 for all of the drugs reported to cause in vivo interactions with more than a 5-fold increase in the AUC. These findings indicate that the degree of in vivo interaction can be predicted based on the $[I]_{in,max,u} / K_i$ ratio.

The relationship between the $[I]_{max,u} / K_i$ ratio and the degree of in vivo interaction is summarized in **Table 4**. The calculated $[I]_{max,u} / K_i$ ratios were less than 0.25 for about 90% of the drugs and about 30% of the drugs in this category were reported to cause in vivo interactions with more than a 1.25-fold increase in the AUC. Furthermore, the $[I]_{max,u} / K_i$ ratio was calculated to be less than 0.25 for half of the drugs reported to cause in vivo interactions with more than a 5-fold increase in the AUC. Therefore, predictions using $[I]_{max,u}$ instead of $[I]_{in,max,u}$ may cause substantial underestimation of in vivo interactions.

Table 3. Relationship between $[I]_{in,max,u} / K_i$ ratio and the degree of in vivo interaction

$[I]_{in,max,u} / K_i$	in vivo AUC %							Total
	100	< 125	< 150	< 200	< 500	≥ 500	No data	
0 to 0.1	5	1	2	2	2		30	42
0.1 to 0.25	1		1	3	2		11	18
0.25 to 0.5	2			1	1		2	6
0.5 to 1.0	1		2	3	2		8	16
1.0 to 2.0		1			3		7	11
2.0 to 3.0					2	1		3
3.0 to 5.0		1			2	2	1	6
5.0 to 10.0								0
10.0 or more					4	3	2	9
Total	9	3	5	9	18	6	61	111

ble 1. Calculation of $[I]_u/K_i$ values from reported parameters and clinically observed AUC increase in vivo

