quantitative and systematic information on CYP reaction profiles not only for new drugs but also for drugs currently on the market.

In general, for the determination of CR or f_m values *in vitro*, drugs are incubated with human hepatic microsomes in the absence and presence of a CYP isozyme-selective inhibitor or antibody. The contribution of a particular CYP enzyme is calculated from the reduced metabolizing activity produced by the addition of an inhibitor or antibody (Newton et al., 1995; Rodrigues, 1999; Tucker et al., 2001). In this determination, the metabolizing activity is measured by a reduction in the parent drug or by an increase in the particular metabolite (Tucker et al., 2001). As an alternative method, drugs are incubated with the recombinant CYP enzymes obtained after transfection of cDNA and the contribution ratio is calculated by considering the expression level of CYP enzymes in the human liver, which is referred to as the relative activity factor (RAF) method (Yamazaki et al., 1997; Venkatakrishnan et al., 1998; McGinnity et al., 2000; Uttamsingh et al., 2005).

In order to determine the CR values precisely, it is also important to perform experiments at low substrate concentrations to keep the kinetics within a linear range. When the metabolizing rate is measured by production of a particular metabolite, the simultaneous production of other metabolites mediated by the same enzyme also needs to be considered carefully. In some situations, the decrease in the parent compound would be a better index than the production of a metabolite. It has been repeatedly pointed out that the unbound drug concentrations in the incubation medium should be checked for accuracy as discussed in Section 5-2 (Wienkers and Heath, 2005; Brown et al., 2006).

In the present article, the CR values of CYP3A4 substrates calculated from results of *in vitro* experiments were plotted against *in vivo* CR values (Fig. 5). In this analysis, some of substrates possess inhibitory and/or induction potential for CYP enzymes. Irrespective of the fact that significant variations were observed, 29 of 38 *in vitro* data sets (78%) were within a range of 50~200% of the *in vivo* observations, suggesting that CR is predictable from *in vitro* experiments (Fig. 5). It is worthy of note that all *in vivo* CR values outside this range were lower than *in vitro* CR values (Fig. 5). In addition, the drugs in this group include protease inhibitors for HIV treatment and carbamazepine, which themselves are substrates of CYP3A4 but also possess inhibitory and/or induction potential for CYP enzymes. It is plausible that, in some of these data, poor correlations between *in vitro* and *in vivo* CR values would be due to these complicated factors. Although further analysis remains to be performed,

the results of this analysis suggested the feasibility of the prediction of CR values of CYP substrates from *in vitro* experiments if the inhibitory and/or induction potentials of CYP are minimum. Although only a limited number of data sets evaluated by the RAF method was used in the present analysis, it provided unbiased predictions of *in vivo* CR values (Fig. 5), suggesting the suitability of the method.

6. Significance of intestinal metabolism in prediction of DDIs

6-1. General issues

It has been well established that a number of drugs are metabolized extensively in the intestine as well as in the liver (Wu et al., 1995; Holtbecker et al., 1996; Thummel et al., 1996; Lampen et al., 1998). Actually, most of the intestinal metabolism is attributed to CYP3A4, although expression of other CYPs, such as CYP2C9 and CYP2J2, has been reported in the intestine (Ding and Kaminsky, 2003; Paine et al., 2006a). (Thummel et al., 1996) analyzed the disposition of a typical substrate of CYP3A4, midazolam, and its metabolite during surgery on liver transplant recipients. The analysis during the anhepatic phase indicated that intraduodenaly administered midazolam was metabolized in enterocytes with an extraction ratio of 0.43. In another set of experiments, they administered midazolam to healthy volunteers intravenously or orally and found that the oral bioavailability was 0.30-0.44 (Thummel et al., 1996). Collectively, the extraction ratios of midazolam in the intestine and liver were determined as 0.43 and 0.47, respectively, suggesting that the small intestine, along with the liver, plays an important role in the first-pass effect of drugs (Thummel et al., 1996).

We also have to consider a synergistic action by P-gp with CYP3A4 in the detoxification of xenobiotics in the intestine. By transporting a broad range of drugs from the enterocytes back to the intestinal lumen, P-gp is responsible for reducing the fraction of drug absorbed (Saitoh and Aungst, 1995; Wacher et al., 1998; Benet et al., 1999; Suzuki and Sugiyama, 2000). In addition, P-gp may have functional significance in preventing the saturation of metabolizing enzyme in enterocytes by eliminating the drug molecules into the lumen. Synergistic role of CYP3A4 and P-gp is supported by the overlapping substrate specificity, similar tissue distribution and induction profiles. In fact, differentiation of the contribution of P-gp from that of CYP3A4 is not clear and not practical in most instances (Suzuki and Sugiyama, 2000). It is worthy of note that the expression level of CYP3A4 in the human intestine is only 1/100 of that in the liver (Paine et al., 1997; Yang et al., 2004). In turn, under an

assumption of the clearance theory, if the first-pass extraction ratios of the intestine and the liver are comparable as reported for midazolam, the AUC of substrate drugs around the small intestinal CYP3A4 should be 100-fold higher than that around the hepatic CYP3A4. This high AUC value of midazolam in the intestinal enterocytes compared to that in the hepatocytes may be explained by the longer exposure of midazolam, which at least partly may be ascribed to efflux transport and intestinal micro-circulation caused by P-gp.

CYP3A4 and/or P-gp-related DDIs also take place in the intestine (Fuhr, 1998; Saito et al., 2005a). It has been shown that grapefruit juice increases the bioavailability of felodipine to 206% due to inhibition of CYP3A4 in the intestine; grapefruit juice causes a significant increase in the bioavailability when the drug is given orally, but not when given intravenously. The inhibition is irreversible and takes several days to recover (Kupferschmidt et al., 1995; Lilja et al., 2000). Several components of grapefruit juice were proposed as responsible for the inhibition (Ameer and Weintraub, 1997), and it was shown that furanocoumarins, such as 6',7'-dihydroxybergamottin, caused significant mechanism-based inhibition of CYP3A4 (Schmiedlin-Ren et al., 1997). Indeed, the inhibition potential of grapefruit juice is abolished when furanocoumarins were removed by absorption on to resins (Paine et al., 2006b). The mechanism-based inhibition of grapefruit juice may induce post-transcriptional regulation of CYP3A4 since the expression level was reduced without a reduction in mRNA (Bailey et al., 1998). It should be noted that drinking large amounts of grapefruit juice caused changes of hepatic CYP3A4 together with intestinal CYP3A4 (Veronese et al., 2003).

