

Risk Assessment of Mechanism-based Inactivation in Drug-Drug Interactions

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Short communication

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Abbreviations: DDI, drug-drug interactions; FDA, Food and Drug Administration; P450, cytochrome P450; TDI, time-dependent inhibition; MBI, mechanism-based inactivation; AUC, area under the curve; CL_{int} , intrinsic clearance; f_m , the fraction of total clearance of the drug to which the affected P450 enzyme contributes.

Abstract

Drug-drug interactions (DDIs) that occur via mechanism-based inactivation (MBI) of cytochrome P450 (P450) are of serious concern. Although several predictive models have been published, early risk assessment of MBIs is still challenging. For reversible inhibitors, the DDI risk categorization using $[I]/K_i$ ($[I]$, the inhibitor concentration; K_i , the inhibition constant) is widely used in drug discovery and development. Although a simple and reliable methodology such as $[I]/K_i$ categorization for reversible inhibitors would be useful for MBIs, comprehensive analysis of an analogous measure reflecting in vitro potency for inactivation has not been reported. The aim of this study was to evaluate whether the term λ/k_{deg} (λ , first order inactivation rate at a given MBI concentration; k_{deg} , enzyme degradation rate constant) would be useful in the prediction of the in vivo DDI risk of MBIs. Twenty-one MBIs with both in vivo AUC change of marker substrates and in vitro MBI parameters were identified in the literature and analyzed. The results of this analysis show that in vivo DDIs with > 2-fold change of object drug AUC can be identified with the cutoff value of $\lambda/k_{deg} = 1$, where unbound steady-state C_{max} is used for inhibitor concentration. However, the use of total C_{max} led to great over prediction of DDI risk. The risk assessment using λ/k_{deg} coupled with unbound C_{max} can be useful for the DDI risk evaluation via MBI in drug discovery and development.

Introduction

Inhibitory drug-drug interactions (DDIs) are of serious concern in drug development because they can lead to restricted use or withdrawal of drugs from the market (Huang and Lesko, 2004; Wienkers and Heath, 2005). The clinical relevance of mechanism-based inactivators (MBIs) is illustrated by the fact that 24 (19%) of the identified 129 P450 inhibitors on the U.S. market and 38% of the known strong inhibitors are MBIs of cytochrome P450 enzymes (P450) (Isoherranen et al., 2009). Eight (33%) of the 24 MBIs caused strong interactions in vivo. The Pharmaceutical Research and Manufacturers of America (PhRMA) recently summarized the industry practices used in MBI measurements and recommended practical methods for in vitro MBI assays and for prediction of in vivo DDIs using in vitro data (Grimm et al., 2009). However, as described in the article, while several mathematical models for MBI predictions have been presented, quantitative prediction of in vivo DDIs is still challenging. For reversible inhibitors, the DDI risk categorization using $[I]/K_i$ ($[I]$, the inhibitor concentration; K_i , the inhibition constant) is widely used and accepted in drug discovery and development. For irreversible inhibitors, the FDA draft guidance for industry of drug interactions studies released in February 2012

(<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/>

UCM292362.pdf) recommends calculating an R-value equal to $\{k_{\text{inact}} \cdot [I] / (K_I + [I]) + k_{\text{deg}}\} / k_{\text{deg}}$ to assess the in vivo DDI risk of MBIs. In this equation, $[I]$ is the inactivator concentration calculated from the total (free and bound) systemic inhibitor concentration, k_{inact} is the maximum inactivation rate, K_I is the inactivator concentration when the rate of inactivation reaches half of k_{inact} and k_{deg} is the rate constant for enzyme degradation in vivo. If this R-value is >1.1 (or 11 for CYP3A inhibition in the gut) the investigational drug is considered to be a likely P450 inhibitor in vivo and further evaluation is necessary. How well this R-value reflects the magnitude of in vivo DDI risk and whether false positives and false negatives are common has not been reported.

