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Systematic prediction of pharmacokinetic drug-drug interactions caused by changes in cytochrome P450 activity

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Abstract

The aim of the present paper was to overview the current status of the methods used to predict the magnitude of pharmacokinetic drug-drug interactions (DDIs) which are caused by changes in cytochrome P450 (CYP) activity with an emphasis on a method using *in vivo* information. In addition, more than hundred representative CYP substrate, inhibitor and inducer drugs involved in significant pharmacokinetic DDIs were selected from the literature and are listed. Although the magnitude of DDIs has been conventionally predicted based on *in vitro* experiments, their predictability is restricted due to several difficulties, including a precise determination of the unbound inhibitor concentrations around the enzyme and a reliable *in vitro* measurement of the inhibition constant (K_i). Alternatively, a simple and reliable method has been recently proposed for systematic prediction of the magnitude of DDIs based on information fully available from *in vivo* clinical studies. The new *in vivo*-based method would be applicable to the adjustment of dose regimens in actual pharmacotherapy situations. In this review, theoretical and quantitative relationships between the *in vivo*- and the *in vitro*-based prediction methods are considered. One of interesting outcomes of the consideration is that *in vitro* K_i divided by daily dose of 20 L (2~200 L, when variability is considered) may be a pragmatic index which predicts significant *in vivo* DDIs. In addition to these *in vitro*-*in vivo* relationships, it will be emphasized that the magnitude of DDIs can be potentiated or abolished as a consequence of the polymorphism of CYP enzymes. In the last part of the article, the relevance of the inclusion of the *in vivo*-based method into the process of new drug development is discussed for good prediction of *in vivo* DDIs.

Keywords:
drug-drug interactions, CYP3A4, area under the curve (AUC), pharmacogenetics, clinical pharmacokinetics, *in vitro-in vivo* correlation

Abbreviations:
AUC, area under the plasma or serum drug concentration curve
CL_{int}, intrinsic clearance
$CL_{int,g}$, intrinsic clearance of enterocytes
CR, contribution ratio of the clearance pathway to oral clearance
CR_h, contribution ratio of hepatic clearance to oral clearance
$CR_{h,i}$, contribution ratio of hepatic clearance in pathway i to oral clearance
CR_{snps}, contribution ratio of a CYP enzyme subject to polymorphism
CYP, cytochrome P450
D, dose
DDI, drug-drug interaction
EC_{50}, concentrations of inductive drugs at the half maximum induction
E_g, intestinal extraction ratio
EM, extensive metabolizer
E_{max}, maximum induction ratio
F_a, absorption ratio
F_g, intestinal availability
f_m, fraction of metabolism
f_u, unbound fraction in plasma
FDA, Food and Drug Administration of the United States
HIV, human immunodeficiency virus
$I_{h,u}$, unbound concentrations of causative drugs in hepatocytes
IC, increase in clearance due to the induction
IM, intermediate metabolizer
I_{pv}, total (unbound and bound) plasma concentrations of causative drugs in portal vein
$I_{pv,u}$, unbound plasma concentrations of causative drugs in the portal vein
IR, inhibition ratio of the clearance pathway
IR_g, inhibition ratio of intestinal clearance

IR_h, inhibition ratio of hepatic clearance

IR', adjusted IR value considering the deviated dose of causative drugs

$I_{sys,u}$, mean unbound plasma concentrations in the systemic circulation

I_{sys}, mean total (unbound and bound) plasma concentrations in the systemic circulation

$I_{sys,max}$, maximum total plasma concentrations in the systemic circulation unbound

I_u, unbound concentrations of causative drugs at the interaction site

J_u, unbound concentrations of inductive drugs at the induction site

k_a, rate constant for absorption

k_{deg}, rate constants for the degeneration of enzyme

K_i, inhibition constant

k_{inact}, rate constants for the inactivation of enzyme

K_m, Michaelis constant

PC_{snps}, personal change in a clearance pathway subject to polymorphism

P-gp, P-glycoprotein

PM, poor metabolizer

Q_h, hepatic plasma flow rate

R, induction ratio

RAF, relative activity factor

SNP(s), single nucleotide polymorphism(s)

UM, ultra-rapid metabolizer

α, fold change in dose

Superscript of o, indication that the parameter is defined based on the simple model

Superscript of *, indication that the parameter is altered by drug-drug interactions and/or polymorphisms

Abbreviations used in Appendix

$CL_{Cr,impaired}$, creatine clearance in patients with impaired renal function

$CL_{Cr,normal}$, creatine clearances in patients with normal renal function

$CR_{h,i}$, contribution ratio of hepatic clearance in pathway i to oral clearance

$CL_{int,h}$ hepatic intrinsic clearance

$CL_{int,h,i}$, hepatic intrinsic clearance in pathway i

CL_{oral}, oral clearance (total clearance / bioavailability)

$CL_{oral,-x}$, altered *in vivo* oral clearance when the clearance in pathway x is absent.