inhibitor	CYP isozyme	K_i (μ M)	$[I]_{max}$ (μ M)	Dose (μ mol)	kaDFa/Qh (μ M)	fu	$[I]_{in,max,u}$ (μ M)	$[I]_{in,max,u}/K_i$	$[I]_{max,u}/K_i$	$[I]_{max}/K_i$	In vivo AUC %	Reference No. *
iodarone	2D6	52.7	1.81	930	57.7	0.037	2.2	0.042	0.0013	0.0344	137-160	9
	3A4	47	1.81	930	57.7	0.037	2.2	0.047	0.0014	0.0386	121-220	10
nitriptyline	2D6	4	0.558	956	59.4	0.036	2.2	0.539	0.0050	0.139	No data	
ffeine	1A2	580	46.3	1545	96.0	0.64	91.0	0.157	0.0511	0.0798	120-130	11
rbamazepine	3A4	422	27.5	847	52.6	0.3	24.1	0.057	0.0196	0.0653	Induction	
rvedilol	2D6	No data	0.0649	49.2	3.1	0.05	0.16				No data	
loroquine	2D6	15.3	0.4001	938	58.2	0.39	22.9	1.49	0.0102	0.0262	No data	
lorpheniramine	2D6	25.6	0.029	22	1.4	0.28	0.39	0.015	0.0003	0.0011	No data	
lorzoxazone	2E1	300	40.3	2358	146	0.04	7.5	0.025	0.0054	0.134	No data	
netidine	1A2	600	9.6	1585	98.5	0.8	86.4	0.144	0.0128	0.0160	150	12
	2D6	607	9.6	1585	98.5	0.8	86.4	0.142	0.0127	0.0158	160	13
	3A4	36	9.6	1585	98.5	0.8	86.4	2.40	0.213	0.267	150-220	14
rofloxacin	1A2	150	5.96	648	40.2	0.8	37	0.247	0.0318	0.0397	160-190	15
alopram	2D6	5.1	0.311	99	6.1	0.5	3.22	0.631	0.0305	0.0610	74-155	16
demethyl-)	2D6	1.3	0.114			0.5			0.0438	0.0877		
mpiramine	2D6	2.2	2.19	712	44	0.03	1.4	0.633	0.0299	0.995	No data	
trimazole	2A6	0.22	3.3	2900	180	No data						
	2C9	1.1	3.3	2900	180	No data						
	2E1	12	3.3	2900	180	No data						
	3A4	0.25	3.3	2900	180	No data						
zapine	2D6	4	10.5	2754	171	0.03	5.4	1.36	0.0790	2.63	No data	
caine	2D6	0.074									No data	
deine	2D6	1459	0.2	67	4.2	0.93	4.1	0.003	0.0001	0.0001	No data	
	3A4	276	0.2	67	4.2	0.93	4.1	0.015	0.0007	0.0008	No data	
rtisone	3A4	2.5	0.083	55	3.4	0.1	0.35	0.14	0.0033	0.0332	No data	
clophosphamide	3A4	510	16.1	857	53.2	0.87	60.3	0.118	0.0274	0.0315	No data	
closporine	3A4	0.34	0.447	582	36.2	0.12	4.4	12.9	0.158	1.32	No data	
psone	2E1	4	8.5	805	50.0	0.3	17.6	4.39	0.638	2.13	No data	
	2C9, 3A4	52.6	8.5	805	50.0	0.3	17.6	0.334	0.0485	0.162	100	17
xtromethorphan	2D6	2.8										
	3A4	286										
izepam	3A4	50	0.973	35	2.2	0.032	0.10	0.0020	0.0006	0.0195	100-130	18
lofenac	2C9	119	4.803	169	10.5	0.03	0.46	0.0039	0.0012	0.0404	No data	
iazem	3A4	51	0.289	434	27.0	0.3	8.18	0.160	0.0017	0.0057	100	19
henhydramine	2D6	2.5	0.0235	171	10.6	0.26	2.77	1.11	0.0024	0.0094	No data	
sulfiram	2E1	mechanism-based inhibition										
oxacin	1A2	150	12.8	1152	71.5	0.8	67.5	0.450	0.0683	0.0853	450	20
thromycin	(3A4)	complex formation										
radiol	1A1	1.1	0.0006	5.5	0.34	0.035	0.012	0.011	0.00002	0.0005	100	21
anol	2E1	3500	10130	652000	40497	No data					171	
notidine	3A4	>1000	0.356	119	7.36	0.8	6.2	<0.00062	0.0003	0.0004	No data	
cainide	2D6	0.954	0.179	121	7.5	0.59	4.53	4.75	0.1107	0.188	1160	22
conazole	2C9	8	7.12	327	20.3	0.88	24.1	3.01	0.783	0.890	284	23
	3A4	1.3	7.12	327	20.3	0.88	24.1	18.5	4.82	5.48	111-440	24
oxetine	2D6	0.92	0.2	194	12.0	0.06	0.735	0.799	0.0130	0.217	190	25
vastatin	2C9	0.05	0.2	186	11.5	0.02	0.23	4.69	0.0800	4.00	120	26
voxamine	1A2	0.07	0.113	314	19.5	0.33	6.48	92.5	0.533	1.61	340	27
	2D6	1.3	0.113	314	19.5	0.33	6.48	4.98	0.0287	0.0869	120-360	28
	3A4	8.2	0.113	314	19.5	0.33	6.48	0.790	0.0045	0.0138	200	29
enclamide	3A4	78	0.237	3.54	0.22	0.01	0.0046	0.00006	0.00003	0.0030	157	30
loperidol	2D6	1	0.0051	13	0.8	0.125	0.10	0.104	0.0006	0.0051	No data	
ed haloperidol)	2D6	2	0.0013			0.28			0.0002	0.0007		
xobarbital	2C19	860	30.1	2116	131	0.534	86.3	0.100	0.0187	0.0350	No data	
ipofen	2C9	300	339	5817	361	0.01	7.00	0.023	0.0113	1.13	No data	
ipramine	2C19	24.7	0.464	947	58.8	0.05	2.96	0.120	0.0009	0.0188	No data	
	2D6	48.3	0.464	947	58.8	0.05	2.96	0.061	0.0005	0.0096	No data	
Jesipramine)	2D6	6.07	0.117			0.09			0.0017	0.0193		
linavir	3A4	0.17	12.6	1303	81.0	0.39	36.5	215	28.9	74.1	100-300	31
erferon- α 3A, 1A2	down-regulation											
aconazole	3A4	0.16	0.385	283	17.6	0.0275	0.49	3.09	0.0662	2.41	145-2700	32
toconazole	3A4	0.006	6.6	376	23.4	0.01	0.300	50.0	11.0	1100	830	33
ocaine	1A2	362	25.6	i.v.		0.3	7.68	0.021	0.0212	0.0707	100	34
	2D6	80	25.6	i.v.		0.3	7.68	0.096	0.0960	0.320	No data	
	3A4	120	25.6	i.v.		0.3	7.68	0.064	0.0640	0.213	No data	