A recent review showed that 13 (42%) of 31 in vitro MBIs were neither moderate nor potent inhibitors in vivo (VandenBrink and Isoherranen, 2010). This suggests that drugs classified as in vitro MBIs do not always cause clinically significant DDIs. In another report of subset of MBIs, relatively accurate predictions of in vivo DDIs were reported (Fahmi et al 2009). The published methods for quantitative prediction of in vivo DDIs for MBIs are shown in equations 1 and 2:

$$\text{predicted } \frac{\text{AUC}_i}{\text{AUC}} = \frac{\text{CL}_{\text{int}}}{\text{CL}_{\text{int},i}} = \frac{1}{\left(\frac{f_{\text{mCYP}}}{1 + \left(\frac{[I] \cdot k_{\text{inact}}}{k_{\text{deg}} \cdot ([I] + K_I)} \right)} \right) + (1 - f_{\text{mCYP}})} \quad \text{Eq.(1)}$$

$$\text{predicted } \frac{\text{AUC}_i}{\text{AUC}} = 1 + \frac{\left(\frac{[\text{I}] \cdot k_{\text{inact}}}{[\text{I}] + K_i} \right)}{k_{\text{deg}}} \rightarrow \lambda \quad \text{Eq.(2)}$$

where AUC_i/AUC is the fold increase in probe AUC, $\text{CL}_{\text{int}}/\text{CL}_{\text{int},i}$ is the fold decrease in probe CL_{int} and λ is the apparent first order inactivation rate at a given inhibitor concentration (Mayhew et al., 2000; Grimm et al., 2009). Eq. 2 assumes that the probe is entirely cleared by a single, inhibited pathway ($f_m = 1$) in the liver, and is mathematically equal to the R-value used in the FDA draft guidance for predicting MBI risk for systemic clearance. Eq. 2 can also be written as $\text{AUC}_i/\text{AUC} = 1 + \lambda/k_{\text{deg}}$, which is similar to the $\text{AUC}_i/\text{AUC} = 1 + [\text{I}]/K_i$ used in risk assessment of reversible inhibitors. The aim of this study was to determine whether the λ/k_{deg} - value obtained from in vitro data could be reliably used to identify DDI risk of in vitro MBIs. The correlation between the magnitude of in vivo DDI and the predicted λ/k_{deg} was determined for known in vitro MBIs and the false positive and false negative rates were evaluated.

Materials and Methods

Data collection. The University of Washington Metabolism and Transport Drug Interaction DatabaseTM (MTDI database; <http://www.druginteractioninfo.org>) was queried to identify known P450 mechanism-based inactivators with K_I and k_{inact} values determined using human liver microsomes or recombinant systems, and to retrieve all reported in vivo interactions for the mechanism-based inactivators extracted. From the resulting list of in vivo interaction studies, those conducted with a known marker substrate (FDA Guidance for Industry, 2006) were selected, and the change in object AUC was recorded. The inhibitor concentrations measured in the interaction study were used if available. For studies that did not measure the plasma concentrations of the inhibitor, literature data using the same dosing regimen was used to obtain steady-state C_{max} values for the inactivator. If data for the inactivator was not available at the dose level used in the in vivo interaction studies, the concentrations were dose normalized to obtain predicted C_{max} values for the inactivator. The plasma or serum protein binding data for the inactivators were also collected from the literature. If multiple K_I and k_{inact} values for the inactivator were available, the value used for analysis was chosen according to the following criteria: 1) The K_I and k_{inact} were tested using the same probe as that used in the in vivo DDI study; 2) the study with the lowest microsomal protein concentration was used.

Assessment of the evaluation methodology to predict in vivo DDI risk. The steady-state inactivator concentrations and in vitro k_{inact} , K_i , and k_{deg} values were used to compute the λ/k_{deg} values. Both total and unbound C_{max} at steady state were used for inactivator concentrations because total C_{max} is used in the $[I]/K_i$ risk assessment for reversible inhibitors, while use of the unbound systemic C_{max} rather than total systemic C_{max} or estimated unbound portal C_{max} yielded the most accurate DDI predictions for MBIs in a previous study (Obach et al., 2007). The reported turnover half-life ($t_{1/2}$) of 36-51 h for CYP1A2, 32 h for CYP2B6, 104 h for CYP2C9, 26 h for CYP2C19, 70 h for CYP2D6, and 26-79 h for CYP3A4 were used to calculate k_{deg} (1/min) values (Yang et al., 2008). The median values were used for CYP1A2 and CYP3A4. Based on the λ/k_{deg} from Eq (2), the likelihood that a drug will cause in vivo interactions was classified as likely ($\lambda/k_{\text{deg}} > 1$), possible ($1 > \lambda/k_{\text{deg}} > 0.1$) or remote ($0.1 > \lambda/k_{\text{deg}}$), then compared with actual AUC change of object drug.