CL_r, renal clearance

CL_x, clearance in pathway X

CR_r, contribution ratio of renal clearance to oral clearance

CR_x, contribution ratio of clearance in pathway x to oral clearance

F, bioavailability

F_a, absorption ratio

F_g, intestinal availability

F_h, hepatic availability

f_m, fraction of metabolism

f_u, unbound fraction in plasma

IR_h, inhibition ratio of hepatic clearance

$IR_{h,i}$, inhibition ratio of hepatic clearance in pathway i

IR_r, inhibition ratio of renal clearance

Q_h, hepatic plasma flow rate

X_u, urinary excretion ratio of the unchanged drug after oral administration

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1. Introduction

Pharmacokinetic drug-drug interactions (DDIs) are unfavorable clinical events which are caused by abnormally increased or decreased drug concentrations in the body as a consequence of coadministration of other drug(s) (Sproule et al., 1997; Patsalos and Perucca, 2003; Saito et al., 2005b). They represent one of the major problems in drug development and, even after approval, some drugs have been withdrawn from the market due to DDIs (Diasio, 1998; Estelle and Simons, 1999; Furberg and Pitt, 2001). Nevertheless, DDIs are still observed frequently in current pharmacotherapy, and the prediction of DDIs is of clinical importance for selecting regimens and adjusting doses (Pirmohamed et al., 2004). Many studies have been performed regarding prediction of DDIs using *in vitro* experimental data (Kanamitsu et al., 2000b; Kanamitsu et al., 2000c; Yamano et al., 2001; Ito et al., 2004; Galetin et al., 2005; Ito et al., 2005), and a number of clinically significant DDIs are known to be caused by inhibition or induction of cytochrome P450 (CYP), a family of oxidative drug metabolizing enzyme expressed in the liver and the intestine. On the other hand, little effort has been made to systematically predict DDIs among the wide range of drugs currently in use. The reason for this is that there are many drugs on the market as discussed below.

The number of FDA-approved small molecule drugs registered in DrugBank, a comprehensive drug database, is approximately one thousand (Wishart et al., 2006). The total number of drugs on the market is approximately 2,800 in Japan excluding formulation changes and combination drugs (Kubota, 2001). From these statistics, one can estimate that more than one thousand drugs are routinely used in modern hospitals in advanced countries. Many of these drugs are administered orally and are believed to be metabolized by CYPs (Wienkers and Heath, 2005). Consequently, the number of oral drugs which are being prescribed in hospitals and are potentially subjects of DDI-related CYP metabolism would be 500~1,000.

In Table 1(a-c), representative substrate, inhibitor and inducer drugs of significant pharmacokinetic DDIs were selected from the literature and are listed. We restricted the drugs to those of which noticeable DDIs were actually observed *in vivo* in humans. Accordingly, it is plausible that the plasma AUC of most substrate drugs in this table would be changed more than two-fold when coadministered with the inhibitor or the inducer of the corresponding CYP which is also listed in the table. Although pharmacokinetic changes do not directly relate to clinical significance, this table may be a comprehensive overview of DDIs caused by changes in CYP activity.

There are 86 substrates and 42 inhibitors in the table.

The number of the drugs which significantly inhibit or induce CYP has been roughly estimated to be ~100 from the above analysis. Considering the number of substrate drug of CYP would be 500~1,000, the number of potential combinations of DDIs is 50,000~100,000, which is obviously too many to be verified by clinical studies. The total number of clinical DDI studies reported in the literature is, on the other hand, only several hundred from our own analysis. Therefore, in order to manage various DDIs properly, a general and systematic prediction method is necessary with which we can predict the significance of DDIs for a wide range of drugs.

American Pharma has proposed well-organized procedures for investigation of DDIs during the drug development process (Bjornsson et al., 2003). Recently, the FDA has announced a new draft guidance for DDI studies (Huang et al., 2007) and is preparing a finalized version. These tasks offer clearer insight into DDIs and how issues should be managed during the process of new drug development. Furthermore, there are several comprehensive reviews which describe the use of *in vitro* experimental data for prediction of DDIs for many drugs (Ito et al., 1998a; Ito et al., 1998b; Obach et al., 2005; Wienkers and Heath, 2005). In the present review article, we will consider the advantages and disadvantages of the *in vivo*-based methods to predict the magnitude of DDIs. Since CYP3A4 is the most abundant and important enzyme involved in DDIs as discussed in Section 2 (Wrighton and Stevens, 1992; Guengerich, 1999; Fujita, 2004; Wienkers and Heath, 2005), we focused on this enzyme. However, the concepts described in this review may be also applicable to other CYP enzymes such as CYP2C and 2D6.

