別紙１

厚生労働科学研究費補助金

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薬物体内動態支配因子のファーマコゲノミクスに基づく医薬品開発評価

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総括研究報告書

薬物体内動態支配因子のファーマコゲノミクスに基づく医薬品開発評価

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概要

医療に薬物動態の予測を適用するためには、方法が単純でしかも精度の十分に高いことが肝要である。我々はこの観点から薬物動態の理論を再検討し、個別化医療における薬物動態の予測に必要不可欠な最低限度の情報は、①遺伝子変異や併用薬によるCYPの活性の変化と基質薬の②各CYP分子種への代謝依存度（寄与率）であるとの結論を得た。これまでに本研究においてこの理論を適用し、CYP3A4の阻害を介する薬物間相互作用の精度の良い予測に成功した（Ohno et al. Clin Pharmacokinet 46;681-96, 2007、平成18年度 総括報告書参照）。個別の薬剤の組合せについて薬物間相互作用を予測した報告は多いが、本研究は数百の組合せの相互作用を一括して網羅的に予測した点が特徴である。H19年度には、本方法をこれまで報告例の少なかった誘導を介する薬物間相互作用の予測に適用し、系統的に精度の良い予測が可能であることを示した（Ohno et al. Clin Pharmacokinet *in press*, 2008）[資料１]。本予測の結果から、ある種の薬物では広範な併用薬の体内からの消失が酵素誘導により亢進しており、そのために治療無効の原因になると推定されたが、これまでは多くが見過ごされてきた可能性がある。

遺伝子変異によるCYP活性の変化については、活性の消失するpoor metabolizer(PM)の評価に加えて、活性が減少するintermediate metabolizer(IM)の予測をどのように扱うのかが１つの課題であった。本研究ではH18年度に、東洋人に多いCYP2D6*10 変異による薬物代謝活性を 9個の基質薬について検討し、IMであるにも関わらず、その低下率は73～91%と顕著であることを示した。このために、トロピセトロン、メキシレチンなどで、臨床試験で東洋人において顕著な薬物血中濃度の上昇が起きたと考えられた。さらに最近の結果から、タモキシフェン、クロピドグレル等について、最近の*in vitro*研究によりCYPのIM、あるいはPM由来の肝ミクロソームにおける代謝反応は野生型のextensive metabolizer(EM)と大きく異なり、そのために活性代謝物の生成が抑制されると考えられた。

CYPにSNPsを有する患者においては、基本的な薬物動態が一部の薬で変化するにとどまらず、薬物間相互作用についても、野生型とは著しい違いを生ずると予測されている[資料２]。すなわち、相互作用によって活性の変化するCYPの活性がSNPsによってそもそも失われている場合は、相互作用は消失するのに対し、相互作用によって活性の変化するCYP以外のCYPがSNPsで活性が失われている場合には、予想以上に強い相互作用が生じて危険となる可能性がシミュレートされた[資料２]。

現在、本研究の理論と情報をすべて組み込み、薬物間相互作用とSNPsの変化による動態の予測を多くの薬剤で網羅的に予測するソフトを開発中であり、実動に近い状態にある[資料3]。今後、これを整備して医療の現場に提供することで、より有効で安全な薬物治療を支援する強力なツールとなることを期待している。

今後の活用・提供

本研究で構築した薬物動態予測のための方法論は、単純で精度が比較的高いことに加え、必要なパラメータを*in vivo*の臨床試験の結果からでも得られる点が、既存の方法に対するアドバンテージである。そのために、*in vitro*データの発表されていない薬物を含む多くの薬剤の動態変化を網羅的かつ定量的に予測できる。一方で *in vitro* のデータを予測に積極的に利用するとともに、そ

の*in vivo*予測の信頼度を検証することも可能であり、将来は医療で直接利用するほかに、新薬認可申請のガイドラインや添付文書のPGxおよび薬物間相互作用の記述に影響を与えると期待される[資料４]（Pharmacol Ther誌よりinvite）。さらに、新薬開発時にCYPにSNPsを有する患者におけるや薬物間相互作用による動態変化が適切に予測可能となり、候補品の選択を効率的に実施可能となる。