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mephenytoin	2C19	162	1.8	687	42.7	0.18	8.01	0.049	0.0020	0.0113	No data	
thadone	2C19	50	0.293	32	2.0	0.11	0.25	0.00507	0.0006	0.0059	100	35
	2D6	2.5	0.293	32	2.0	0.11	0.25	0.101	0.0129	0.117	No data	
ethylphenidate	2D6	15	0.080	171	10.6	0.88	9.4	0.629	0.0047	0.0053	No data	
ethylprednisolone	3A4	210	26.5	2671	166	0.22	42.3	0.202	0.0278	0.126	100-230	36
toprolol	2D6	18	0.181	175	11	0.11	1.2	0.068	0.0011	0.0100	No data	
trondazole	1A1, 1A2	166	35.1	2921	181	0.9	195	1.17	0.190	0.211	No data	
xileline	2D6	30	1.9	698	43.3	0.57	25.8	0.86	0.0361	0.0633	No data	
conazole	1A2	4	1.129	601	37.3	0.02	0.769	0.19	0.0056	0.282	No data	
	2A6	0.42	1.129	601	37.3	0.02	0.769	1.83	0.0538	2.69	No data	
	2C9	0.5	1.129	601	37.3	0.02	0.769	1.54	0.0452	2.26	300	37
	2E1	4	1.129	601	37.3	0.02	0.769	0.19	0.0056	0.282	No data	
	3A4	0.84	1.129	601	37.3	0.02	0.769	0.915	0.0269	1.34	No data	
dazolam	3A4	10.5	0.179	41	2.6	0.0212	0.058	0.00555	0.0004	0.0170	No data	
fazodone	1A2	31	4.26	426	26.5	0.02	0.614	0.020	0.0027	0.137	No data	
	2D6	18	4.26	426	26.5	0.02	0.614	0.034	0.0047	0.237	136	38
	3A4	0.6	4.26	426	26.5	0.02	0.614	1.02	0.142	7.10	200-400	39
ardipine	3A4	8	98.8	39	2.4	0.11	11.1	1.39	1.36	12.4	110-360	40
edipine	3A4	10	0.228	29	1.8	0.045	0.091	0.00910	0.0010	0.0228	100-120	41
rethindrone	3A4	>100	0.263	17	1.0	(1)	1.26	<0.013	0.0026	0.0026	No data	
reprozole	2C19	2	0.43	116	7.19	0.05	0.38	0.19	0.0108	0.215	220	42
roxetine	1A2	0.24	0.194	94	5.9	0.05	0.30	1.26	0.0404	0.808	No data	
	2C19	7.5	0.194	94	5.9	0.05	0.30	0.040	0.0013	0.0259	No data	
	2D6	0.15	0.194	94	5.9	0.05	0.30	2.02	0.0647	1.29	200-2100	43
	3A4	36.7	0.194	94	5.9	0.05	0.30	0.00800	0.0003	0.0053	No data	
enacetin	2D6	4	45.8	5580	347	0.7	275	68.7	8.02	11.5	No data	
enytin	2C9	17	23.2	1189	73.9	0.1	9.7	0.571	0.136	1.36	Induction	
oxicam	2C9	7	63.9	43	2.6	0.077	5.12	0.732	0.703	9.13	147	44
ednisone	3A4	190	0.418	139	8.7	0.25	2.3	0.012	0.0006	0.0022	100	45
ednisolone	3A4	125	4.77	166	10.3	0.1	1.51	0.012	0.0038	0.0382	No data	
oguanil	3A4	>200	1.53	689	42.8	0.25	11.1	<0.055	0.0019	0.0077	No data	
opafenone	2D6	0.58	0.000397	794	49.3	0.027	1.3	2.30	0.00002	0.0007	210	46