2. Significance of DDIs caused by changes in CYP3A4 activity

In general, substrates of CYP3A4 are neutral to cationic compounds of relatively large molecular weight. Many drugs are substrates of CYP3A4, and representatives of these include 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (simvastatin and lovastatin), bezodiazepines (midazolam and triazolam) and a number of calcium channel blockers. It has been recognized that CYP3A4 is the most important drug metabolizing enzyme and is involved in the metabolism of approximately half of the drugs on the market (Rendic and Di Carlo, 1997; Rogers et al., 2002). Other important CYPs involved in drug metabolism include CYP2D6, CYP2C9, and CYP2C19 (Williams et al., 2004). To confirm importance of these CYPs in DDIs of medicines on the current market, the DDI section of the Japanese labeling was

searched with each CYP species as a keyword on December 2007. As shown in Table 2, 902 hits were found with CYP3A4 which accounts for 46% of all CYPs. The number of hits was more than 100 for CYP2D6, CYP1A2, CYP2C9 and CYP2C19, and the total of these top five CYPs accounts for 94% of the CYP-related hits within the DDI section. The other CYPs, which contribute to DDIs to minor degrees, are CYP2C8, CYP2E1 and CYP2B6. In the search results of PubMed, a similar trend was observed but greater contributions were observed for CYP2D6 and CYP2C19, probably due to greater interest in these enzymes because of significant polymorphism. It is also notable that the number of substrates and inhibitors are more for CYP3A4 than those for other CYPs in Table 1.

From these considerations, it seems reasonable to conclude that the role of CYP3A4 is particularly important as a mechanism of DDIs associated with pharmacokinetic changes. As will be discussed in Section 6, CYP3A4 is also expressed in the small intestine to reduce the oral bioavailability (Ding and Kaminsky, 2003; Paine et al., 2006a). In addition, we have to consider the role of MDR1 P-glycoprotein (P-gp), an efflux transporter, whose substrate specificity resembles that of CYP3A4, in reducing the oral bioavailability (Suzuki and Sugiyama, 2000).

3. Prediction of DDIs resulting from inhibition of CYP3A4

It has been reported that the plasma AUCs of some CYP3A4 substrate drugs is increased several- to more than ten-fold when the enzyme activity is strongly inhibited (Varhe et al., 1994; Neuvonen and Jalava, 1996; Olkkola et al., 1996; Neuvonen et al., 1998; Heinig et al., 1999). Representative inhibitors of CYP3A4 include azoles (ketoconazole, itraconazole and voriconazole), and macrolide/ketolide antibiotics (clarithromycin, telithromycin and erythromycin). Some of these strong inhibitors display irreversible inhibition kinetics (Kanamitsu et al., 2000a; Galetin et al., 2006). The DDIs of azole antifungals would be unavoidable because their mechanism of pharmacological action involves inhibition of CYP enzymes in fungals (Lepesheva and Waterman, 2007). Many efforts have been devoted to predict the DDIs of these strong inhibitors from *in vitro* (Kanamitsu et al., 2000b; Yamano et al., 2001; Ito et al., 2004; Galetin et al., 2005) and *in vivo* data (Ohno et al., 2007).

To start with a prediction of pharmacokinetics, one would need to consider whether information on time-dependent changes of DDI is required. To predict time-dependent changes, it is necessary, in general, to construct a pharmacokinetic model composed of differential equations, and subsequently to solve the equations

using a numeric technique (Jolivette and Ekins, 2007; Rostami-Hodjegan and Tucker, 2007). The prediction of time-dependent changes is helpful when the dosing schedule needs to be simulated because of large daily fluctuations in the blood drug concentrations (Kanamitsu et al., 2000a; Ito et al., 2003). In addition, when the steady-state is reached after weeks of treatment due to slow kinetic processes, such as mechanism-based inhibition or enzyme induction, it is advantageous to predict time-dependent changes in these processes (Kanamitsu et al., 2000a; Ito et al., 2003). In particular, when the causative drug has both inhibitory and inductive potentials, the inhibitory effects may be greater than the inductive effects during the initial phase, but will be reversed later (Mikus et al., 2006; Liu et al., 2007) and, consequently, time-dependent changes might be critical.

Nevertheless, except for these particular situations, a time-independent approach to the prediction is frequently sufficient to adjust dosage or to select suitable regimens. When time-dependent change does not need to be considered, the theory and the prediction procedures are simplified greatly; the mean concentrations of the causative drug at the target site at steady-state are the only pharmacokinetics information necessary for prediction. In this paper, the descriptions will focus on the time-independent predictions considering general clinical needs and applicability to a wide range of drugs.

3.1. In vitro-based prediction of inhibitory DDIs

3.1.1. General issues

In order to predict the increase in the AUC of substrate drugs by CYP3A4 inhibition, *in vitro* kinetic data are frequently used in the literature (Kanamitsu et al., 2000b; Kanamitsu et al., 2000c; Yamano et al., 2001; Ito et al., 2004; Galetin et al., 2005; Ito et al., 2005). These methods generally involve two steps. The first step is a determination of the pharmacokinetics of the causative drug, and the second step is a prediction of the changes in concentrations of the victim drug based on altered CYP activity with the assumed interaction mechanism. The predictability of the *in vitro*-based method is restricted due to several difficulties, including a precise determination of the unbound inhibitor concentrations around the enzyme and a reliable *in vitro* measurement of the inhibition constant (K_i), even for a simple situation of competitive inhibition by unchanged drug. For more general situations, consideration should also be given to irreversible inhibition, an altered expression level of the enzyme, and a contribution of the metabolites to the DDIs.

