AUC was used when more than one article was available for the same interaction set (Table I). In these cases, however, significant deviation was observed in the AUC increase between or among reports. For the analysis, we often combined the data of clinical studies with different doses of the inhibitor. Since the lower doses frequently gave more AUC increase of a substrate, it is possible that the deviation of the inhibitor dose may not largely affect the results, as far as the dose of inhibitor is set within the therapeutic range. The extent of this deviation is shown in Fig. 1A, which was prepared in the same style as Fig. 1B. Each circle and vertical bar in Fig. 1A represents the mean + S.D. reported in each article shown by the underlines in Table I. If the S.D. values were not reported in articles listed in Tables I and II, the reported mean values are shown by squares. As shown by the dotted lines in Fig. 1A, for most of the articles, the increase in AUC of substrate drugs caused by drug inhibition deviated by 67-150% of the algebraic mean values. The predictions within 50-200% of the observed AUC increase in Fig. 1B were regarded as successful, since the corresponding variation of AUC in Fig. 1A (the estimation set) was within this range.

Then, we reorganized the data shown in Fig. 1 to indicate the relationships between the IR_{3A4} values and the increase in AUC of each substrate (Fig. 2). It was found that the AUC increased steeply as the IR_{3A4} value increased for highly CYP3A4-dependent substrates, such as simvastatin, lovastatin and buspirone, whereas only minimal increases were observed for poor CYP3A4 substrates, such as zolpidem and cerivastatin (Fig. 2). In the same manner, potent inhibitors, such as azole antifungals, increased the blood levels of a number of CYP3A4 substrates markedly, whereas no or only very minor increases were observed for weak inhibitors, such as azithromycin, gatifloxacin and fluoxetine (Fig. 3).

Finally, the data were reorganized to show that the increase in AUC in 251 kinds of drug interactions between 14 substrate drugs and 18 inhibitors could be predicted (Fig. 4, note that telithromycin is included both among the substrates and inhibitors). The nomogram in Fig. 4 indicates that a very marked increase in the AUC is anticipated when substrate drugs with high CR_{3A4} values were administered with potent inhibitors with high IR_{3A4} values.

DISCUSSION

CYP3A4 is the most important drug-metabolizing enzyme, which oxidizes

preferentially relatively large, lipophilic, and neutral to basic molecules. Therefore, CYP3A4 is recognized as a key enzyme which determines the clearance of various drugs and, in some cases, has a major effect on their safety and efficacy. Although no major polymorphism in the CYP3A4 gene has been identified, marked inter-individual differences have been reported in the activity of CYP3A4.[31] One possible reason for such differences in the activity is that CYP3A4 is inducible by various diets and drugs, such as rifampicin and carbamazepin, *via* the mechanism mediated by PXR.[32-34] Furthermore, CYP3A4 is the predominant metabolizing enzyme not only in the liver but also in the intestine. It has been reported that intestinal metabolism is the major factor determining the bioavailability of some drugs.[16,35-37] However, as far as we know, nobody has succeeded in predicting the extent of the first-pass effect on metabolism by intestinal CYP3A4 from *in vitro* data. Although there are some established methods to determine the activity of CYP3A4 *in vivo*, including evaluation of the metabolic ratio of selective substrates (midazolam, testosterone and cortisol) and the erythromycin breathe test, it has been reported that these methods do not offer consistent results,[38] possibly due to differences in the organ of metabolism (liver or intestine) and/or the presence of multiple recognition sites in the CYP3A4 molecule.[39]

In spite of these issues regarding the *in vivo* evaluation of CYP3A4 activity, the current rather simple method gave satisfactory predictions in most cases. The following issues may contribute to this success. Firstly, uncertain factors were avoided since the current method primarily relies on an overall *in vivo* evaluation. For example, the present method satisfactorily predicted drug interactions with mechanism-based inhibitors such as azithromycin, clarithromycin, diltiazem, erythromycin, and roxythiromycin (Fig.3), which frequently exhibit complicated kinetics. Accurate predictions have been achieved recently from *in vitro* data for mechanism-based inhibitors by sophisticated analysis. For the successful prediction, it has been reported that evaluation of the unbound fraction of the drug in the incubation medium is important.[13,40] Moreover, the analysis requires a turnover rate of the metabolizing enzyme and a rate constant for the irreversible reaction, both of them are not easy to estimate from *in vitro* experiments.

Secondly, we used simvastatin as a selective substrate and ketoconazole and itraconazole as selective inhibitors of CYP3A4, although these drugs are not absolutely specific for CYP3A4. For example, although we assumed that the CR_{3A4} value of simvastatin is 1.0, this drug is also metabolized by CYP2C8 to a minor extent.[27] Ketoconazole is a well known selective inhibitor of CYP3A4, but this drug also inhibits

the activities of CYP2C8,[41] 2C9[42] and MDR1,[43] which may also affect the disposition of substrate drugs analyzed in the present study. In spite of these defects, the success in the prediction of drug interactions with the present method (Fig. 1) suggests that CYP3A4 plays a crucial role in most of the drug interactions analyzed in the present study.

A number of probe drugs have been used to study the activity of CYP3A4, including midazolam, nifedipine, simvastatin, and erythromycin.[44] Among them, it is generally recognized that the most reliable probe drug is midazolam for CYP3A4.[45,46] The plasma AUC of midazolam is increased significantly by coadministration of various CYP3A4 inhibitors (Figs. 1, 2 and 4). In our preliminary analysis, we found that the rank order of the AUC increase of typical substrates, such as simvastatin, lovastatin and buspirone, by a series of inhibitors was generally in good agreement with the rank order of the AUC increase of midazolam produced by these inhibitors. These results suggest that the extent of CYP3A4 inhibition after administration of each inhibitor is almost the same among substrates. From this analysis, we hypothesized that calculation of AUC increases from IR_{3A4} values should be possible.