opranolol	1A2	2	0.524	406	25.2	0.1	2.6	1.29	0.0262	0.262	No data	
	2D6	5	0.524	406	25.2	0.1	2.6	0.515	0.0105	0.105	100	46
inidine	2D6	0.06	4.1	617	38.3	0.15	6.4	106	10.3	68.3	290	47
itidine	2A6	>200	3.11	855	53.1	0.7	39.4	<0.197	0.0109	0.0156	No data	
navir	2D6	4.84	15.5	832	51.7	0.006	0.40	0.083	0.0192	3.20	245	48
	3A4	0.017	15.5	832	51.7	0.006	0.40	23.7	5.47	912	74-13000	49
quinavir	3A4	0.7	0.378	894	55.6	0.02	1.12	1.60	0.0108	0.540	110	49
rtaline	1A2	8.8	0.0715	292	18.1	0.02	0.36	0.041	0.0002	0.0081	No data	
	2C19	2	0.0715	292	18.1	0.02	0.36	0.182	0.0007	0.0358	No data	
	2D6	0.7	0.0715	292	18.1	0.02	0.36	0.520	0.0020	0.1021	123-250	50
	3A4	23.8	0.0715	292	18.1	0.02	0.36	0.015	0.0001	0.0030	No data	
lfaphenazole	2C9	0.1	70	1591	98.8	0.32	54.0	540	224	700	530	51
lfipyrarzone	2C9	230	81.6	1236	76.8	0.02	3.17	0.014	0.0071	0.355	170	52
sulfide)	2C9	17	6.43			(1)			0.378	0.378		
sulfone)	2C9	73	4.75			(1)			0.0651	0.0651		
rolimus	3A4	8.09	0.0102	12.8	0.793	0.23	0.18	0.023	0.0003	0.0013	No data	
noxifen	3A4	10	0.404	53.8	3.34	0.02	0.0750	0.0075	0.0008	0.0404	No data	
col	3A4	18	4.88	i.v.		0.27	1.32	0.073	0.0732	0.271	No data	
oxicam	2C9	80	14.9	69.1	4.29	0.01	0.19	0.0024	0.0019	0.186	No data	
fenadine	2D6	3.6	0.636	127.2	7.90	0.009	0.0768	0.021	0.0016	0.177	No data	
	3A4	25	0.636	127.2	7.90	0.009	0.0768	0.0031	0.0002	0.0254	100	53
ophylline	1A2	280	74.5	2220	138	0.456	96.8	0.346	0.121	0.266	No data	
oridazine	2D6	0.75	1.003	270	16.8	0.024	0.43	0.568	0.0321	1.34	135-193	54
olol	2D6	18	0.14	23	1.4	0.44	0.69	0.039	0.0034	0.0078	No data	
butamide	2C9	100	407	7398	459	0.04	34.6	0.346	0.163	4.07	100	55
azolam	3A4	8.19	0.007	0.728	0.045	0.292	0.0153	0.0019	0.0003	0.0009	No data	
nethoprim	3A4	>100	25	1550	96.3	0.653	79	<0.79	0.163	0.250	No data	
nlafaxine	3A4	40	1.8	812	50.4	0.73	38.1	0.953	0.0329	0.0450	136	56
o-desmethyl-)	3A4	40	1.8			0.7			0.0315	0.0450		
rapamil	2D6	6.04	0.88	264	16.4	0.1	1.73	0.286	0.0146	0.146	No data	
irfarin	2C9	2.2	9.73	81	5.0	0.12	1.77	0.805	0.531	4.42	No data	
	2C19	60.7	9.73	81	5.0	0.12	1.77	0.029	0.0192	0.160	No data	
nisamide	3A4	274	13.7	943	58.6	0.5	36.1	0.132	0.0250	0.0499	No data	