研究の実施経過

我々は、CYPの活性変化による薬物動態変化に関して、典型的な薬物間相互作用の臨床試験の報告からCYP分子種の基質薬のクリアランスへの寄与率と阻害薬の阻害率あるいは誘導剤による見かけのクリアランス増加を算出する方法を検討した。例としてCYP3A4の阻害による相互作用の場合は、阻害剤の併用による経口投与時の基質薬の血中濃度曲線下面積（AUC）の変化率は式1で表される。

$$R_{inhibition} = \frac{AUC_{+inhibitor}}{AUC_{control}} = \frac{1}{1 - CR_{CYP3A4} \cdot IR_{CYP3A4}} \quad \cdots\cdot式1$$

ここで、CR_{CYP3A4}は*in vivo*におけるCYP3A4の基質の経口クリアランスへの寄与率、IR_{CYP3A4}は阻害剤のCYP3A4の阻害率を表す。式1は今までの確立された薬物速度論のセオリーに基づきながら、*in vivo*で観察できる要因にパラメータを単純化したものと要約できる。

式１に基づけば、AUCの変化率とIRから基質のCRが、またAUCの変化率とCRから阻害剤のIRが算出できる。我々は、イトラコナゾール、ケトコナゾール、ボリコナゾールなどのCYP3A4の典型的な阻害剤との相互作用による各基質薬のAUCの変化率から、各基質薬のCR_{CYP3A4}を算出した。同様にミダゾラムなどのCYP3A4の典型的な基質薬との相互作用試験の結果からIR_{CYP3A4}を算出した。78文献から113の相互作用試験の報告を抽出し、そのうち53の相互作用試験から基質薬14剤のCR_{CYP3A4}と阻害薬18剤のIR_{CYP3A4}が算出された。これらのパラメータと式１を用いて、残りの60の相互作用試験におけるAUC変化率の報告値と予測値との関係を検証したところ、57試験（95%）で報告値の50-200%の範囲で予測は正確であった。この予測方法はCYP2D6やCYP2C9などの他のCYP酵素を介した相互作用、複数のCYP分子種の阻害による相互作用でも適応可能であり、CYP3A4と同様の予測精度が得られた。一方、CYP3A4の誘導に基づく相互作用による、経口投与時の基質薬のAUCの変化率は式2で表すことができる。

$$R_{induction} = \frac{AUC_{+inducer}}{AUC_{control}} = \frac{1}{1 + CR_{CYP3A4} \cdot IC_{CYP3A4}} \quad \cdots\cdot式2$$

ここで、IC_{CYP3A4}はCYP3A4の誘導による基質薬のクリアランスの増加を表す。ミダゾラムなどのCYP3A4の典型的な基質薬との相互作用試験の結果から式2によって、IC_{CYP3A4}を算出した。37文献から42の相互作用試験の報告を抽出し、そのうち10の相互作用試験から誘導剤7剤のIR_{CYP3A4}が算出された。これらのパラメータと式2を用いて、残りの32の相互作用試験におけるAUC変化率の報告値と予測値との関係を検証したところ、すべての試験（100%）で報告値の20%の誤差の範囲で予測することに成功した。

タモキシフェンは古くから乳癌の治療に広く用いられる薬剤であるが、近年、その生理活性はCYP2D6を介して生成する代謝物エンドキシフェンに由来することが判明した。一方、クロピドグレルは最近日本で上市された抗血小板薬であるが、活性発現にはCYPによる代謝が必要とされる。活性代謝物生成に関わるCYP分子種は特定されていないが、CYP2C19の活性低下する患者では効果が減弱することが報告されている。CYP2D6および2C19はSNPsによって日本人で活性が大きく変動することから、生理活性と活性代謝物の血中濃度、およびPGxの関係を臨床試験で確認することは、個別化医療のために大変重要である。

我々の研究室では、昨年末に高感度分析装置LC-MS/MSが導入され、薬物を高感度で分析する手段が確保された。現在、*in vitro*の代謝実験で活性代謝物の生成を確認するとともに、臨床試験のための分析法の確立を進めている。

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

General framework for the prediction of oral drug interaction s 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, P-glycoprotein) and organic anion transporting polypeptides (OATPs, SLCOs), were inducible by

various drugs and supplements.[19] If a substrate drug is extensively eliminated by inducible metabolizing enzymes or transporters other than CYP3A4, the present method would misestimate the magnitude of DDI. Furthermore, the interactions with protease inhibitors for treatment of HIV were excluded, because those drugs exhibited a marked potential to induce and inhibit CYP3A4 simultaneously *in vivo*.[20-22] In spite of these limitations, the present study is the first method which can predict the magnitude of inductive DDIs between multiple drugs in a systematic manner.