In this section, we would like to consider potential prediction errors produced by ignoring intestinal metabolism in the *in vivo*-based method using a Monte-Carlo simulation.

6-2. Theoretical considerations

The ratio of intestinal intrinsic clearance in the absence and presence of inhibitors can be determined by Eq. 14 (Wang et al., 2004).

$$\frac{F_g^*}{F_g} = \frac{1}{F_g + (1 - F_g) \times \dfrac{CL_{int,g}^*}{CL_{int,g}}} = \frac{1}{1 - E_g \cdot IR_g} \qquad (14)$$

where $CL_{int,g}$ is the intrinsic clearance of enterocytes, E_g is the intestinal extraction ratio given by $1-F_g$, and IR_g is the intestinal inhibition ratio given by $1-CL^*_{int,g}/CL_{int,g}$. Equation 14 assumes a well-stirred model for enterocytes in the presence of a hypothetical drug flow through the cells regulated by diffusion and transport. In this section, only metabolism by CYP3A4 is considered in the intestine for simplicity. The exact model herein assumed that intestinal and hepatic extractions together play a role in the first-pass effect of an oral drug. In the exact model, the overall AUC will be increased in the presence of inhibitor by a ratio which can be calculated from Eq. 15 obtained by multiplying Eq. 7 by Eq 14:

$$\frac{AUC^*}{AUC} = \frac{1}{(1 - CR_h \cdot IR_h)(1 - E_g \cdot IR_g)} \tag{15}$$

where CR_h and IR_h are the contribution ratio and the inhibition ratio of hepatic clearance, respectively. In contrast, under an assumption of the simple model, the intestinal metabolism was mathematically not considered as discussed in the previous sections. The AUC changes can be calculated from Eq. 4 in the simple model. The exact model is more accurate, but not applicable to many drugs, since we do not know the E_g and IR_g values for many drugs. Thus, we can compare the predicted changes in AUC between the simple and exact models with Eqs. 4 and 15, respectively.

In general, CR and IR are calculated from an AUC change when a potent inhibitor or a selective substrate, respectively, is coadministered (Ohno et al., 2007). Accordingly, by substituting IRs in Eqs. 4 and 15 with 1, we can obtain relationships between CRs for the simple and the exact models:

$$CR^\circ = CR_h + E_g(1 - CR_h) \tag{16}$$

The superscript of o denotes the parameter of the simple model. Similarly, substitutions of CRs in Eqs. 4 and 15 with 1 yield relationships between IRs:

$$IR^\circ = IR_h + E_g \cdot IR_g(1 - IR_h) \tag{17}$$

From Eqs. 16 and 17, it is apparent that CR and IR of the simple model overestimate

CR_h and IR_h of the exact model, respectively, when the intestinal metabolism is evident. To compare the prediction ability of the two models, the magnitudes of DDIs were simulated by generating CR_h, IR_h, E_g, and IR_g values randomly. Based on the generated parameters, the changes in AUC were calculated with the exact model using Eq. 15 and plotted against predictions from the simple model (Eq. 4), in which the CR and IR values were calculated from Eqs. 16 and 17, respectively.

As shown in Fig. 6A, when the E_g values were generated randomly with a range of 0 to 1, the simple model overestimated the AUC increase considerably. However, when the range of E_g was limited to less than 0.6, the magnitude of overestimation was reduced markedly (Fig. 6B).

These results may support the suitability of our analysis method described by Ohno et al. (2007). In a series of analyses of the CR and IR values in Table 3 for CYP3A4, midazolam was used for calculation of the IR values for most situations (Table 3; (Ohno et al., 2007)). E_g of midazolam was determined to be 0.43 which is less than 0.6. Furthermore, when a moderate correlation was introduced between the intestinal and hepatic inhibition ratios ($r = 0.6$ between IR_g and IR_h), the tendency towards overestimation largely disappeared (Fig. 6C). Moderate correlation between IR_g and IR_h seems reasonable, since the molecular species of the target enzyme (*i.e.* CYP3A4) is the same in the intestine and the liver. From these simulations, it was suggested that the AUC increase can be predicted by the simple *in vivo*-based model which assumes only hepatic metabolism with acceptable accuracies, when E_g of the test substrate is moderate and a moderate correlation exists between the inhibition ratios in the intestine and the liver.

7. Consideration on genomic polymorphisms

7-1. General issues

The impact of polymorphism of CYP enzymes on pharmacokinetics and pharmacotherapy is widely recognized and has been extensively reviewed (Rodrigues and Rushmore, 2002; Ingelman-Sundberg, 2004; Ingelman-Sundberg et al., 2007; Kirchheiner and Seeringer, 2007). With respect to CYP3A enzymes, it is believed that considerable variability between subjects in the expression of CYP3A4 is not regulated by genetic polymorphism to a notable degree (Floyd et al., 2003; Haufroid et al., 2004; Wong et al., 2004). In contrast to CYP3A4, the expression of CYP3A5 is highly dependent on genetic polymorphism. It has been shown that the contribution of SNPs in CYP3A5 to the overall clearance is moderate for many drugs due to its analogous