3.1.2. Prediction of pharmacokinetics of causative drugs

It should be noted that the unbound drug concentrations of the causative drug at the target site, *i.e.* in hepatocytes and enterocytes, need to be determined for the quantitative prediction of DDIs (Wienkers and Heath, 2005). Based on the hypothesis of simple diffusion, the unbound concentrations of causative drugs in hepatocytes ($I_{h,u}$) are equal to the unbound concentrations of causative drugs in the portal vein ($I_{pv,u} = f_u \cdot I_{pv}$), which can be calculated by adding the systemic unbound drug concentrations ($f_u \cdot I_{sys}$) and increased concentrations by absorption as described by Eq. 1 (Kanamitsu et al., 2000a).

$$I_{h,u} = f_u \cdot I_{pv} = f_u (I_{sys} + \frac{D \cdot k_a \cdot F_a}{Q_h}) \quad (1)$$

where f_u, D, k_a, F_a, and Q_h are the unbound fraction in plasma, dose, rate constant for absorption (or gastric emptying rate as its maximum), absorption ratio, and hepatic plasma flow, respectively. When the drug is taken up into hepatocytes in a concentrative manner, the tissue-to-blood unbound concentration ratio needs to be determined. In Section 5, the determination of drug concentrations in hepatocytes will be discussed again.

3.1.3. Determination of inhibitory effect of causative drugs in vitro

3.1.3.1. Reversible inhibition

Reversible inhibition is the most likely mechanism of changes in CYP activity in the body. Reversible inhibitions of CYP are usually categorized as competitive, non-competitive, and mixed types (Ito et al., 1998b). Any substrates of CYP cause competitive inhibition of each other at high concentrations if they share the same recognition site on the enzyme. If the recognition site is different, non-competitive inhibition would take place.

For both competitive and non-competitive inhibition, if the plasma concentrations of a substrate are low enough compared with the Michael's constant of the metabolism, K_m, its intrinsic clearance (CL_{int}) decreases with a ratio of

$$\frac{CL_{int}}{CL^*_{int}} = 1 + \frac{I_u}{K_i} \quad (2)$$

where I_u represents the unbound inhibitor concentrations at the interaction site. In the following analysis, * denotes the parameter altered by the DDIs and/or polymorphism. As discussed in section 3-1-2, I_u may be substituted by $C_{h,u}$ defined by Eq. 1 when the hepatic active transport of the causative drug is negligible.

Equation 2 is the fundamental for the prediction of an inhibitory DDIs (Ito et al., 1998b), since, based on the well-stirred model, exposure of the drug after oral dosing is given in inverse proportion to the hepatic intrinsic clearance (Pang and Rowland, 1977), and AUC of the victim drug, which is cleared by hepatic metabolism, will increase in accordance with this ratio. Therefore, determination of the K_i values is essential for the *in vitro*-based prediction. In many situations, the K_i values are calculated from the IC_{50} under the assumption of competitive inhibition, although this is invalid for non-competitive inhibition. Some *in vitro* inhibitions of CYP3A4 activity show complicated atypical kinetics including auto-inhibition and auto-activation, and these phenomena have been explained by models involving multi-substrate recognition sites (Niwa et al., 2008b). Although this is a plausible explanation considering the broad substrate specificity and the relatively large substrate recognition sites of CYP3A4 (Yano et al., 2004), the number of *in vivo* demonstrations of atypical kinetics is small (Houston and Galetin, 2005) and, consequently, these complicated models have been applied in the prediction of *in vivo* DDIs in limited studies (Egnell et al., 2005; Henshall et al., 2008). This point will be discussed later in Section 5-2.

One of the problems associated with the quantitative prediction of DDIs is that the reported Ki values in the literature are often very variable from one study to another. Possible explanations for this include the binding to microsomal proteins and/or adsorption to the container of inhibitor drugs in the *in vitro* reaction system (Brown et al., 2006). It has been recognized that the rate of *in vitro* metabolism of some lipophilic substrates of CYP3A4 and CYP2C9 are increased by addition of albumin to the incubation medium (Baba et al., 2002; Matsumoto et al., 2002), and such phenomena would act against the unbound fraction theory. Recently, an interesting mechanism was reported for some CYP2C9-mediated interactions (Rowland et al., 2008). During *in vitro* incubation of human liver microsomes, unbound fatty acids are released from the membranes and these fatty acids potently inhibit the activity of CYP2C9. Since albumin binds strongly to the fatty acids, addition of albumin facilitated the metabolism by lowering the unbound concentrations of the fatty acids. Considering the complexity of the situation, evaluation of *in vitro* kinetics of

CYP metabolism should be carried out with great care (Baranczewski et al., 2006).