It has often been reported that *in vitro Ki* values vary significantly among CYP3A4 substrates used,[10] which contradicts our hypothesis that the IR_{3A4} value is the same for any substrate. For example, nifedipine was allocated to a different group from midazolam and triazolam by a cluster analysis of the victim profile of *in vitro* drug interactions.[47] However, as represented in Fig.2, no clear discrepancy was observed for the predicted AUC increases of any particular substrate assuming a single IR_{3A4} value for each inhibitor. It is therefore possible that the *in vivo Ki* value of each inhibitor is not affected by the substrate drugs analyzed in the present study. This result may be due to the fact that the number of available drug interaction studies is limited for each inhibitor. Accordingly, we should be cautious in predicting the increase in AUC for a novel substrate drug by using the IR_{3A4} values determined in the present study.

In the validation process of the present study, the method provided successful predictions in 57 out of 60 cases. Telithromycin is a particular example of an accurate prediction. The CR_{3A4} value of telithromycin was calculated to be 0.49 from the results of an interaction study with ketoconazole. The AUC increase produced by interaction with itraconazole, which has an IR_{3A4} value of 0.95, was predicted to be 1.85, which was in good agreement with the observed increase of 1.60. Telithromycin also acts as an inhibitor of CYP3A4. The IR_{3A4} value of telithromycin was 0.91 and an

AUC increase of simvastatin produced by interaction of telithromycin was predicted to be 11.1, which was also in good agreement with the observed increase of 10.8.

In contrast, we had difficulties in predicting 3 reports of drug interactions; i.e., cyclosporin-voriconazole, triazolam-itraconazole, and one of two reports for a triazolam-erythromycin interaction. In article #63 in Table II, it was reported that the AUC of cyclosporin was increased 1.70-fold by the administration of voriconazole, whereas we predicted a 4.61-fold increase (Figs. 1B and 4). In the same manner, although the AUC of triazolam was reported to be increased 27.1-fold by the administration of itraconazole (article #71 in Table II), we predicted an 8.85-fold increase (Figs. 1B and 4). Concerning the interaction between triazolam and erythromycin, there was a deviation in the increase in AUC of triazolam by erythromycin. In article #31 in Table II, a 3.65-fold increase was reported whereas a 2.06-fold increase was reported in article #61. Our prediction was 4.32-fold (Figs. 1B and 4) and was in accord with the former article. The reason for these deviations is unknown. Further studies will help to investigate whether there was some mechanistic reason or whether there was simply some unavoidable variability. The factors that need to be considered include the contributions by other metabolizing enzymes and transporters, and the variety of enzyme kinetics of CYP3A4 inhibition.

In the present study, midazolam, itraconazole and ketoconazole were used as a standard substrate or an inhibitor because they are used most commonly in interaction studies. As a result, overall AUC increases were successfully predicted, indicating that the standard drugs were selected appropriately. It may be possible to use other commonly used substrates of CYP3A4 such as simvastatin, lovastatin and buspirone to calculate IR_{3A4} values because no deviation was observed in the predictability of AUC increase for these substrate drugs when coadministered with wide range of inhibitors (Fig. 2).

To prioritize drug interaction studies during the course of drug development, Obach et al. have recently proposed a rank-order approach in which the mechanism of possible interactions is explored by *in vitro* experiments and then the most probable interactions are evaluated *in vivo* using typical substrates or inhibitors.[48] The results of the present study support their approach. If a drug interaction study is carefully designed using the appropriate standard drugs, significant interactions *via* CYP3A4 will not be missed. In addition, the extent of CYP3A4-mediated interactions between many other drugs will be able to be predicted using the current method, as suggested by the results in Fig. 4.

In conclusion, we have constructed a general framework for prediction of the increase in AUC which is mediated by CYP3A4. The precision and robustness of the method have been demonstrated satisfactorily. Several standard substrates and inhibitors are proposed for the evaluation of drug interactions *via* CYP3A4. This method would be applicable (1) to prioritize clinical trials to investigate drug interactions during the course of drug development, and (2) to estimate the clinical significance of unknown drug interactions.

Reference list

1 Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab Rev* 1997; **29**: 413-580

2 Rogers JF, Nafziger AN, Bertino JS Jr. Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. *Am J Med* 2002; **113**: 746-50

3 Furberg CD, Pitt B. Withdrawal of cerivastatin from the world market. *Curr Control Trials Cardiovasc Med* 2001; **2**: 205-7

4 Estelle F, Simons R. H1-receptor antagonists: safety issues. *Ann Allergy Asthma Immunol* 1999; **83**:481-8

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

6 Kanamitsu S, Ito K, Sugiyama Y. Quantitative prediction of *in vivo* drug-drug interactions from *in vitro* data based on physiological pharmacokinetics: use of maximum unbound concentration of inhibitor at the inlet to the liver. *Pharm Res* 2000; **17**: 336-43

7 Kanamitsu S, Ito K, Green CE, et al. Prediction of *in vivo* interaction between triazolam and erythromycin based on *in vitro* studies using human liver microsomes and recombinant human CYP3A4. *Pharm Res* 2000; **17**: 419-26

8 Yamano K, Yamamoto K, Katashima M, et al. Prediction of midazolam-CYP3A inhibitors interaction in the human liver from *in vivo/in vitro* absorption, distribution, and metabolism data. *Drug Metab Dispos* 2001; **29**: 443-52

9 Ito K, Brown HS, Houston JB. Database analyses for the prediction of *in vivo* drug-drug interactions from *in vitro* data. *Br J Clin Pharmacol* 2004; **57**: 473-86

10 Galetin A, Ito K, Hallifax D, et al. CYP3A4 substrate selection and substitution in the prediction of potential drug-drug interactions. *J Pharmacol Exp Ther* 2005; **314**: 180-90

11 Ito K, Hallifax D, Obach RS, et al. Impact of parallel pathways of drug elimination and multiple cytochrome P450 involvement on drug-drug interactions: CYP2D6 paradigm. *Drug Metab Dispos* 2005; **33**: 837-44