substrate specificity with CYP3A4 (Lamba et al., 2002; Floyd et al., 2003; Williams et al., 2002; Galetin et al., 2004). Collectively, based on our currently available information, clinical significance of the genotyping of CYP3A enzymes would be controversial. In contrast to the comparable metabolizing activities of CYP3A4 and CYP3A5, recent studies have shown that inhibition of CYP3A5 is significantly less compared with CYP3A4 in a number of instances (Spizhovyi et al., 1983; Gibbs et al., 1999; Patki et al., 2003; McConn et al., 2004; Wang et al., 2005; Niwa et al., 2008a). One of such examples is the inhibition by raloxifen. Raloxifene is a mechanism-based inhibitor of CYP3A4, and it has been shown recently that the compound is metabolically activated and then covalently bound to Cys239 of CYP3A4 (Baer et al., 2007). However, raloxifene is not a mechanism-based inhibitor of CYP3A5 due to lack of cysteine in the region of CYP3A5 compatible to that of CYP3A4 (Pearson et al., 2007). Concerning the CYP isozymes other than CYP3A4/5, CYP2D6, CYP2C9 and CYP2C19 are representative polymorphic enzymes and individualized pharmacotherapy is being considered for drugs like tamoxifen, warfarin and omeprazole, respectively (Beverage et al., 2007; Furuta et al., 2007; Caraco et al., 2008).

In this section, we would like to discuss the effect of SNPs on the pharmacokinetic changes of victim drugs exhibiting multiple metabolic pathways following the co-administration of causative drugs.

7-2. Effect of SNPs on the pharmacokinetic DDIs

When the activity of a metabolizing enzyme is altered by the presence of SNPs, the AUC of blood drug concentrations after oral dosing will be changed according to Eq. 18.

$$\frac{AUC^{*}}{AUC} = \frac{1}{1 + PC_{snps} \cdot CR_{snps}} \tag{18}$$

where CR_{snps} is the contribution ratio of a CYP enzyme subject to polymorphism (such as CYP2C9, 2C19, and 2D6), and PC_{snps} is the personal change in a clearance pathway subject to polymorphism which is defined as $\frac{CL_{int}^{*}}{CL_{int}} - 1$, where CL_{int} denotes the CL_{int} in the wild type subjects and CL_{int}^{*} denotes the CL_{int} for each genotyped subjects. Consequently, PC_{snps} equals -1 for a poor metabolizer (PM), $-1 < PC_{snps} < 0$ for an intermediate metabolizer (IM), 0 for an extensive metabolizer (EM) and a positive

value for an ultra-rapid metabolizer (UM). Equation 18 was derived based on similar considerations to those for Eq. 4. In this section, the intestinal metabolism is not considered for simplification. The presence of SNPs markedly affects the magnitude of DDIs. In subjects having a variety of SNPs, changes in AUC associated with coadministration of inhibitor drugs are determined by Eq. 19 in relation to the AUC of the EM without coadministration of the inhibitor drug:

$$\frac{AUC^*}{AUC} = \frac{1}{1 + CR_{snps}(PC_{snps} - IR + PC_{snps} \cdot IR)} \tag{19}$$

Equation 19 is relevant when the polymorphic enzyme is inhibited by coadministration. In Fig. 7B, the changes in AUC in this situation were simulated. In the absence of an inhibitor, AUCs of a substrate drug in PM and IM are higher than those in EM and UM. In the presence of the inhibitor, the magnitude of changes caused by the DDI is more evident in EM and UM compared with PM and IM in this situation. The actual DDIs of omeprazole and moclobemide (Yu et al., 2001; Cho et al., 2002), and omeprazole/lansoprazole and fluvoxamine have been reported in EM and PM of CYP2C19 (Yasui-Furukori et al., 2004a; Yasui-Furukori et al., 2004b). In these studies, AUC of the CYP2C19 substrate, omeprazole, lansoprazole or moclobemide, was changed by coadministration of the inhibitor of CYP2C19, omeprazole, moclobemide or fluvoxamine, in EM, but not in PM. Analogous outcomes were reported for DDIs of diphenhydramine and metoprolol (Hamelin et al., 2000), quinidine and venlafaxine (Eap et al., 2003), diphenhydramine and venlafaxine (Lessard et al., 2001), and fluoxetine and risperidone (Bondolfi et al., 2002) in subjects having a variety of SNPs of CYP2D6. In these studies, metoprolol, venlafaxine, and risperidone were victim drugs of the DDI associated with inhibition of CYP2D6.

In contrast to the DDIs in PM and IM in the above, the situation changes greatly for substrate drugs which are metabolized by both a polymorphic enzyme and another enzyme subjected to DDIs. The changes in AUC in SNPs subjects concomitantly receiving such substrate and inhibitor drugs is described by Eq. 20:

$$\frac{AUC^*}{AUC} = \frac{1}{1 + PC_{snps} \cdot CR_{snps} - CR \cdot IR} \tag{20}$$

The simulated AUC changes in this situation are demonstrated in Fig. 7C. In the

absence of an inhibitor, AUC is higher in PM and IM than in EM and UM which is the same situation presented in Fig. 7B. However, in the presence of the inhibitor, the AUC increases produced by DDI is more evident in PM and IM, which is the opposite situation to that of Fig. 7B. Actual DDI has been reported for the combination of ritonavir, a potent inhibitor of CYP3A4, and voriconazole which is metabolized by both CYP3A4 and polymorphic CYP2C19 (Michalets, 1998). Indeed, in the absence of ritonavir, the AUC of voriconazole was significantly higher in PM of CYP2C19 whereas it increased much more in PM compared with EM following coadministration of ritonavir; the $AUC_{0\text{-infinite}}$ of voriconazole in PM and EM receiving the coadministration of ritonavir was 1.5- and 26-fold higher, respectively, than the AUC of voriconazole in EM subjects without receiving coadministration of ritonavir (Michalets, 1998).

Even more complicated situations have been reported regarding SNPs and DDIs. The changes in AUC of voriconazole described above are relevant only for a single dose of ritonavir. After multiple doses of ritonavir, the AUC of concomitantly dosed voriconazole was markedly reduced in many subjects presumably due to induction of CYP2C19 by ritonavir (Michalets, 1998). However, as an evident exception, the AUC of voriconazole was markedly increased after consecutive doses of ritonavir only in one subject in this study. Although genotyping was not carried out in this study, it may be possible that this subject was a PM of CYP2C19 (Michalets, 1998).