Results and Discussion

Twenty-one inactivators with complete in vivo and in vitro data were identified and the λ/k_{deg} values were calculated. Since several inhibitors had multiple DDI studies, a total of 160 in vivo studies were analyzed. Figure 1 shows the correlation between predicted risk (λ/k_{deg}) and the in vivo AUC change for all DDI studies analyzed. The analysis using unbound inhibitor concentrations and all reported in vivo DDI studies is shown in Figure 1-A and B. The relationship between greatest observed in vivo DDI (the maximum in vivo DDI risk) with a given inhibitor, and the predicted λ/k_{deg} with accepted P450 marker probes is shown in Figure 1-C and D. The in vivo studies and in vitro parameters used are summarized in Table 1. The DDIs with > 2-fold AUC change of object drugs could be identified using a λ/k_{deg} cutoff value of 1 and unbound inactivator C_{max} (Figure 1-C and D). The use of total C_{max} in the λ/k_{deg} calculation resulted in exaggerated risk prediction, and an increase in the number of false positives (Figure 1-E). These results suggest that unbound C_{max} rather than total C_{max} would be appropriate for DDI risk assessment with MBIs.

This analysis shows the effect of probe sensitivity in observed DDI risk. When all 160 in vivo studies were included, 58 (36%) were categorized into the zone between AUC change < 2-fold and $\lambda/k_{deg} > 1$. This demonstrates an over-prediction of the in vivo risk with many substrates.

Some inactivators distributed between the different zones from low to high DDI risk mainly due to different probes used and variable probe sensitivity (Figure 1-B). This is not unexpected, as it is known that the f_m of object drugs as well as the F_g of CYP3A4 substrates are important factors affecting the magnitude of in vivo DDIs. For example for diltiazem, a 1.5-fold and a 3.8-fold increase in AUC of quinidine ($f_{mCYP3A4} = 0.76$) and midazolam ($f_{mCYP3A4} = 0.94$) were observed, respectively, despite the fact that dosing regimens in both studies were similar (Laganiere et al., 1996; Backman et al., 1994). For paroxetine, a 1.7-fold and a 5.2-fold increase in AUC of imipramine ($f_{mCYP2D6} = 0.46$) and desipramine ($f_{mCYP2D6} = 0.88$) were observed, respectively (Albers et al., 1996; Alderman et al., 1997). This demonstrates, that simple risk analysis does not take into account the effect of multiple clearance pathways, genetic polymorphisms and polytherapy on the magnitude of the DDIs observed in individual patients.

When the in vivo data was analyzed for individual MBIs using only the largest observed in vivo interactions (Figure 1-C and -D), the portion of datapoints falling in the zone between AUC change < 2 -fold and $\lambda/k_{deg} > 1$ was decreased (5 of 21 (24%)) but still showed a significant false-positive rate. Using the R-value of 1.1 which is equivalent to a λ/k_{deg} cutoff of >0.1 the false positive rate was 38% demonstrating a significant overprediction of DDI risk even when unbound C_{max} was used. The false positives included the two CYP2B6 MBIs, clopidogrel and