12 Isoherranen N, Kunze KL, Allen KE, et al. Role of itraconazole metabolites in CYP3A4 inhibition. *Drug Metab Dispos* 2004; **32**: 1121-31

13 Galetin A, Burt H, Gibbons L, et al. Prediction of time-dependent CYP3A4 drug-drug interactions: impact of enzyme degradation, parallel elimination pathways, and intestinal inhibition. *Drug Metab Dispos* 2006; **34**: 166-75
14 Lennernas H. Human jejunal effective permeability and its correlation with preclinical drug absorption models. *J Pharm Pharmacol* 1997; **49**: 627-38
15 Paine MF, Khalighi M, Fisher JM, et al. Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* 1997; **283**: 1552-62
16 Thummel KE, O'Shea D, Paine MF, et al. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. *Clin Pharmacol Ther* 1996; **59**: 491-502
17 Saitoh H, Aungst BJ. Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine. *Pharm Res* 1995; **12**: 1304-10
18 Wacher VJ, Silverman JA, Zhang Y, et al. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* 1998; **87**: 1322-30
19 Bnet LZ, Izumi T, Zhang Y, et al. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *J Control Release* 1999; **62**: 25-31
20 Suzuki H, Sugiyama Y. Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. *Eur J Pharm Sci* 2000; **12**: 3-12
21 Brady JM, Cherrington NJ, Hartley DP, et al. Tissue distribution and chemical induction of multiple drug resistance genes in rats. *Drug Metab Dispos* 2002; **30**: 838-44
22 Korzekwa KR, Krishnamachary NSM, Ogai A, et al. Evaluation of atypical cytochrome P450 kinetics with two-substrates-models: Evidence that multiple substrates can simultaneously bind to cytochrome P450 active sites *Biochemistry* 1998; **37**: 4137-47
23 Wang RW, Newton DJ, Liu N, et al. Human cytochrome P-450 3A4: *In vitro* drug-drug interaction patterns are substrate-dependent. *Drug Metab Dispos* 2000; **28**: 360-6
24 Mayhew BS, Jones DR, Hall SD. An *in vitro* model for predicting *in vivo* inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation. *Drug Metab Dispos* 2000; **28**: 1031-7
25 Wang YH, Jones DR, Jones DR, Hall SD. Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites. *Drug Metab Dispos* 2004; **32**: 259-66
26 Wang YH, Jones DR, Hall SD. Differential mechanism-based inhibition of CYP3A4 and CYP3A5 by verapamil. *Drug Metab Dispos* 2005; **33**: 664-71
27 Ahmad AB, Bennett PN, Rowland M. Models of hepatic drug clearance: discrimination between the 'well stirred' and 'parallel-tube' models. *J Pharm Pharmacol* 1983; **35**: 219-24
28 Brown HS, Galetin A, Hallifax D, et al. Prediction of *in vivo* drug-drug interactions from *in vitro* data : factors affecting prototypic drug-drug interactions involving CYP2C9, CYP2D6 and CYP3A4. *Clin Pharmacokinet* 2006; **45**: 1035-50
29 Brown HS, Ito K, Galetin A, Houston JB. 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* 2005; **60**: 508-18

30 Prueksaritanont T, Ma B, Yu N. The human hepatic metabolism of simvastatin hydroxy acid is mediated primarily by CYP3A, and not CYP2D6. *Br J Clin Pharmacol* 2003; **56**: 120-4
31 Lamba JK, Lin YS, Schuetz EG, et al. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 2002; **54**: 1271-94
32 Villikka K, Kivisto KT, Backman JT, et al. Triazolam is ineffective in patients taking rifampin. *Clin Pharmacol Ther* 1997; **61**: 8-14
33 Villikka K, Kivisto KT, Maenpaa H, et al. Cytochrome P450-inducing antiepileptics increase the clearance of vincristine in patients with brain tumors. *Clin Pharmacol Ther* 1999; **66**: 589-93
34 Gibson GG, Plant NJ, Swales KE, et al. Receptor-dependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica* 2002; **32**: 165-206
35 Wu CY, Benet LZ, Hebert MF, et al. Differentiation of absorption and first-pass gut and hepatic metabolism in humans: studies with cyclosporine. *Clin Pharmacol Ther* 1995; **58**: 492-7
36 Lampen A, Zhang Y, Hackbarth I, et al. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. *J Pharmacol Exp Ther* 1998; **285**: 1104-12
37 Holtbecker N, Fromm MF, Kroemer HK, et al. The nifedipine-rifampin interaction. Evidence for induction of gut wall metabolism. *Drug Metab Dispos* 1996; **24**: 1121-3
38 Kinirons MT, O'Shea D, Kim RB, et al. Failure of erythromycin breath test to correlate with midazolam clearance as a probe of cytochrome P4503A. *Clin Pharmacol Ther* 1999; **66**: 224-31
39 Tanaka E, Kurata N, Yasuhara H. How useful is the "cocktail approach" for evaluating human hepatic drug metabolizing capacity using cytochrome P450 phenotyping probes *in vivo*? *J Clin Pharm Ther* 2003; **28**: 157-65
40 Obach RS, Walsky RL, Venkatakrishnan K. Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions. *Drug Metab Dispos* 2007; **35**: 246-55
41 Ong CE, Coulter S, Birkett DJ, et al. The xenobiotic inhibitor profile of cytochrome P4502C8. *Br J Clin Pharmacol* 2000; **50**: 573-80
42 Park JY, Kim KA, Shin JG, et al. Effect of ketoconazole on the pharmacokinetics of rosiglitazone in healthy subjects. *Br J Clin Pharmacol* 2004; **58**: 397-402
43 Siegsmund MJ, Cardarelli C, Aksentijevich I, et al. Ketoconazole effectively reverses multidrug resistance in highly resistant KB cells. *J Urol* 1994; **151**: 485-91
44 Watkins PB. Noninvasive test of CYP3A enzymes. *Pharmacogenetics* 1994; **4**: 171-84
45 Bjornsson TD, Callaghan JT, Einolf HJ, et al. The conduct of *in vitro* and *in vivo* drug-drug interaction studies: a PhRMA perspective. *J Clin Pharmacol* 2003; **43**: 443-69
46 Chung E, Pharm D, Nafziger AN. Comparison of midazolam and simvastatin as cytochrome P450 3A probes. *Clin Pharmacol Ther* 2006; **79**: 350-61
47 Kenworthy KE, Bloomer JC, Clarke SE, et al. CYP3A4 drug interactions: correlation of 10 *in vitro* probe substrates. *Br J Clin Pharmacol* 1999; **48**: 716-27
48 Obach RS, Walsky RL, Venkatakrishnan K, et al. The utility of *in vitro* cytochrome P450 inhibition data in the prediction of drug-drug interactions. *J Pharmacol Exp Ther* 2006; **316**: 336-48