3.1.3.2. Mechanism-based inhibition

In the process of oxidative metabolism of drugs by CYP enzymes, oxygen is transferred from the heme to the drug and, subsequently, a reactive molecular species is generated in some situations, such as an epoxide or radical, which can bind covalently to the heme or the enzyme (Silverman, 1995; Kent et al., 2001; Venkatakrishnan et al., 2007). As a consequence, the CYP enzyme is inactivated permanently. This is a typical mechanism of irreversible binding and mechanism-based inhibition. The CYP is also inhibited strongly by substrates, such as macrolide antibiotics and diltiazem, which form relatively stable complexes with the heme (Lin and Lu, 1998). In these situations, the inhibition is classified as quasi-irreversible since the binding is not covalent. Irreversible and quasi-irreversible inactivation proceeds in a time- and concentration-dependent manner, and is detected as pre-incubation time-dependent inhibition during *in vitro* experiments. The inactivation is enhanced typically for several days *in vivo*, and reaches equilibrium between the inactivation and the generation rates of the enzyme (Takanaga et al., 2000). The maximum and steady-state change of the intrinsic clearance is calculated by

$$\frac{CL_{int}}{CL_{int}^{*}} = 1 + \frac{k_{deg} \cdot I_u}{k_{inact}(I_u + K_i)} \qquad (3)$$

where k_{deg} and k_{inact} are the rate constants for the degeneration and inactivation of the enzyme, respectively (Ito et al., 1998b; Venkatakrishnan et al., 2007). Among these parameters, k_{inact} is a characteristic of the target CYP enzyme and independent of the inhibitor used. Therefore, the values of K_i and k_{deg} need to be evaluated in an *in vitro* experiment for the prediction of mechanism-based inhibition. Mechanism-based inhibition of macrolides *in vivo* in humans has been successfully predicted from *in vitro* data based on this type of model (Kanamitsu et al., 2000b; Polasek and Miners, 2006).

3.2. In vivo-based prediction of inhibitory DDIs

3.2.1. General issues

In spite of the considerable success of *in vitro*-based prediction of DDIs, there have remained serious issues which limit its precision and applicability as discussed in

Section 3-1-1. Alternatively, a simple and reliable method has been recently proposed for systematic prediction of the magnitude of DDIs based on information readily available from *in vivo* clinical studies.

In Fig. 1, AUC increases of CYP3A4 substrates collectable from the literature were plotted against inhibitor drugs of CYP3A4 used clinically. The analysis is based on 113 *in vivo* studies reported in 78 articles published over the period 1983 to 2006 (Ohno et al., 2007). Although small numbers of substrates and inhibitors were removed due to the minor contribution or low selectivity for CYP3A4, we collected as many studies as possible if the report of a clinical DDI study included information on the increase in AUC. From the plot in Fig.1, it appears plausible to classify all the inhibitors and the substrates by the strength of inhibition and sensitivity to inhibition, respectively. In other words, the inhibitors and the substrates could be ranked in order. For example, azole antifungal agents, such as ketoconazole and itraconazole, increase the AUC of many substrate drugs most potently, followed by some macrolide antibiotics (Fig. 1). In addition, the AUC of simvastatin is markedly increased by itraconazole, suggesting the large contribution of CYP3A4 in the metabolism of this statin (Fig. 1). In contrast, the AUC increase of terithromycin by itraconazole is only two-fold due to the low contribution of CYP3A4 in the metabolism of this antibiotic (Fig. 1). Collectively, the magnitude of increase in AUC of victim drugs in the presence of inhibitors may be ranked by considering the contribution of CYP3A4 to the metabolism of the victim drugs (Fig. 1). These data suggest that we can determine two parameters representing the strength of inhibition and sensitivity to inhibition for each drug and, once these parameters are related to AUC changes appropriately, we can approximate the AUC changes for all the possible combinations of substrates and inhibitors using these parameters. Based on this concept, we have developed an *in vivo*-based method for the general prediction of AUC changes associated with inhibition of CYP3A4 (Ohno et al., 2007).

In this prediction method, it was assumed that DDIs occur in the liver, although it has been widely recognized that intestinal metabolism and efflux transport play a significant role in the first-pass clearance of oral drugs. This assumption was provisionally made due to the fact that it is practically impossible to separate the contributions between intestinal and hepatic metabolism for many drugs and, thus, they should be considered in combination. The contributions are hardly distinguishable because the same CYP species, CYP3A4, acts extensively in the intestine and the liver and, in addition, the substrate specificities of CYP3A4 and an

important transporter, P-gp, are similar. The contribution of the intestinal metabolism will be further considered in Section 6.

3.2.2. Determination of parameters and precision of prediction

The basic assumption of the *in vivo*-based method is that the magnitude of change in AUC in various DDIs is determined by two parameters, *i.e.* CR and IR (Fig. 1). The CR is the contribution ratio of the target metabolizing enzyme to the oral clearance of a substrate drug, and the IR is the inhibition ratio of the enzyme caused by an inhibitor drug. It is expected that the sensitivity of a substrate drug to various DDIs is determined by the CR value. In addition, we assumed that the IR value is constant over time and various substrates and, thus, entirely dependent on the inhibitor. Under these assumptions, Eq. 4 was derived which relates the change in AUC and these parameters for DDIs (Ohno et al., 2007):

$$\frac{AUC^*}{AUC} = \frac{1}{1 - CR \cdot IR} \tag{4}$$

Although Eq. 4 was previously introduced in our report (Ohno et al., 2007), its more generalized derivation with a consideration of renal clearance is described in the Appendix. Under an assumption of reversible inhibition, Eq. 4 can be rewritten using K_i and fraction of metabolism (f_m) which are more frequently used in the literature. However, we avoided the use of fm because ambiguity remains in its definition for non-metabolizing clearances. In addition, it should be noted that Eq. 4 also covers situations of irreversible inhibition and even inhibition by metabolites.