The synergism of DDIs and SNPs could be clinically very dangerous in some situations. Codeine is metabolized by polymorphic CYP2D6 to yield pharmacologically active morphine. Therefore, the efficacy of codeine is expected to be less in PM of CYP2D6 compared with EM. On the other hand, coadministration of an inhibitor of CYP3A4 potentiates the efficacy of codeine because codeine is metabolized to inactive metabolites by CYP3A4, and morphine is deactivated also by CYP3A4 (Caraco et al., 1996; Projean et al., 2003). Life-threatening opioid intoxication was found in a patient after he received small doses of codeine (Gasche et al., 2004). Genotyping showed that the patient was a UM of CYP2D6, thus it was suggested that the excessive production and the reduced clearance of morphine synergistically operated on this severe adverse event.

Based on these considerations, it is recommended that volunteers enrolled in clinical DDI studies are genotyped for the major polymorphic metabolizing enzymes and transporters, since the magnitude of any interactions could be fundamentally changed depending on the genotypes as discussed in this session. In some instances, determination of CRs for multiple CYP species involved in the metabolism of substrate

drugs may be important as discussed above. The CR values determined from *in vitro* experiments will be of immense value in predicting SNPs-dependent DDIs in a clinical setting. If such SNPs-dependent DDIs are anticipated, it may be better to perform *in vitro* experiments using hepatic microsome preparations obtained from subjects having the corresponding SNPs, if possible.

8. Limitations of the *in vivo*-based prediction method

Although the *in vivo*-based prediction methods have been successfully applied to many drugs for both inhibitory and inductive DDIs related to drug metabolism, it should be noted that there are two categories of DDIs in which the method did not work properly. The first category is DDIs caused by mechanism(s) other than the activity change of the metabolizing enzymes. For example, cyclosporine A was excluded from the prediction as an inhibitor of CYP3A4, although it is widely recognized as a substrate and an inhibitor of CYP3A4. This is because cyclosporine inhibits several important transporters, such as P-glycoprotein or organic anion transporting polypeptide 1B1 (OATP1B1/SLC21A6). Indeed, the administration of cyclosporine A results in significant DDIs with poor substrates of CYP3A4, such as cerivastatin, *via* a transporter-mediated mechanism (Shitara et al., 2004; Shitara and Sugiyama, 2006). In order to apply the *in vivo*-based method to DDIs caused by changes entirely in transporter activity, theoretical extensions would be necessary since drug transport is a reversible process in principle and different from usual irreversible metabolism.

The second category is DDIs caused by drugs which simultaneously inhibit and induce metabolic enzymes as discussed in Sections 3 and 5-3. In some instances, the inhibition is associated with a mechanism-based mechanism, which may lead to the nonlinear and time-dependent pharmacokinetics of both victim and causative drugs. Typical drugs in this group are protease inhibitors used for the treatment of HIV infection, such as ritonavir and nelfinavir. Indeed, these drugs increased the AUC of triazolam (Greenblatt et al., 2000), sildenafil (Muirhead et al., 2000), and simvastatin (Fichtenbaum et al., 2002), but reduced the AUC of ethinylestradiol (Ouellet et al., 1998), zidovudine (Cato et al., 1998), and voriconazole (Liu et al., 2007). For analysis of DDIs of this group, construction of a more precise *in vivo* / *in vitro* pharmacokinetic model would be required in which the time profiles of the disposition of causative drugs as well as the changes in activity of metabolizing enzymes are also taken into account.

Currently, the *in vivo*-based method has been extensively applied to DDIs

caused by changes only in CYP3A4 activity. Further studies are underway in our laboratory to apply the method to hepatic metabolizing enzymes other than CYP3A4.

9. Application to the clinical development of novel drug candidates

In a new drug development process, a number of drug interaction studies need to be carried out with drugs which may interrupt the detoxification pathway of the drug candidate. The number of clinical drug interaction studies has increased in the last decade and the burden of such experiments has become significant for pharmaceutical companies. Nevertheless, the information obtained in these studies is insufficient to predict many drug interactions that may occur in clinical settings. Considering the precision and broad applicability of the *in vivo*-based DDI prediction method, it may be useful in the novel drug development process to construct a strategy for drug interaction studies. Our proposals for the strategy may be summarized as follows.

For drug candidates which may inhibit or induce CYP enzymes, the following procedure may be used for the quantitative prediction of their ability to alter the pharmacokinetics of various victim drugs in patients.

- An *in vitro* study should be conducted to evaluate Ki values for significant reversible inhibition of CYPs if observed. The potential for irreversible inhibition should also be investigated. As far as the potential for induction is concerned, studies with human-derived cells will be useful. These studies are already being carried out routinely in many pharmaceutical companies. However, we would like to point out here that irreversible inhibition, particularly caused by metabolites, is not always carefully investigated compared with reversible inhibition.
- A clinical study will be conducted to evaluate inhibitory or inductive DDIs, if their potential has been suggested by *in vitro* experiments. We would like to propose the criterion of a Ki/dose of 2L for conducting clinical DDI studies for reversible inhibition based on the analysis presented in Fig. 4F. The pharmacokinetics of a standard CYP substrate with a known CR (such as midazolam and simvastatin for CYP3A4; Table 3) would be used before and after consecutive dosing of a drug candidate, typically for 5 to 10 days. The dose of the substrate drug may need to be reduced due to safety concerns when a significant increase in the AUC is anticipated.
- From such *in vivo* studies, the IR and/or IC value for this compound will be calculated. If considerable inhibition or induction is observed in a clinical study, it should be recommended to confirm the DDI potential with an additional typical substrate with a known CR (Table 3), and the results should be compared with the previous value. Once reliable IR or IC values are obtained, we can predict the

magnitude of AUC changes of many CYP substrates with known CR values using Eq. 4 or Eq. 10.