Table I. Relationships between investigational drugs and article index which reports clinical drug interaction studies involving CYP3A4 used as the data source

inhibitor	substrate													
	alprazolam	atrovastatin	buspirone	cerivastain	cyclosporin	felodipine	lovastatin	midazolam	nifedipine	nisoldipine	simvastatin	telithromycin	triazolam	zolpidem
azithromycin	-	4	-	-	-	-	-	11, 77, 78	-	-	-	-	31	-
cimetidine	1, 62	-	-	55	-	-	-	19, 21	42, 43	69	-	-	1, 18, 25, 62	36
clarithromicin	-	4, 37	-	-	-	-	-	27, 77		-	37	-	31	-
diltiazem	-	-	49	-	5, 23	-	7	8	67	-	54	-	47, 72	-
erythromycin	75	66	45	55	24, 34	12	-	58, 78	-	-	39	-	31, 61	52
fluconazole	-	-	-	-	16	-	-	57	-	-	-	-	70	32
fluoxetine	29, 51	-	-	-	-	-	-	48	-	-	-	-	74	3
fluvoxamine	22	-	50	-	-	-	-	48	-	-	-	-	-	-
gatifloxacin	-	-	-	-	-	-	-	28	-	-	-	-	-	-
itraconazole	76	40, 53	45, 46	41, 53	-	38	44, 56	2, 10, 57, 59	-	-	56	6, 65	71	32
ketoconazole	30	-	-	-	15, 23, 26	-	-	17, 48, 59, 68	-	35	-	6	30, 71, 73	32
nefazodone	33	-	-	-	-	-	-	48	-	-	-	-	13	-
ranitidine	-	-	-	-	-	-	-	19, 20, 21	42, 43	-	-	-	-	36
roxithromycine	-	-	-	-	-	-	14	9	-	-	-	-	-	-
saquinavir	-	-	-	-	-	-	-	60	-	-	-	-	-	-
telithromycin	-	-	-	-	-	-	-	6	-	-	6	-	-	-
verapamil	-	-	49	-	-	-	-	8	-	-	37, 39	-	-	-
voriconazole	-	-	-	-	63	-	-	64	-	-	-	-	-	-

Refer to Table II for each article.
The underlined indexes indicate that the results of the corresponding report were used to calculate the ratio of contribution of CYP3A4 to oral clearance (CR_{3A4}) and time-averaged apparent inhibition ratio (IR_{3A4}).
The indexes without underline indicate that the results of the corresponding report were used to evaluate the propriety of the present method.

Table II. List of articles for clinical drug interaction studies involving CYP3A4 used as the data source

index	article
1	Abernethy et al., Psychopharmacology 1983;80:275-8
2	Ahonen et al., Br J Clin Pharmacol 1995;40:270-2
3	Allard et al., Drug Metab Dis 1998;26:617-22
4	Amsden et al., J Clin Pharmacol 2002;42:444-9
5	Asberg et al., Eur J Clin Pharmacol 1999;55:383-7
6	Aventis. Ketek (telithromycin) Tablets. 2005. (www.fda.gov/cder/foi/label/2005/21144s001,003lbl.pdf)
7	Azie et al., Clin Pharmacol Ther 1998;64:369-77
8	Backman et al., Br J Clin Pharmacol 1994;37:221-5
9	Backman et al., Eur J Clin Pharmacol 1994;46:551-5
10	Backman et al., Eur J Clin Pharmacol 1998;54:53-8
11	Backman et al., Int J Clin Pharmacol Ther 1995;33:356-9
12	Bailey et al., Clin Pharmacol Ther 1996;60:25-33
13	Barbhaiya et al., J Clin Psychopharmacol 1995;15:320-6
14	Bucher et al., Eur J Clin Pharmacol 2002;57:787-91
15	Butman et al., J Heart Lung Transplant 1991;10:351-8
16	Canafax et al., Transplantation 1991;51:1014-8
17	Chung et al., Clin Pharmacol Ther 2006;79:350-61
18	Cox et al., Biopharm Drug Dispos 1986;7:567-75
19	Elliott et al., Eur J Anaesthesiol 1984;1:245-51
20	Elwood et al., Br J Clin Pharmacol 1983;15:743-5
21	Fee et al., Clin Pharmacol Ther 1987;41:80-4
22	Fleishaker & Hulst, Eur J Clin Pharmacol 1994;46:35-9
23	Foradori et al., Transplant Proc 1998;30:1685-7
24	Freeman et al., Br J Clin Pharmacol 1987;23:776-8
25	Friedman et al., J Clin Pharmacol 1988,28:228-33
26	Gomez et al., Clin Pharmacol Ther 1995;58:15-9
27	Gorski et al., Clin Pharmacol Ther 1998;64:133-43
28	Grasela et al., Pharmacotherapy 2000;20:330-5
29	Greenblatt et al., Clin Pharmacol Ther 1992;52:479-86
30	Greenblatt et al., Clin Pharmacol Ther 1998;64:237-47
31	Greenblatt et al., Clin Pharmacol Ther 1998;64:278-85
32	Greenblatt et al., Clin Pharmacol Ther 1998;64:661-71
33	Greene et al., J Clin Psychopharmacol 1995;15:399-408
34	Gupta et al., Br J Clin Pharmacol 1989;27:475-81
35	Helnig et al., Eur J Clin Pharmacol 1999;55:57-60
36	Hulhoven et al., Int J Clin Pharm Res 1988;8:477-83
37	Jacobson, Am J Cardiol 2004;94:1140-6
38	Jalava et al., Clin Pharmacol Ther 1997;61:410-5
39	Kantola et al., Clin Pharmacol Ther 1998;64:177-82
40	Kantola et al., Clin Pharmacol Ther 1998;64:58-65