ticlopidine. The overprediction with these two MBIs is likely due to low f_m of the probe bupropion by CYP2B6 and the contribution of alternative elimination pathways (reduction to threohydrobupropion and erythrohydrobupropion) (Faucette et al., 2000; Reese et al., 2008). Hence clopidogrel and ticlopidine are expected to cause more potent DDIs with a higher $f_{mCYP2B6}$ substrate. Indeed, ticlopidine was a weaker MBI for CYP2C19 than CYP2B6 in vitro, but in vivo a 6-fold increase in AUC of omeprazole (CYP2C19 probe) was observed. Zileuton, which had a λ/k_{deg} value > 1 , suggesting a significant DDI risk in vivo, resulted in a weak interaction in vivo (1.92-fold increase in AUC of theophylline). In addition to CYP1A2, theophylline is also cleared by CYP3A4, CYP2E1 and renal clearance, suggesting that use of a higher f_m probe such as caffeine would result in a correct risk categorization. The fact that tadalafil induces CYP3A in vitro (Ring et al., 2005) and most likely in vivo, is a likely reason for over-prediction of CYP3A4 inhibition in vivo. The reasons for the overprediction of CYP3A4 DDI risk by amiodarone and fluoxetine are unknown.

In conclusion, the results show that the use of λ/k_{deg} with unbound steady-state C_{max} can be useful for identifying high DDI risk compounds, but this method is not applicable for accurate quantitative prediction. The presented approach does not account for probe f_m by inhibited pathway, gut extraction of the probe, possible simultaneous induction and competitive inhibition

by the inhibitor. Therefore, it is less accurate for predicting DDI magnitude than other existing static methods (Fahmi et al 2009) or physiologically based modeling. However, the presented method is expected to provide the highest risk estimate in comparison to other methods for a potential MBI unless significant gut extraction of the object drug exists. More compounds with weak in vivo interactions and known in vitro MBIs need to be identified to determine the false positive/negative rates of this method. Since this dataset includes the currently known in vivo inhibitors that are MBIs, it would be especially important to obtain data from in vivo DDI studies of compounds in development that are MBIs to further demonstrate the utility of this method. This evaluation methodology is simple and could be used in drug discovery and development for risk assessment of MBIs without accounting for probe specific values such f_m and gut metabolism.

Authorship Contribution

Participated in Research Design: Fujioka, Kunze, Isoherranen

Conducted Experiments: Fujioka

Contributed New Reagents or analytic tools: None

Performed Data analysis: Fujioka, Kunze, Isoherranen

Wrote or contributed to the writing of the manuscript: Fujioka, Kunze, Isoherranen

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Footnotes

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Legends for figures

Figure 1. DDI risk assessment for the identified MBIs. All 160 in vivo DDI studies for the 21 inactivators were analyzed using calculated λ/k_{deg} coupled with unbound steady-state C_{max} (A). The relationship between λ/k_{deg} and AUC change for individual inactivators are categorized as shown in (B). The numbers after each MBI indicate the number of in vivo studies conducted within that category. In vivo studies with the most sensitive probes for the inactivated P450 and highest in vivo interactions for the 21 inactivators were analyzed using λ/k_{deg} coupled with unbound steady-state C_{max} (C), and the relationship between λ/k_{deg} and AUC change for individual inactivators is shown for different risk categories in (D). All 160 in vivo DDI studies for the 21 inactivators were analyzed using λ/k_{deg} coupled with total steady-state C_{max} (E). A λ/k_{deg} of 1 predicts a two-fold AUC increase in vivo for an ideal probe substrate.

Table 1. In vivo and invitro data used for risk assessment:

Inactivator	CYP	Inactivator dose	Object drug	AUC change of object drug (observed)	K_I (μM)	k_{inact} (/min)	λ/k_{deg} (total C_{max})	λ/k_{deg} (unbound C_{max})	References
amiodarone	3A4	400 mg, q.d., 4 d	simvastatin acid	1.8	42	0.02	7.8*	0.3*	Becquemont et al., 2007; Shoaf et al., 2005; Mori et al., 2009
amprenavir	3A4	1200 mg, b.i.d., 10 d	rifabutin	2.9	0.3	0.73	3268	2876	Polk et al., 2001; Ernest et al., 2005
cimetidine	2D6	300 mg, q.i.d., 6 d	imipramine	2.7	77	0.03	15	12	Wells et al., 1986; Kosoglou et al., 2000; Madeira et al., 2004
clarithromycin	3A4	500 mg, b.i.d., 9 d	simvastatin	10	5.5	0.07	133	36	Jacobson, 2004; van Haarst et al., 1998; Mayhew et al., 2000
clopidogrel	2B6	75 mg, b.i.d., 4 d	bupropion	1.4	1.4	1.9	78	4.7	Turpeinen et al., 2005; Kim et al., 2008; Walsky and Obach, 2007
dasatinib	3A4	100 mg, single	simvastatin	1.2	6.3	0.03	5.8	0.4	Product Label; Li et al. 2009
diltiazem	3A4	60 mg, t.i.d., 2 d	buspirone	5.3	3.7	0.07	37.6	11	Lamberg et al., 1998; Shum et al., 1996; Zhang et al., 2009
erythromycin	3A4	500 mg, t.i.d., 2 d	simvastatin	6.2	11	0.05	57.0	12	Kantola et al., 1998; Olkkola et al., 1993; McConn et al., 2004
fluoxetine	3A4	20 mg, q.d., 21 d	alprazolam	1.3	5.3	0.02	3.8	0.2	Hall et al., 2003; Harvey and Preskorn, 2001; Mayhew et al., 2000
isoniazid	3A4	90 mg, b.i.d., 4 d	triazolam	1.5	228	0.08	9.7*	9.7*	Ochs et al., 1983; Dattani et al., 2004; Wen et al., 2002
mibefradil	3A4	100 mg, single	midazolam	8.9	2.3	0.40	553	4.0	Veronese et al., 2003; Welker et al., 1998
nelfinavir	3A4	1250 mg, b.i.d., 14 d	simvastatin	6.1	0.48	0.22	952	77	Hsyu et al., 2001; Fang et al., 2008; Ernest et al., 2005
paroxetine	2D6	20 mg, q.d., 17 d	atomoxetine	7.1	3.6	0.13	25	1.3	Belle et al., 2002; Perloff et al., 2009
ritonavir	3A4	600 mg, single	saquinavir	112	0.038	0.29	1315	913	Hsu et al., 1998; Luo et al., 2003
rofecoxib	1A2	25 mg, q.d., 4 d	tizanidine	12	4.8	0.07	44	6.7	Backman et al., 2006; Karjalainen et al., 2006
saquinavir	3A4	1200 mg, t.i.d., 5 d	midazolam	5.2	0.2	0.31	1223	102	Palkama et al., 1999; Cook et al., 2004; Ernest et al., 2005
tadalafil	3A4	20 mg, q.d., 14 d	lovastatin	1.12	12	0.21	76*	4.9*	Ring et al., 2005; Wrishko et al., 2008
ticlopidine	2B6	250 mg, b.i.d., 4 d	bupropion	1.6	0.3	0.43	1067*	174*	Turpeinen et al., 2005; Lu et al., 2006;

									Walsky and Obach, 2007
ticlopidine	2C19	200 mg, q.d., 8 d	omeprazole	6.2	9.2	0.25	108	2.7	Ieiri et al., 2005; Lu et al., 2006; Atkinson et al., 2005
tienilic acid	2C9	250 mg, q.d., 19 d	(S)-warfarin	2.9	12.5	0.13	846	30	O'Reilly, 1982; Dubb et al., 1979; Hutzler et al., 2009
verapamil	3A4	80 mg, t.i.d., 2 d	simvastatin	4.7	4.6	0.43	195	21	Kantola et al., 1998; Johnson et al., 2001; Wang et al., 2004
zileuton	1A2	800 mg, b.i.d., 5 d	theophylline	1.9	117	0.04	16	1.2	Granneman et al., 1995; Lu et al., 2003