For drug candidates which are metabolized by CYPs in *in vitro* experiments, the following procedure may be used for the quantitative prediction of changes in the AUCs produced by coadministered inhibitors and inducers.

- The CR value of drug candidates should be determined from *in vitro* studies using human liver microsomes or expressed enzymes. To determine the CR, clearances other than those due to CYP metabolism should also be considered.
- If the determined CR value is significant (typically more than 0.2), a clinical pharmacokinetics study will be performed to confirm the CR value *in vivo*. For inhibitory DDI studies, a standard inhibitor of CYP, typically itraconazole or ketoconazole for CYP3A4, would be coadministered to examine the increase in AUC. For inductive DDI studies, a standard inducer, typically rifampicin for CYP3A4, would be coadministered to examine the reduction in AUC.
- From these results, the CR values of a candidate drug can be calculated. If the CR evaluated *in vivo* is fairly high (typically more than 0.5), an additional clinically study would be recommended for confirmation with another typical inhibitor or inducer, and the results should be compared with the predicted value.
- Theoretically, it is important to know the contributions of all major elimination pathways for each drug candidate. Therefore, it is recommended to continue the evaluation of CR values for each elimination pathway, until their sum becomes almost one (typically more than 0.7).

By applying and extending these methods, it will be possible to predict almost all clinically significant pharmacokinetic DDIs *via* drug metabolism in a quantitative manner in the future.

10. Conclusion

Traditional *in vitro*-based prediction methods of DDIs are very important to minimize the potential disadvantages of new drug candidates during the preclinical development phase. Furthermore, it is necessary to fully understand the mechanisms of DDIs observed in clinical studies. However, the predictability of the *in vitro*-based methods is limited, and therefore, *in vivo* clinical studies are required to evaluate potential DDIs quantitatively. The *in vivo*-based prediction method would shed light on this situation because it is applicable to many drugs with acceptable precision. This method may be useful in predicting the magnitude of DDIs in clinical situations and in designing clinical studies during the course of novel drug development. The severity of DDIs is potentially multiplied markedly in the presence of a genetic

polymorphism in the drug metabolizing enzymes. Pharmacogenomic testing should be widely used in clinical studies in which DDIs are examined. The prediction of DDIs could play an essential role in alerting clinical personnel to these situations, since it is not always possible to evaluate DDIs which can occur in only a small population of genotypes by clinical studies.

Appendix

Theoretical considerations

The oral clearance, CL_{oral}, of drugs is described by Eq. A1 (Ohno et al., 2007).

$$F_a \cdot F_g \cdot CL_{oral} = f_u \cdot CL_{int,h} + \frac{CL_r}{F_h} \quad \text{(A1)}$$

where F_a, F_g, F_h, f_u, $CL_{int,h}$ and CL_r are the absorption ratio, intestinal availability, hepatic availability, unbound fraction in the blood, hepatic intrinsic clearance and renal clearance, respectively. For simplicity, F_g is regarded as 1.0 in the present analysis. The significance of intestinal metabolism was discussed in Section 6 of the main text in detail. Based on the hypothesis of the well-stirred model, Eq. A1 is rewritten as follows.

$$F_a CL_{oral} = f_u \cdot CL_{int,h} + \frac{f_u \cdot CL_{int,h} + Q_h}{Q_h} CL_r$$

$$= (1 + \frac{CL_r}{Q_h}) f_u \cdot CL_{int,h} + CL_r \quad \text{(A2)}$$

The contribution ratio of clearance in pathway x to oral clearance (CR_x) is defined by

$$CR_x = \frac{CL_x}{CL_{oral}} = \frac{CL_{oral} - CL_{oral,-x}}{CL_{oral}} \quad \text{(A3)}$$

where $CL_{oral,-x}$ is an altered *in vivo* oral clearance when the clearance in pathway x (CL_x) is absent. This situation corresponds to when the enzyme is genetically deficient or its activity is suppressed completely by a selective inhibitor. We can assume that the hepatic intrinsic clearance is the sum of several pathways of hepatic intrinsic clearances ($CL_{int,h,i}$, i = 1 to n) with Eq. A4.

$$CL_{int,h} = \sum_{i=1}^{n} CL_{int,h,i} \qquad \text{(i =1 to n)} \quad \text{(A4)}$$

Based on Eq. A2 to A4, the contribution ratio of the hepatic clearance in pathway i ($CR_{h,i}$) and that of renal clearance (CR_r) are obtained as Eqs. A5 and A6, respectively.

$$CR_{h,i} = \frac{\{(1+\frac{CL_r}{Q_h})f_u \cdot CL_{int,h} + CL_r\} - \{(1+\frac{CL_r}{Q_h})f_u \cdot (CL_{int,h} - CL_{int,h,i}) + CL_r\}}{F_a \cdot CL_{oral}}$$
$$= \frac{f_u \cdot (1+CL_r/Q_h) \cdot CL_{int,h,i}}{F_a \cdot CL_{oral}} = \frac{1+\frac{CL_r}{Q_h}}{1+\frac{CL_r}{Q_h}+\frac{CL_r}{f_u \cdot CL_{int,h}}} \cdot f_m \quad \text{(A5)}$$

$$CR_r = \frac{\{(1+\frac{CL_r}{Q_h})f_u \cdot CL_{int,h} + CL_r\} - f_u \cdot CL_{int,h}}{F_a \cdot CL_{oral}}$$
$$= \frac{CL_r}{F_a \cdot F_h \cdot CL_{oral}} = \frac{X_u}{F} \quad \text{(A6)}$$

where f_m, F and X_u are the fraction metabolized by the enzyme (Brown et al., 2005; Ito et al., 2005), the bioavailability, and the urinary excretion ratio of the unchanged drug after oral dosing, respectively. Note that the definition of $CR_{h,i}$ is the same as f_m, which is expressed as $CL_{int,h,i}/CL_{int,h}$, only when CL_r can be ignored compared with Q_h and f_u $CL_{int,h}$ according to Eq. A5. Furthermore, the definition of CR_r is the same as the fraction of drug excreted unchanged which is used in the Giusti-Hayton equation for calculating the dosage of drugs in patients with decreased renal function(Giusti and Hayton, 1973). Here, we assume a DDI situation where $CL_{int,h,i}$ and CL_r are altered due to coadministration of an inhibitor or inducer drug with apparent *in vivo* inhibition ratios of $IR_{h,i}$ and IR_r respectively. We assume the IRs are constant and not time-dependent. It could be considered as a weighed time-average of the actual fluctuating inhibition ratios. In the Giusti-Hayton equation, IR_r is estimated from creatinine clearance with Eq. A7.