Table II. List of articles for clinical drug interaction studies involving CYP3A4 used as the data source (continued)

index	article
41	Kantola et al., Eur J Clin Pharmacol 1999;54:851-5
42	Khan et al., Br J Clin Pharmacol 1991;32:519-22
43	Kirch et al., Arch Toxicol Suppl 1984;7:256-9
44	Kivisto et al., Br J Clin Pharmacol 1998;46:49-53
45	Kivisto et al., Clin Pharmacol Ther 1997;62:348-54
46	Kivisto et al., Pharmacol Toxicol 1999;84:94-7
47	Kosuge et al., Br J Clin Pharmacol 1997;43:367-72
48	Lam et al., J Clin Pharmacol 2003;43:1274-82
49	Lamberg et al., Clin Pharmacol Ther 1998;63:640-5
50	Lamberg et al., Eur J Clin Pharmacol 1998;54:761-6
51	Lasher et al., Psychopharmacology (Berl) 1991;104:323-7
52	Luurila et al., Eur J Clin Pharmacol 1998;54:163-166
53	Mazzu et al., Clin Pharmacol Ther 2000;68:391-400
54	Mousa et al., Clin Pharmacol Ther 2000;67:267-74
55	Muck et al., Eur J Clin Pharmacol 1998;53:469-73
56	Neuvonen & Jalava, Clin Pharmacol Ther 1996;60:54-61
57	Olkkola et al., Anesth Analg 1996;82:511-6
58	Olkkola et al., Clin Pharmacol Ther 1993;53:298-305
59	Olkkola et al., Clin Pharmacol Ther 1994;55:481-5
60	Palkama et al., Clin Pharmacol Ther 1999;66:33-9
61	Phillips et al., J Clin Psychopharmacol 1986;6:297-9
62	Pourbaix et al., Int J Clin Pharmacol Ther Toxicol 1985;23:447-51
63	Romero et al., Clin Pharmacol Ther 2002;71:226-34
64	Saari et al., Clin Pharmacol Ther 2006;79:362-70
65	Shi et al., Pharmacotherapy 2005;25:42-51
66	Siedlik et al., J Clin Pharmacol 1999;39:501-4
67	Tateishi et al., J Clin Pharmacol 1989;29:994-7
68	Tsunoda et al., Clin Pharmacol Ther 1999;66:461-71
69	van Harten et al., Clin Pharmacol Ther 1988;43:332-41
70	Varhe et al., Br J Clin Pharmacol 1996;42:465-70
71	Varhe et al., Clin Pharmacol Ther 1994;56:601-7
72	Varhe et al., Clin Pharmacol Ther 1996;59:369-75
73	von Moltke et al., J Pharmacol Exp Ther 1996;276:370-9
74	Wright et al., Pharmacotherapy 1992;12:103-6
75	Yasui et al., Clin Pharmacol Ther 1996;59:514-9
76	Yasui et al., Psychopharmacology (Berl) 1998;139:269-73
77	Yeates et al., Int J Clin Pharmacol Ther 1996;34:400-5
78	Zimmermann et al., Arzneimittelforschung 1996;46:213-7

Table III. Calculated ratios of the contribution of CYP3A4 to the oral clearance (CR_{3A4}) of substrates

Substrate	CR_{3A4}
Simvastatin	1.00
Lovastatin	1.00
Buspirone	0.99
Nisoldipine	0.96
Triazolam	0.93
Midazolam	0.92
Felodipine	0.89
Cyclosporin	0.80
Nifedipine	0.78
Alprazolam	0.75
Atorvastatin	0.68
Telithromycin	0.49
Zolpidem	0.40
Cerivastatin	0.18

Table IV. Calculated ratios of the time-averaged apparent inhibition ratio of CYP3A4 (IR_{3A4}) for inhibitors

Inhibitor	daily dose	IR_{3A4}
Ketoconazole	200-400mg	1.00
Voriconazole	400mg	0.98
Itraconazole	100-200mg	0.95
Telithromycin	800mg	0.91
Clarithromycin	500-1000mg	0.88
Saquinavir	3600mg	0.88
Nefazodone	400mg	0.85
Erythromycin	1000-2000mg	0.82
Diltiazem	90-270mg	0.80
Fluconazole	200mg	0.79
Verapamil	240mg-480mg	0.71
Cimetidine	800-1200mg	0.44
Ranitidine	300-600mg	0.37
Roxithromycin	300mg	0.35
Fluvoxamine	100mg-200mg	0.30
Azithromycin	250-500mg	0.11
Gatifloxacin	400mg	0.08
Fluoxetine	20-60mg	0.00