*References for the largest in vivo interaction are listed. Others are available from the authors on request.

Table 4. Relationship between $[I]_{\max,u}/K_i$ ratio and the degree of in vivo interaction

$[I]_{\max,u}/K_i$	in vivo AUC %							Total
	100	< 125	< 150	< 200	< 500	≥ 500	No data	
0 to 0.1	8	3	4	8	10	2	53	88
0.1 to 0.25	1				2	1	5	9
0.25 to 0.5				1				1
0.5 to 1.0			1		2		2	5
1.0 to 2.0					1			1
2.0 to 3.0								0
3.0 to 5.0					1			1
5.0 to 10.0						1	1	2
10.0 or more					2	2		4
Total	9	3	5	9	18	6	61	111

Table 5. Relationship between $[I]_{\max}/K_i$ ratio and the degree of in vivo interaction

$[I]_{\max}/K_i$	in vivo AUC %							Total
	100	< 125	< 150	< 200	< 500	≥ 500	No data	
0 to 0.1	6	1	3	6	5		33	54
0.1 to 0.25	2		1	1	3	1	10	18
0.25 to 0.5				1	1		7	9
0.5 to 1.0		1			1		2	4
1.0 to 2.0				1	1	1	4	7
2.0 to 3.0					1	1	3	5
3.0 to 5.0	1	1			1		1	4
5.0 to 10.0			1		2			3
10.0 or more					3	3	1	7
Total	9	3	5	9	18	6	61	111

Finally, **Table 5** shows the relationship between the $[I]_{\max}/K_i$ ratio and the degree of in vivo interaction. The calculated $[I]_{\max}/K_i$ ratios were less than 0.25 for about 65% of the drugs, and about 30% of the drugs in this category were reported to cause in vivo interactions with more than a 1.25-fold increase in the AUC. Results in Table 5 indicate that predictions based only on $[I]_{\max}$ can lead to both overestimation and underestimation of in vivo interactions.

Figure 2 shows the comparison of predictions using $[I]_{\text{in,max,u}}$, $[I]_{\text{max,u}}$, and $[I]_{\text{max}}$. The inhibitor-CYP isozyme combinations were classified according to the $[I]_u/K_i$ ratios and the reported degree of in vivo interactions. When the $[I]_{\text{max,u}}$ was used, the number of combinations for which the $[I]_u/K_i$ ratio was calculated to be less than 0.25 or 0.1 was larger than when the $[I]_{\text{in,max,u}}$ was used. Furthermore, as shown in the closed column, the possibility of false-negative predictions was greatly increased when the $[I]_{\text{max,u}}$ was used in the prediction.

In conclusion, the findings in the present study indicate that the $[I]_{\text{in,max,u}}/K_i$ ratios can be used to predict the possibility of drugs causing in vivo drug-drug interactions, in addition to $[I]_{\text{max,u}}/K_i$ or $[I]_{\text{max}}/K_i$ ratios. The possibility of underestimation of in vivo interactions (possibility of false-negative prediction) is greater when the $[I]_{\text{max,u}}/K_i$ or $[I]_{\text{max}}/K_i$ ratios are used in the prediction compared with using the $[I]_{\text{in,max,u}}/K_i$ ratio.

Figure 2. Comparison of the prediction of in vivo interactions using 3 kinds of inhibitor concentration. The inhibitor-CYP isozyme combinations for which $[I]_u/K_i$ values were calculated to be lower than 0.25 (A) or 0.1 (B) were classified according to the reported increase in the AUC: more than 1.25-fold increase (closed column), less than 1.25-fold increase (hatched column), and no data available (dotted column).

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