For clarification, the IR value relates to the K_i of reversible inhibition and of mechanism-based inhibition with Eqs. 5 and 6, respectively.

$$IR = \frac{I_u}{I_u + K_i} \tag{5}$$

$$IR = \frac{I_u}{I_u + \frac{k_{inact}}{k_{deg}}(I_u + K_i)} \tag{6}$$

From Eq. 4, CR can be obtained if IR and the AUC increase are known, and IR can be obtained if CR and the AUC increase are known. Indeed, the CR and IR values were

evaluated for many substrates and inhibitors relying entirely on clinical data without using any *in vitro* experimental data (Table 3). Once the CR and IR were evaluated, the magnitude of changes in AUC caused by any analogous DDIs between many drug combinations can be approximated according to Eq. 4.

In these analyses, the parameter CR is dose-independent, since linear pharmacokinetics of the victim drug is assumed. However, theoretically, IR depends on the dose of the causative drug. All the IR values in Table 3 were calculated from clinical data at usual therapeutic doses which do not vary markedly. If an adjustment is necessary for the IR value due to a deviated dose of the inhibitor drug, Eqs. 7 and 8 provide the adjusted value, IR', with an α-fold dose change for reversible and irreversible inhibition, respectively. Equations 7 and 8 were derived from Eqs. 5 and 6, respectively, assuming linear pharmacokinetics of the inhibitor drug.

$$IR' = \frac{\alpha \cdot IR}{(\alpha - 1)IR + 1} \tag{7}$$

$$IR' = \frac{\alpha \cdot IR}{(\alpha - 1)(\frac{k_{inact}}{k_{deg}} + 1)IR + 1} \tag{8}$$

In order to demonstrate the precision of the *in vivo*-based prediction, the magnitude of the increase in AUC by a drug interaction was predicted for the 60 combinations which correspond to clinical studies not used for calculation of CR and IR, and the results were compared with the observed values (Ohno et al., 2007). We found that the predictions agreed successfully with the observed increases in AUC within 67-150% for 50 clinical studies (83%), and within 50-200% for 57 clinical studies (95%) (Ohno et al., 2007). By applying Eq. 4, the magnitudes of the various inhibitory DDIs can be predicted systematically (Fig. 2).

4. Prediction of DDIs resulting from induction of CYP3A4

4-1. General issues

Induction of CYP enzymes has been initially studied in relation to activation of procarcinogens (Popper et al., 1973). As a consequence of a series of extensive studies, the roles of nuclear receptors in enzyme induction have been clarified and our knowledge of the mechanisms of induction has been greatly advanced (Lehmann et al., 1998; Michalets, 1998; Goodwin et al., 1999). Induction of CYP potentially reduces the

blood concentrations of substrate drugs to less than 1/10, which obviously results in ineffective pharmacotherapy (Niemi et al., 2003). CYP3A4 is one of the most inducible CYP isozymes (Guengerich, 1999), and the role of nuclear receptors (pregrane X receptor / farnesoid X receptor) has been widely recognized in the induction mechanisms (Lehmann et al., 1998; Michalets, 1998; Goodwin et al., 1999). Rifampicin is one of the most potent drug inducers of CYP3A4 in current therapeutic use(Ohno et al., 2008). Results of biopsy studies indicated that the hepatic content of CYP3A4 is approximately 3- to 5-fold higher after administration of rifampicin (Combalbert et al., 1989; Ged et al., 1989). In the same manner, rifampicin also induced CYP3A4 and P-gp in the intestine by approximately 3.3- to 4.4-fold (Greiner et al., 1999; Glaeser et al., 2005) and 3.5- to 4.2- fold (Westphal et al., 2000; Hamman et al., 2001; Glaeser et al., 2005), respectively. The synergistic effect of the induction of both CYP3A4 and P-gp both in the intestine and in the liver may be responsible for the marked reduction in clearance of many drugs coadministered with rifampicin.