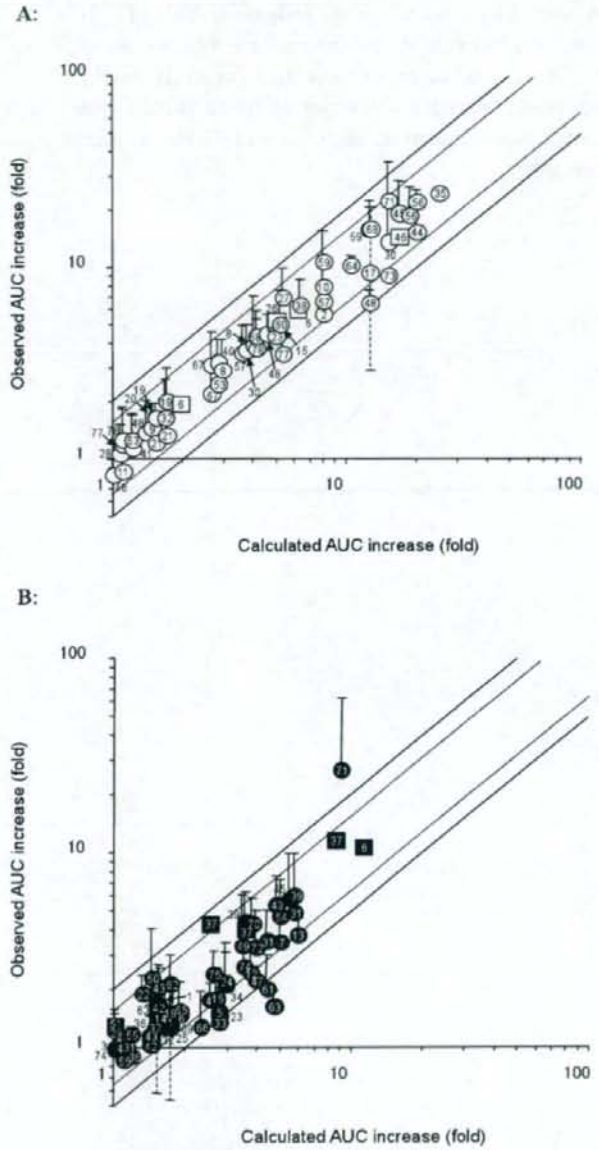

Figure 1 ····· Relationship between the observed and calculated increase in AUC by drug interactions. Using the CR_{3A4} and IR_{3A4} values shown in Table III and IV, respectively, the increase in AUC of substrate drugs by drug interactions reported in 60 clinical studies, indicated by the indexes without underline in Table I, was predicted with Eq. 11 (Panel B). Panel A was prepared in the same style as Panel B, for the purpose of demonstrating the deviation of AUC values among 53 clinical studies, the mean values of which were used to determine the CR_{3A4} and IR_{3A4} values.

The data source for Panel A is indicated by the underlined indexes in Table I. In Panels A and B, each circle and vertical bar represents the mean + S.D. values of subjects reported in each article. A dashed bar represents the range. If the S.D. values or the ranges were not reported in articles, the reported mean values were shown by squares. Solid and dotted lines represent 50-200% and 67-150% ranges, respectively, of the calculated increase.

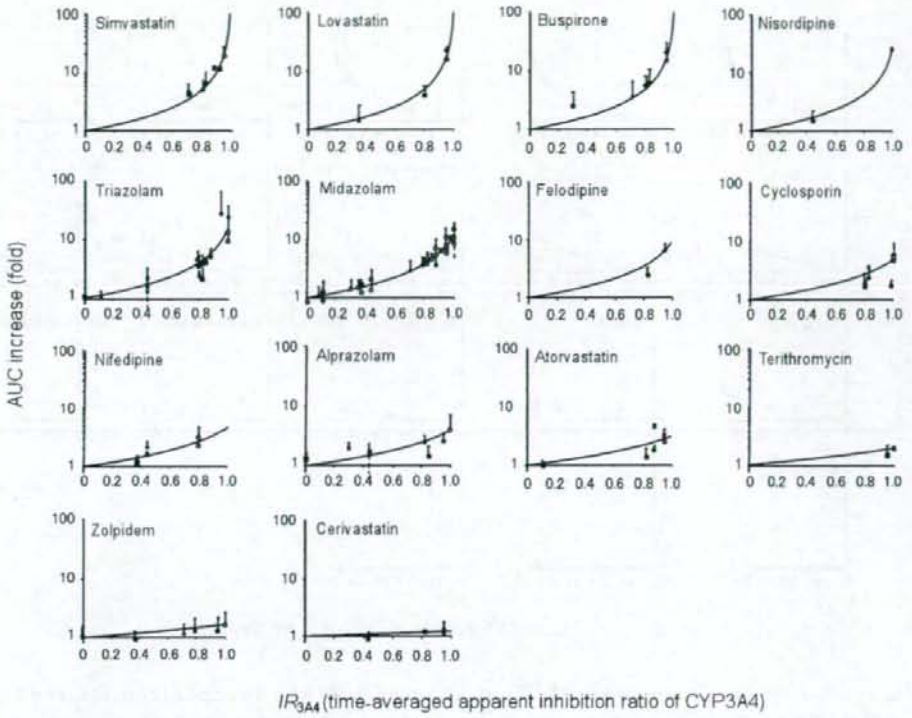

Figure 2 ····· Increase in the AUC reorganized for each substrate drug as a function of *IR* of inhibitors. Data shown in Fig. 1 were reorganized to show the increase in AUC of each substrate drug as a function of the IR_{3A4} values of inhibitors. Open and closed symbols represent the data set shown in Figs. 1A and 1B, respectively. See legends to Fig. 1 for details.