$$IR_r \approx 1 - \frac{CL_{Cr,impaired}}{CL_{Cr,normal}} \quad \text{(A7)}$$

where $CL_{Cr,impaired}$ and $CL_{Cr,normal}$ are creatine clearances in the impaired and normal patients, respectively. In the following equations, asterisks denote parameters altered by DDI. Altered oral clearance can be calculated by Eq. A8, which was derived from Eqs. A2 to A6.

$$F_a \cdot CL^*_{oral} = CL_r(1 - IR_r) + (1 + \frac{CL_r(1 - IR_r)}{Q_h}) f_u \cdot \sum_{i=1}^{n} CL_{int,h,i}(1 - IR_{h,i})$$

$$= F_a \cdot CL_{oral} - \frac{CL_r \cdot IR_r}{F_h} - (1 + \frac{CL_r(1 - IR_r)}{Q_h}) f_u \cdot \sum_{i=1}^{n} CL_{int,h,i} \cdot IR_{h,i}$$

$$= F_a \cdot CL_{oral}\{1 - CR_r \cdot IR_r - (1 - \frac{CL_r \cdot IR_r}{Q_h + CL_r}) \sum_{i=1}^{n} CR_{h,i} \cdot IR_{h,i}\} \quad \text{(A8)}$$

From Eq. A8, changes in the AUC of substrates with altered hepatic and/or renal clearance are given by Eq. A9

$$\frac{AUC^*}{AUC} = \frac{CL_{oral}}{CL^*_{oral}} = |1 - CR_r \cdot IR_r - (1 - \frac{CL_r \cdot IR_r}{Q_h + CL_r}) \cdot \sum_{i=1}^{n} CR_{h,i} \cdot IR_{h,i}|^{-1} \quad \text{(A9)}$$

Equation A9 is simplified to Eq. A10 in situations where $IR_r = 0$ or $IR_{h,i} = 0$; i.e. either hepatic or renal clearance of the drug is not altered.

$$\frac{AUC^*}{AUC} = |1 - CR_r \cdot IR_r - \sum_{i=1}^{n} CR_{h,i} \cdot IR_{h,i}|^{-1} \quad \text{(A10)}$$

Indeed, the difference between Eqs. A9 and A10 is apparent in situations where a drug is eliminated *via* the hepatic and renal pathways to similar magnitudes and both pathways are markedly inhibited at the same time. Other than these exceptional situations, it would be reasonable to hypothesize $1 >> \frac{CL_r \cdot IR_r}{Q_h + CL_r}$ and/or $0 \approx \sum_{i=1}^{n} CR_{h,i} \cdot IR_{h,i}$ in Eq. A9. Equation A10 is similar to Eq. 4 used in the previous study (Ohno et al., 2007), but is applicable to various hepatic metabolisms and is also valid when the renal clearance is significant.

References

Ahonen J, Olkkola KT and Neuvonen PJ (1995) Effect of itraconazole and terbinafine on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers. *Br J Clin Pharmacol* **40**:270-272.

Alderman J, Preskorn SH, Greenblatt DJ, Harrison W, Penenberg D, Allison J and Chung M (1997) Desipramine pharmacokinetics when coadministered with paroxetine or sertraline in extensive metabolizers. *J Clin Psychopharmacol* **17**:284-291.

Ameer B and Weintraub RA (1997) Drug interactions with grapefruit juice. *Clin Pharmacokinet* **33**:103-121.

Baba T, Touchi A, Ito K, Yamaguchi Y, Yamazoe Y, Ohno Y and Sugiyama Y (2002) Effects of serum albumin and liver cytosol on CYP2C9- and CYP3A4-mediated drug metabolism. *Drug Metab Pharmacokinet* **17**:522-531.

Back DJ, Tjia J, Monig H, Ohnhaus EE and Park BK (1988) Selective inhibition of drug oxidation after simultaneous administration of two probe drugs, antipyrine and tolbutamide. *Eur J Clin Pharmacol* **34**:157-163.

Backman JT, Karjalainen MJ, Neuvonen M, Laitila J and Neuvonen PJ (2006) Rofecoxib is a potent inhibitor of cytochrome P450 1A2: studies with tizanidine and caffeine in healthy subjects. *Br J Clin Pharmacol* **62**:345-357.

Backman JT, Kivisto KT, Olkkola KT and Neuvonen PJ (1998) The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. *Eur J Clin Pharmacol* **54**:53-58.

Backman JT, Kyrklund C, Neuvonen M and Neuvonen PJ (2002) Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* **72**:685-691.

Backman JT, Olkkola KT, Aranko K, Himberg JJ and Neuvonen PJ (1994) Dose of midazolam should be reduced during diltiazem and verapamil treatments. *Br J Clin Pharmacol* **37**:221-225.

Backman JT, Olkkola KT and Neuvonen PJ (1996) Rifampin drastically reduces plasma concentrations and effects of oral midazolam. *Clin Pharmacol Ther* **59**:7-13.

Baer BR, Wienkers LC and Rock DA (2007) Time-dependent inactivation of P450 3A4 by raloxifene: identification of Cys239 as the site of apoprotein alkylation. *Chem Res Toxicol* **20**:954-964.