4-2. In vitro-based prediction of inductive DDIs

To replicate the induction of metabolizing enzymes *in vitro*, human hepatocytes and immortalized liver cells (Li et al., 1997; Kostrubsky et al., 1999; Ripp et al., 2006) are frequently used. Currently, cryopreserved human hepatocytes are commercially available, although their viability is variable. In principle, the potential of enzyme induction should be evaluated by the increase in the target metabolizing activity. However, due to the difficulty in replicating physiological potent metabolizing activity in these cells for a sufficiently period, levels of immnoreactive enzyme protein or its mRNA are often determined as surrogates (Lin, 2006). For screening of the induction potential of new drug candidates, a semi-quantitative approach, such as a determination of a rank order for the increase in the mRNA, has been frequently applied (Sinz et al., 2008). However, reports of successful quantitative prediction of enzyme induction are limited. The E_{max} model expressed by the following equation was used for the quantitative prediction of enzyme induction (Kato et al., 2005):

$$R = 1 + \frac{E_{max} \cdot J_u}{EC_{50} + J_u} \quad (9)$$

where R, J_u, E_{max} and EC_{50} represent the induction ratio, unbound inducer

concentrations at the induction site, maximum induction ratio and concentrations at the half maximum induction, respectively. Prediction of CYP induction from *in vitro* experiments has recently been reviewed in detail (Hewitt et al., 2007).

4-3. In vivo-based prediction of inductive DDIs

The *in vivo*-based method was applied to predict CYP3A4-mediated inductive DDIs (Ohno et al., 2007). Kinetic analysis revealed that the reduction in the plasma AUC of a substrate of CYP3A4 produced by consecutive administration of an inducer of CYP3A4 could be approximated by Eq. 10 (Ohno et al., 2007).

$$\frac{AUC^*}{AUC} = \frac{1}{1 + IC \cdot IR} \tag{10}$$

where IC is the apparent increase in clearance due to the induction. The IC value is related to the *in vitro* parameters by Eq. 11.

$$IC = \frac{E_{max} \cdot J_u}{EC_{50} + J_u} \tag{11}$$

Many inductive DDIs have been successfully predicted in a quantitative manner with the *in vivo*-based method; indeed, the magnitude of the deviation between the mean values of the observed and predicted AUCs of all substrate drugs was less than 20% of the AUCs of the respective substrate drugs before administration of inducers (Ohno et al., 2007). By applying Eq. 10, the magnitudes of various inductive DDIs can be predicted systematically (Fig. 3). We found that the apparent clearance for CYP3A4 in humans was increased by 8.7-, 5.7-, 4.0-, 2.4- and 2.2-fold after repeated administration of rifampicin, phenytoin, carbamazepine, efavirenz and St John's wort, respectively, at clinically conventional doses (Table 4; Ohno et al., 2007).

From Eqs. 10 and 11, the dose-dependence of the IC value with an α-fold change of dose is derived from Eq. 12 assuming linear pharmacokinetics of the inducer drug.

$$IC' = \frac{\alpha \cdot IC}{(\alpha - 1)IC + E_{max}} \tag{12}$$

Based on AUC changes of a substrate drug associated with different doses of an

inducer drug, the E_{max} value can be evaluable using Eq. 12.

5. *In vitro-in vivo* relationships of DDIs

5-1. General issues

Although it might sound paradoxical, the characteristics of the *in vivo*-based method offer a unique opportunity to examine relationships between *in vitro* and *in vivo* experimental data in a comprehensive manner. In some *in vitro*-based prediction studies, *in vivo* information has already been incorporated such as adjusting factors to bridge quantitative gaps between *in vitro* and *in vivo* data (Einolf, 2007). In other situations, the liver-to-plasma concentration ratio of inhibitors is determined as a ratio of K_i values obtained from *in vitro* and *in vivo* experiments (Kato et al., 2008). The strategies used in these studies are logical, however, it should be noted that the observed predictability did not represent pure *in vitro-in vivo* relationships. In this section, IR and CR values are used to compare the *in vitro* and *in vivo* evaluations of DDIs.

5-2. Comparison of inhibition ratio (IR)

The IR values for hepatic clearance are related to the *in vitro* K_i by Eq. 5 when competitive, non-competitive, or mixed type inhibition is involved (Ito et al., 1998b). Accordingly, the relationship between I_u/K_i and IR is obtained as:

$$\frac{I_u}{K_i} = \frac{IR}{1 - IR} \tag{13}$$

It should be noted that the term on the right side of Eq. 13 is obtained entirely from *in vivo* observations whilst the K_i term on the left side term is an *in vitro* measure. Therefore, with a suitable approximation of I_u, it is possible to compare *in vitro* and *in vivo* observations directly.

It has been reported that the use of $I_{pv,u}$ (Eq. 1) as I_u, even although theoretically relevant, failed to explain the *in vivo* DDI appropriately from *in vitro* K_i values (Ito et al., 2004). Here, concerning the IR values determined *in vitro* and *in vivo*, we performed analysis using comprehensive data reported in the literature (Ito et al., 2004). In Fig. 4, IR/(1 – IR) values were plotted against five kinds of parameters according to the analysis by (Ito et al., 2004); these include mean unbound concentrations in the systemic circulation divided by K_i ($I_{sys,u}$ / K_i; Fig. 4A), mean total