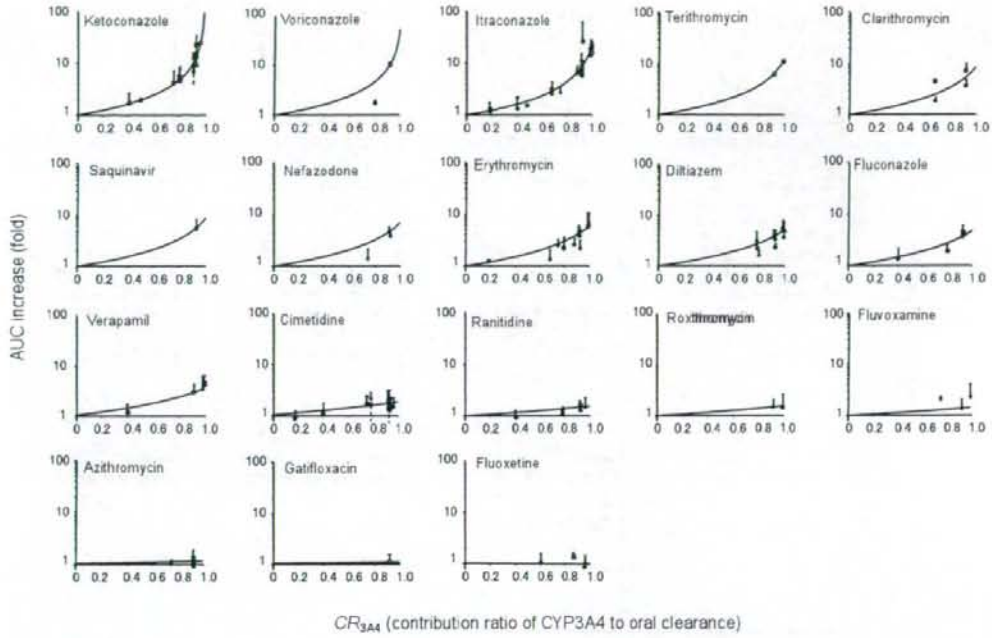

Figure 3 ----- Increase in the AUC of substrate drugs reorganized for each inhibitor as a function of the *CR* values of substrate drugs. Data shown in Fig. 1 were reorganized for each inhibitor to show the increase in AUC of each substrate drug as a function of the CR_{3A4} values of substrate drugs. Daily doses of inhibitors are indicated in parentheses. Open and closed symbols represent the data set shown in Figs. 1A and 1B, respectively. See legends to Fig. 1 for details.

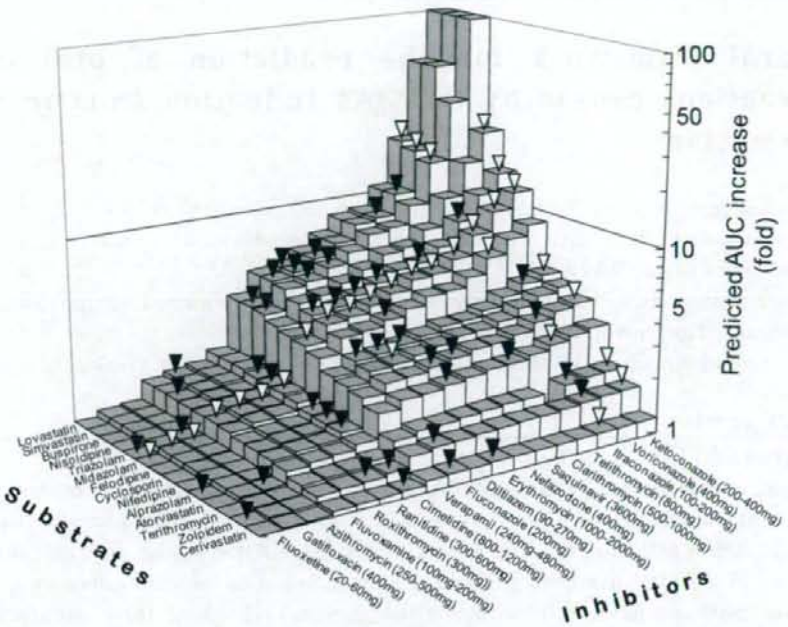

Figure 4 ····· Predicted increase in the AUC of substrate drugs by various drug interactions. The increase in AUC of substrate drugs by various drug interactions was predicted according to the CR_{3A4} and IR_{3A4} values shown in Table III amd IV, respectively. Open and closed arrows show the data set shown in Figs. 1A and 1B, respectively.

別紙3　資料1
(Ohno et al. Clinical Pharmacokinetics, 2008, *in press*)

General framework for the prediction of oral drug interactions caused by CYP3A4 induction from *in vivo* information

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[ABSTRACT]

Background:

Induction of cytochrome P450 (CYP) 3A4 potentially reduces the blood concentrations of substrate drugs to less than 1/10, which results in ineffective pharmacotherapy. Although the prediction of drug-drug-interactions (DDIs) which are mediated by induction of CYP3A4 has been performed mainly based on *in vitro* information, such previous methods have met with limited success regarding their accuracy and applicability. Therefore, a realistic method for the prediction of CYP3A4-mediated inductive DDIs is of major clinical importance.

Objective:

The objective of the present study is to construct a robust and accurate method for the prediction of CYP3A4-mediated inductive DDIs. We developed a method to quantitatively predict the inductive DDIs based on the principle which was applied for predictions of inhibitory DDI in the previous report (Y. Ohno et al., Clin Pharmacokinet 2007;46:681-96). A unique character of this principle is that the extent of alterations in the plasma AUC is predicted based on *in vivo* information from minimal clinical studies without using *in vitro* data.

Data sources:

The analysis is based on 42 DDI studies in humans reported in 37 published articles over the period 1983 to 2007.

Methods:

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 the equation, $1 / (1 + CR_{CYP3A4} \times IC_{CYP3A4})$, where CR_{CYP3A4} is the apparent ratio of the contribution of CYP3A4 to the oral clearance of a substrate, and IC_{CYP3A4} is the apparent increase in clearance due to the induced CYP3A4. Using this equation, IC_{CYP3A4} was calculated for seven inducers (bosentan, carbamazepine, efavirenz, phenytoin, pioglitazone, rifampicin, and St. John's Wort) based on the reduction in the plasma AUC of a coadministered standard substrate of CYP3A4, such as simvastatin, in 10 DDI studies. CR_{CYP3A4} was calculated for 22 substrates based on

the previously reported method from inhibitory DDI studies with a potent CYP3A4 inhibitor, such as itraconazole or ketokonazole.