Bailey DG, Malcolm J, Arnold O and Spence JD (1998) Grapefruit juice-drug interactions. *Br J Clin Pharmacol* **46**:101-110.

Baranczewski P, Stanczak A, Sundberg K, Svensson R, Wallin A, Jansson J, Garberg P and Postlind H (2006) Introduction to in vitro estimation of metabolic stability and drug interactions of new chemical entities in drug discovery and development. *Pharmacol Rep* **58**:453-472.

Bashir H, Motl S, Metzger ML, Howard SC, Kaste S, Krasin MP and Hudson MM (2006) Itraconazole-enhanced chemotherapy toxicity in a patient with Hodgkin lymphoma. *J Pediatr Hematol Oncol* **28**:33-35.

Baumann P, Jonzier-Perey M, Koeb L, Kupfer A, Tinguely D and Schopf J (1986) Amitriptyline pharmacokinetics and clinical response: II. Metabolic polymorphism

assessed by hydroxylation of debrisoquine and mephenytoin. *Int Clin Psychopharmacol* **1**:102-112.

Becquemont L, Mouajjah S, Escaffre O, Beaune P, Funck-Brentano C and Jaillon P (1999) Cytochrome P-450 3A4 and 2C8 are involved in zopiclone metabolism. *Drug Metab Dispos* **27**:1068-1073.

Benet LZ, Izumi T, Zhang Y, Silverman JA and Wacher VJ (1999) Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *J Control Release* **62**:25-31.

Bergstrom RF, Peyton AL and Lemberger L (1992) Quantification and mechanism of the fluoxetine and tricyclic antidepressant interaction. *Clin Pharmacol Ther* **51**:239-248.

Beverage JN, Sissung TM, Sion AM, Danesi R and Figg WD (2007) CYP2D6 polymorphisms and the impact on tamoxifen therapy. *J Pharm Sci* **96**:2224-2231.

Bjornsson TD, Callaghan JT, Einolf HJ, Fischer V, Gan L, Grimm S, Kao J, King SP, Miwa G, Ni L, Kumar G, McLeod J, Obach RS, Roberts S, Roe A, Shah A, Snikeris F, Sullivan JT, Tweedie D, Vega JM, Walsh J and Wrighton SA (2003) The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metab Dispos* **31**:815-832.

Black DJ, Kunze KL, Wienkers LC, Gidal BE, Seaton TL, McDonnell ND, Evans JS, Bauwens JE and Trager WF (1996) Warfarin-fluconazole. II. A metabolically based drug interaction: in vivo studies. *Drug Metab Dispos* **24**:422-428.

Blum RA, Wilton JH, Hilligoss DM, Gardner MJ, Henry EB, Harrison NJ and Schentag JJ (1991) Effect of fluconazole on the disposition of phenytoin. *Clin Pharmacol Ther* **49**:420-425.

Boberg M, Angerbauer R, Fey P, Kanhai WK, Karl W, Kern A, Ploschke J and Radtke M (1997) Metabolism of cerivastatin by human liver microsomes in vitro. Characterization of primary metabolic pathways and of cytochrome P450 isozymes involved. *Drug Metab Dispos* **25**:321-331.

Bohets H, Lavrijsen K, Hendrickx J, van Houdt J, van Genechten V, Verboven P, Meuldermans W and Heykants J (2000) Identification of the cytochrome P450 enzymes involved in the metabolism of cisapride: in vitro studies of potential co-medication interactions. *Br J Pharmacol* **129**:1655-1667.

Bohme A, Ganser A and Hoelzer D (1995) Aggravation of vincristine-induced neurotoxicity by itraconazole in the treatment of adult ALL. *Ann Hematol* **71**:311-312.

Bohmer GM, Drollmann A, Gleiter CH and Nave R (2008) Effect of coadministered

ketoconazole, a strong cytochrome P450 3A4 enzyme inhibitor, on the pharmacokinetics of ciclesonide and its active metabolite desisobutyryl-ciclesonide. *Clin Pharmacokinet* **47**:343-349.

Bondolfi G, Eap CB, Bertschy G, Zullino D, Vermeulen A and Baumann P (2002) The effect of fluoxetine on the pharmacokinetics and safety of risperidone in psychotic patients. *Pharmacopsychiatry* **35**:50-56.

Bosque E (2001) Possible drug interaction between itraconazole and vinorelbine tartrate leading to death after one dose of chemotherapy. *Ann Intern Med* **134**:427.

Brosen K and Gram LF (1989) Quinidine inhibits the 2-hydroxylation of imipramine and desipramine but not the demethylation of imipramine. *Eur J Clin Pharmacol* **37**:155-160.

Brosen K, Hansen JG, Nielsen KK, Sindrup SH and Gram LF (1993) Inhibition by paroxetine of desipramine metabolism in extensive but not in poor metabolizers of sparteine. *Eur J Clin Pharmacol* **44**:349-355.

Brown HS, Galetin A, Hallifax D and Houston JB (2006) Prediction of in vivo drug-drug interactions from in vitro data : factors affecting prototypic drug-drug interactions involving CYP2C9, CYP2D6 and CYP3A4. *Clin Pharmacokinet* **45**:1035-1050.

Brown HS, Ito K, Galetin A and Houston JB (2005) Prediction of in vivo drug-drug interactions from in vitro data: impact of incorporating parallel pathways of drug elimination and inhibitor absorption rate constant. *Br J Clin Pharmacol* **60**:508-518.

Brynne N, Svanstrom C, Aberg-Wistedt A, Hallen B and Bertilsson L (1999) Fluoxetine inhibits the metabolism of tolterodine-pharmacokinetic implications and proposed clinical relevance. *Br J Clin Pharmacol* **48**:553-563.

Butman SM, Wild JC, Nolan PE, Fagan TC, Finley PR, Hicks MJ, Mackie MJ and Copeland JG, 3rd (1991) Prospective study of the safety and financial benefit of ketoconazole as adjunctive therapy to cyclosporine after heart transplantation. *J Heart Lung Transplant* **10**:351-358.