(unbound and bound) concentrations in the systemic circulation divided by K_i (I_{sys} / K_i; Fig. 4B), maximum total concentrations in the systemic circulation divided by K_i ($I_{sys,max}$ / K_i; Fig. 4C), unbound concentrations in the portal vein (Eq. 1) divided by K_i ($I_{pv,u}$ / K_i; Fig. 4D), and total concentrations in the portal vein divided by K_i (I_{pv} / K_i; Fig. 4E). The vertical position of the symbols in Fig. 4 presents IR/(1-IR) calculated from DDIs with standard substrate drugs, such as midazolam, whereas the horizontal position presents the geometric mean calculated by the reported K_i values. The K_i values of inhibitors were collected from *in vitro* experiments using a variety of substrate drugs in the literatures listed in the legend to Fig 4. Here, we have to note that, in many reports, the binding of inhibitors to microsomal proteins and/or adsorption to the container of inhibitor drugs in the *in vitro* reaction systems are not considered. In Fig. 4, dotted lines indicate the consistency between *in vitro* and *in vivo* observations with a precision of 50~200% which was satisfied for most situations with the *in vivo*-based prediction method. Among the parameters investigated, the *in vitro-in vivo* relationships could most reasonably be explained by the theoretically irrelevant I_{pv} (Fig. 4E). When other drug concentrations were used, unacceptable underestimations of the *in vivo* exposure change, ranging from several-fold to several hundred-fold, were always obtained (Figs. 4A-D).

It should be noted that the intra-portal drug concentrations were calculated as a sum of I_{sys} and the concentrations due to newly absorbed drug from the intestine as described in Eq. 1. For many drugs, I_{sys} is significantly lower than the concentrations due to newly absorbed drug. In addition, the latter concentrations adopted in the analysis were completely in proportion to the dose, since the other parameters in Eq. 1, *i.e.* k_a, F_a and Q_h were fixed to 0.1 min^{-1}, 1 and 1,610 ml/min (Ito et al., 2004), respectively, in the present analysis. Collectively, the total intra-portal inhibitor concentrations, I_{pv}, were primarily regulated by the dose. Based on these considerations, IR/(1-IR) was plotted against K_i/dose in Fig. 4F. As expected, the association in Fig. 4F was as good as when I_{pv} was used for the analysis in Fig. 4E.

There would be several reasons to explain the poor association of IR values between *in vitro* and *in vivo*, even if theoretically appropriate $I_{pv,u}$ values were used in the analysis (Fig 4D). It may be due to the methodological restrictions of the experiments, that ignore concentrative uptake of drugs into the hepatocytes and also ignore the contribution of the metabolites to inhibition. In addition, the irreversible and quasi-irreversible inhibitions also need to be taken into considerations.

Although quantitative association between I_{pv} and *in vivo* IR was observed

in Fig. 4E, this relationship may be a pseudo one. Most likely, it may be indicated that a simple K_i/dose (Fig. 4F) is sufficient to predict *in vivo* DDIs from *in vitro* data under the current methodological restrictions. From the analysis presented in Fig. 4F, an empirical criterion of the significant DDI was newly obtained. Since the dimension of the horizontal axis of Fig. 4F is the volume, the analysis indicated that, if the daily dose is dissolved in a volume of 20 L (2~200 L, when variability is considered), and if the resulting drug concentrations are higher than K_i, the drug will cause significant DDIs with the doubled AUC of sensitive substrates (such as midazolam and simvastatin) due to inhibition of CYP3A4. This relationship would be a useful criterion in order to roughly predict *in vivo* DDIs from *in vitro* information. There are reports that a K_i value of 1 μM is appropriate for the detection of potential DDIs (Tucker et al., 2001; Obach et al., 2006). However, we recommend use of the dose together with K_i wherever possible, since the correlation deteriorates if only the K_i value is plotted against IR/(1-IR) (data not shown).

Concerning the comparison of IR values between *in vitro* and *in vivo*, we also have to refer to the previously accepted concept that CYP3A4 has multiple substrate recognition sites and the inhibition profiles of inhibitors are substrate-dependent and even atypical for some instances as discussed in Section 3-1-3-1 (Korzekwa et al., 1998; Wang et al., 2000). The presence of different substrate subgroups for CYP3A4 was suggested based on cluster analysis of CYP3A4 inhibition data (Kenworthy et al., 1999), and further mechanistic studies have been performed (Houston and Galetin, 2003). In their *in vitro* analysis, the inhibition profile of nifedipine was moderately different from that of other substrates (Kenworthy et al., 1999). However, in our *in vivo* analysis, the predictability of nifedipine is similar to other substrates as demonstrated by the small MPE and RMSE values in Table 3. Considering multiple factors of *in vivo* variability, it is plausible that we cannot detect atypical kinetics of CYP3A4 in most clinical settings.

5-3. Comparison of contribution ratio (CR)

The CR values for metabolizing enzymes and/or elimination pathways would be one of the most valuable parameters in clinical pharmacokinetics. Determination of CRs is helpful in quantitatively predicting the probable DDIs in humans. As discussed in Section 7, with CR values, we can also predict pharmacokinetic changes in subjects with a single nucleotide polymorphism (SNP) in CYP genes. To reduce the overall risks of DDIs in the current pharmacotherapy, it is important to obtain