*indicates the inactivator concentration was calculated with dose normalization

References for in vivo AUC change, in vivo inactivator concentration (unless no C_{max} data in the corresponding DDI study), and in vitro MBI parameters in Table 1: Atkinson et al., 2005, *Drug Metab Dispos* **33**: 1637-1647; Backman et al., 2006, *Br J Clin Pharmacol* **62**: 345-357; Becquemont et al., 2007, *Clin Pharmacol Ther* **81**: 679-684; Belle et al., 2002, *J Clin Pharmacol* **42**: 1219-1227; Cook et al., 2004, *Xenobiotica* **34**: 215-228; Dattani et al., 2004, *Eur J Clin Pharmacol* **60**: 679-682; Dubb et al., 1979, *Postgrad Med J* **55 Suppl 3**: 47-57; Ernest et al., 2005, *J Pharmacol Exp Ther* **312**: 583-591; Fang et al., 2008, *Pharmacotherapy* **28**: 42-50; Granneman et al., 1995, *Clin Pharmacokinet* **29 Suppl 2**: 77-83; Hall et al., 2003, *J Clin Psychopharmacol* **23**: 349-357; Harvey and Preskorn, 2001, *J Clin Psychopharmacol* **21**: 161-166; Hsu et al., 1998, *Clin Pharmacol Ther* **63**: 453-464; Hsyu et al., 2001, *Antimicrob Agents Chemother* **45**: 3445-3450; Hutzler et al., 2009, *Drug Metab Dispos* **37**: 59-65; Ieiri et al., 2005, *Pharmacogenet Genomics* **15**: 851-859; Jacobson, 2004, *Am J Cardiol* **94**: 1140-1146; Johnson et al., 2001, *J Clin Pharmacol* **41**: 1248-1256; Kantola et al., 1998, *Clin Pharmacol Ther* **64**: 177-182; Karjalainen et al., 2006, *Drug Metab Dispos* **34**: 2091-2096; Kim et al., 2008, *Clin Pharmacol Ther* **84**: 236-242; Kosoglou et al., 2000, *Br J Clin Pharmacol* **50**: 581-589; Lamberg et al., 1998, *Clin Pharmacol Ther* **63**: 640-645; Li et al., 2009, *Drug Metab Dispos* **37**: 1242-1250; Lu et al., 2003, *Drug Metab Dispos* **31**: 1352-1360; Lu et al., 2006, *J Clin Pharmacol* **46**: 628-634; Luo et al., 2003, *Drug Metab Dispos* **31**: 1170-1175; Madeira et al., 2004, *Drug Metab Dispos* **32**: 460-467; Mayhew et al., 2000, *Drug Metab Dispos* **28**: 1031-1037; McConn et al., 2004, *Drug Metab Dispos* **32**: 1083-1091; Mori et al., 2009, *Xenobiotica* **39**: 415-422; Ochs et al., 1983, *Br J Clin Pharmacol* **16**: 743-746; Olkkola et al., 1993, *Clin Pharmacol Ther* **53**: 298-305; O'Reilly, 1982, *Clin Pharmacol Ther* **32**: 356-361; Palkama et al., 1999, *Clin Pharmacol Ther* **66**: 33-39; Perloff et al., 2009, *Xenobiotica* **39**: 99-112; Polk et al., 2001, *Antimicrob Agents Chemother* **45**: 502-508; Ring et al., 2005, *Clin Pharmacol Ther* **77**: 63-75; Shoaf et al., 2005, *J Cardiovasc Pharmacol Ther* **10**: 165-171; Shum et al., 1996, *J Clin Pharmacol* **36**: 1161-1168; Turpeinen et al., 2005, *Clin Pharmacol Ther* **77**: 553-559; van Haarst et al., 1998, *Clin Pharmacol Ther* **64**: 542-546; Veronese et al., 2003, *J Clin Pharmacol* **43**: 1091-1100; Walsky and Obach, 2007, *Drug Metab Dispos* **35**: 2053-2059; Wang et al., 2004, *Drug Metab Dispos* **32**: 259-266; Welker et al., 1998, *Clin Pharmacokinet* **35**: 405-423; Wells et al., 1986, *Eur J Clin Pharmacol* **31**: 285-290; Wen et al., 2002, *Eur J Clin Pharmacol* **57**: 799-804; Wrishko et al., 2008, *J Clin Pharmacol* **48**: 610-618; Zhang et al., 2009, *Drug Metab Dispos* **37**: 150-160

Figure 1.