Caraco Y, Blotnick S and Muszkat M (2008) CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. *Clin Pharmacol Ther* **83**:460-470.

Caraco Y, Tateishi T, Guengerich FP and Wood AJ (1996) Microsomal codeine N-demethylation: cosegregation with cytochrome P4503A4 activity. *Drug Metab Dispos* **24**:761-764.

Carstensen H, Jacobsen N and Dieperink H (1986) Interaction between cyclosporin A and

phenobarbitone. *Br J Clin Pharmacol* **21**:550-551.

Cato A, 3rd, Qian J, Hsu A, Levy B, Leonard J and Granneman R (1998) Multidose pharmacokinetics of ritonavir and zidovudine in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* **42**:1788-1793.

Cho JY, Yu KS, Jang IJ, Yang BH, Shin SG and Yim DS (2002) Omeprazole hydroxylation is inhibited by a single dose of moclobemide in homozygotic EM genotype for CYP2C19. *Br J Clin Pharmacol* **53**:393-397.

Christensen J, Dupont E and K OS (2002) Cabergoline plasma concentration is increased during concomitant treatment with itraconazole. *Mov Disord* **17**:1360-1362.

Chung E, Nafziger AN, Kazierad DJ and Bertino JS, Jr. (2006) Comparison of midazolam and simvastatin as cytochrome P450 3A probes. *Clin Pharmacol Ther* **79**:350-361.

Clarke SE, Jones BC. (2002). Drug-drug interactions. In AD. Rodrigues (Eds.), *Human cytochromes P450 and their role in metabolism-based drug-drug interactions* (pp. 55-88) USA: Marcel Dekker Inc.

Combalbert J, Fabre I, Fabre G, Dalet I, Derancourt J, Cano JP and Maurel P (1989) Metabolism of cyclosporin A. IV. Purification and identification of the rifampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450IIIA gene subfamily. *Drug Metab Dispos* **17**:197-207.

Cook CS, Berry LM and Burton E (2004) Prediction of in vivo drug interactions with eplerenone in man from in vitro metabolic inhibition data. *Xenobiotica* **34**:215-228.

Dalen P, Dahl ML, Bernal Ruiz ML, Nordin J and Bertilsson L (1998) 10-Hydroxylation of nortriptyline in white persons with 0, 1, 2, 3, and 13 functional CYP2D6 genes. *Clin Pharmacol Ther* **63**:444-452.

Deng LJ, Wang F and Li HD (2005) Effect of gemfibrozil on the pharmacokinetics of pioglitazone. *Eur J Clin Pharmacol* **61**:831-836.

Desta Z, Kerbusch T and Flockhart DA (1999) Effect of clarithromycin on the pharmacokinetics and pharmacodynamics of pimozide in healthy poor and extensive metabolizers of cytochrome P450 2D6 (CYP2D6). *Clin Pharmacol Ther* **65**:10-20.

Diasio RB (1998) Sorivudine and 5-fluorouracil: a clinically significant drug-drug interaction due to inhibition of dihydropyrimidine dehydrogenase. *Br J Clin Pharmacol* **46**:1-4.

Ding X and Kaminsky LS (2003) Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol* **43**:149-173.

Dresser GK, Schwarz UI, Wilkinson GR and Kim RB (2003) Coordinate induction of both cytochrome P4503A and MDR1 by St John's wort in healthy subjects. *Clin Pharmacol Ther* **73**:41-50.

Dutreix C, Peng B, Mehring G, Hayes M, Capdeville R, Pokorny R and Seiberling M (2004) Pharmacokinetic interaction between ketoconazole and imatinib mesylate (Glivec) in healthy subjects. *Cancer Chemother Pharmacol* **54**:290-294.

Eagling VA, Wiltshire H, Whitcombe IW and Back DJ (2002) CYP3A4-mediated hepatic metabolism of the HIV-1 protease inhibitor saquinavir in vitro. *Xenobiotica* **32**:1-17.

Eap CB, Lessard E, Baumann P, Brawand-Amey M, Yessine MA, O'Hara G and Turgeon J (2003) Role of CYP2D6 in the stereoselective disposition of venlafaxine in humans. *Pharmacogenetics* **13**:39-47.

Edeki TI, He H and Wood AJ (1995) Pharmacogenetic explanation for excessive beta-blockade following timolol eye drops. Potential for oral-ophthalmic drug interaction. *JAMA* **274**:1611-1613.

Efthymiopoulos C, Bramer SL, Maroli A and Blum B (1997) Theophylline and warfarin interaction studies with grepafloxacin. *Clin Pharmacokinet* **33 Suppl 1**:39-46.

Egnell AC, Houston JB and Boyer CS (2005) Predictive models of CYP3A4 Heteroactivation: in vitro-in vivo scaling and pharmacophore modeling. *J Pharmacol Exp Ther* **312**:926-937.

Einolf HJ (2007) Comparison of different approaches to predict metabolic drug-drug interactions. *Xenobiotica* **37**:1257-1294.

Elliott P, Dundee JW, Elwood RJ and Collier PS (1984) The influence of H2 receptor antagonists on the plasma concentrations of midazolam and temazepam. *Eur J Anaesthesiol* **1**:245-251.

Erickson DA, Mather G, Trager WF, Levy RH and Keirns JJ (1999) Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. *Drug Metab Dispos* **27**:1488-1495.

Estelle F and Simons R (1999) H1-receptor antagonists: safety issues. *Ann Allergy Asthma Immunol* **83**:481-488.

Fichtenbaum CJ, Gerber JG, Rosenkranz SL, Segal Y, Aberg JA, Blaschke T, Alston B, Fang F, Kosel B and Aweeka F (2002) Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG Study A5047. *AIDS* **16**:569-577.

Firkusny L and Gleiter CH (1994) Maprotiline metabolism appears to co-segregate with the