Results:

The proposed method enabled the prediction of AUC reduction by CYP3A4 induction with any combination of these substrates and inducers (total 154 matches). In order to assess the accuracy of the prediction, the AUC reductions in 32 studies were analyzed. We found that we were able to successfully predict the inductive DDIs in a quantitative manner; 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 respective substrate drugs before administration of inducers. In addition, rifampicin was found to be the most potent inducer among the compounds analyzed in the present study with an IC_{CYP3A4} value of 7.7, followed by phenytoin and carbamazepine with the values of 4.7 and 3.0, respectively. The IC_{CYP3A4} values of the other CYP3A4 inducers analyzed in the present study were approximately 1 or less,which suggests that the AUCs of coadministered drugs may not be reduced to less than approximately 1/2 even if the drug is metabolized solely by CYP3A4.

Conclusion:

By using the method reported in the present study, the susceptibilities of a substrate drug of CYP3A4 to inductive DDIs can be predicted quantitatively. It was indicated that coadministrations of rifampicin, phenytoin and carbamazepine may reduce plasma AUCs to less than half for a broad range of CYP3A4 substrate drugs with a CR_{CYP3A4} greater than 0.13, 0.21 and 0.33, respectively.

[Introduction]

Cytochrome (CYP) 3A4 is the main human metabolizing enzyme in the liver and intestine, and is involved in the metabolism of more than half of the drugs currently on the market.[1, 2] CYP3A4 has also been recognized as a target of clinically significant drug-drug interactions (DDIs). There are two types of DDIs: DDIs which are mediated by inhibition of metabolizing enzymes/transporters and DDIs which are mediated by induction of these proteins. The therapeutic effects as well as the adverse effects of a drug are potentiated with the increased blood concentrations caused by inhibitory DDIs, whereas these effects are usually reduced by the reduced blood concentrations caused by inductive DDIs.[3] If the therapeutic or adverse effects of a drug are attributed to its metabolite which is generated by CYP3A4, the above descriptions on pharmacological effects would be reversed.

Many inhibitory DDIs follow competitive kinetics and are relatively transient, whereas some are due to mechanism-based inhibition and are more long-lasting.[4-6] These direct interactions between inhibitors and metabolizing enzymes can be investigated *in vitro* in a quantitative manner.[7] On the other hand, it has been shown that most of inductive DDIs are raised by the increased transcription of CYP3A4 as a result of signal transductions *via* nuclear receptors, such as pregnane X receptor (PXR) / retinoid X receptor (RXR), and take a few weeks to exert stabilized influence.[8-10] It is not easy to replicate CYP3A4 inductions *in vitro* in a quantitative manner, since multiple factors are involved in its regulation. Accordingly, only a few studies have met with limited success as far as the prediction of *in vivo* CYP3A4 induction from *in*

vitro experiments is concerned.[11, 12]

From a therapeutic viewpoint, there is only limited information about alternations in drug clearance or therapeutic/adverse effects caused by induction of CYP3A4.[13, 14] This is presumably due to a limited number of clinical studies to evaluate inductive DDIs compared with those for inhibitory DDIs during the development of novel drug candidates. Typical examples of drugs capable of inducing CYP3A4 include rifampicin, some of antiepileptic drugs, and efavirenz. Rifampicin is one of the most potent enzyme inducers of CYP3A4 on the market and it reduces plasma drug levels of verapamil and simvastatin to less than 1/10.[15] Considering the broad substrate specificity of CYP3A4, rifampicin would have a significant influence on the clearance of number of drugs. Although many warnings are given in the labeling of rifampicin regarding DDIs, most of them are not quantitative.[16] Accordingly, it is not easy to adjust the regimen or substitute the drug based on such information. In addition to rifampicin, some antiepileptic drugs such as phenytoin and carbamazepine also induce CYP3A4 significantly. The serum concentrations of these drugs have been routinely measured by therapeutic drug monitoring for decades. However, there have been few investigations of their quantitative effects on the clearance of other drugs, and inductive DDIs are far less documented compared with rifampicin. Efavirenz, which can also induce CYP3A4, is a new reverse transcriptase inhibitor used for the treatment of HIV infection which induces CYP3A4.[17] It is difficult to judge appropriateness of coadministration of other drugs with efavirenz due to the current limited information.

Considering the current state of affairs described above, there is a serious clinical need for a reliable method of predicting changes in drug clearance triggered by enzyme induction, particularly for CYP3A4, to avoid the use of ineffectual regimens and to select the most suitable drugs and dosage regimens. The objective of the present study was to offer a method of drug and dosage selection which was both broad and comprehensive. For this purpose, we have applied our previously proposed principle for the prediction of inhibitory DDIs[18] to the prediction of inductive DDIs; in the previous study, we reported a general prediction method for increased AUCs triggered by inhibition of CYP3A4. In this method, we assumed that the extent of the alterations in AUC by coadministered inhibitors is determined by two factors; *i.e.* the contribution of CYP3A4 to the oral clearance of substrate drugs (CR_{CYP3A4}), and the extent of inhibition of CYP3A4 caused by inhibitor drugs (IR_{CYP3A4}).[18] A unique character of the method was that these two factors were calculated entirely from *in vivo* information which had been reported in clinical studies. Although the method developed in the previous study cannot be directly applied to the analysis of inductive DDIs, the basic framework would be applicable by replacing IR_{CYP3A4} by a factor which represents the extent of enzyme induction.

In the present report, we describe a derived new method to predict inductive DDIs in a quantitative manner which is also based on the information from clinical studies. The target interactions were limited to those caused mainly by induction of CYP3A4, although it is well known that a series of metabolizing enzymes and transporters other than CYP3A4, such as CYP1A, CYP2C, uridine diphosphate-glucuronosyltransferases (UGTs), sulfotransferases, MDR1 (ABCB1,