[Methods]

Data source

Information on clinical DDI studies of seven drugs and supplements which are known to induce CYP3A4 (bosentan, carbamazepine, efavirenz, phenytoin, pioglitazone, rifampicin, and St. John's Wort) were collected from the literature. We used information on inducers of CYP3A4 as much as possible, but did not incorporated drugs which exhibit potent induction and inhibition at the same time, such as protease inhibitors for treatment of HIV. On the other hand, efavirenz, a nucleoside reverse transcriptase inhibitor for treatment of HIV, was included as an inducer, although it exhibits both inductive and inhibitory activities *in vitro*.[17, 23] This was because blood concentrations of drugs were reduced following coadministration of efavirenz for all the cases reported in the literature.[24] Concerning the data of St. John's Wort (SJW), studies using high-dose hyperforin extracts (>10 mg day^{-1}) were used.[25] Reductions in the AUC of drug plasma or serum concentrations produced by enzyme induction were quantitatively evaluated. Values of the apparent contribution of CYP3A4 to oral clearance (CR_{CYP3A4}) were extracted for 13 substrate drugs from a previous study.[18] In addition to these 13 substrates, information on inhibitory clinical DDI studies was collected for 9 substrates of CYP3A4 (amitriptyline, etizolam, gefitinib, imatinib, mefloquine, prednisolone, quetiapine, ziprasidone, and zopiclone) to calculate CR_{CYP3A4} in the present study.

Theory

The oral clearance, CL_{oral}, of drugs can be described with Eq. 1.[18]

$$F_a \cdot CL_{oral} = fu \cdot CL_{int(H)} \qquad \text{Eq. 1}$$

where F_a, fu and $CL_{int(H)}$ are the fraction absorbed, unbound fraction in the blood, and hepatic intrinsic clearance of substrates, respectively. Two simplifications were used in the development of Eq. 1. The first one is that the urinary excretion of the unchanged substrate drug is minimal as often the case for lipophilic CYP3A4 substrates. Another simplification is that the contribution of intestinal metabolism was combined with that of hepatic metabolism. The appropriateness of the latter hypothesis will be examined in the Discussion section. We assume two intrinsic metabolic clearances, $CL_{int(CYP3A4)}$ and $CL_{int(others)}$, which represent the metabolism of substrates mediated by CYP3A4 and the sum of other metabolic pathways, respectively.

$$CL_{int(H)} = CL_{int(CYP3A4)} + CL_{int(others)} \qquad \text{Eq. 2}$$

In the following equations, asterisks denote parameters altered by induction of CYP3A4. When the amount of CYP3A4 is increased by inducers with an induction ratio of $R_{induction}$, the $CL_{int(H)}$ of substrates is increased as follows.

$$CL^{*}_{int(H)} = R_{induction} \cdot CL_{int(CYP3A4)} + CL_{int(others)} \qquad \text{Eq. 3}$$

It should be noted that $CL_{int(others)}$ is assumed not to be altered by induction of CYP3A4 under the assumption of Eq. 3. This is one of the basic assumptions of the present method. The apparent ratio of the contribution of CYP3A4 to the oral clearance of a substrate (CR_{CYP3A4}) is defined by Eq.4 where $CL_{oral(-CYP3A4)}$ is an altered *in vivo* oral clearance when $CL_{int(CYP3A4)}$ is blocked completely.[18]

$$CR_{CYP3A4} = \frac{CL_{oral} - CL_{oral(-CYP3A4)}}{CL_{oral}} \qquad \text{Eq. 4}$$

The definition of CR is similar to that of Fm, the fraction metabolized by the enzyme, which has been frequently used in the literature,[26, 27] but a distinct nomenclature has been used to make it clear that the CR values are an apparent estimation from *in vivo* outcomes. Considering that CR_{CYP3A4} is expressed by $CL_{int(CYP3A4)}$ / $CL_{int(H)}$ when the extra-hepatic clearance is minimal, Eq.3 is transformed to Eq. 5.

$$\begin{aligned} CL^{*}_{int(H)} &= (1 + (R_{induction} - 1) \cdot CR_{CYP3A4}) \cdot CL_{int(H)} \\ &= (1 + IC_{CYP3A4} \cdot CR_{CYP3A4}) \cdot CL_{int(H)} \end{aligned} \qquad \text{Eq. 5}$$

where IC_{CYP3A4} is the apparent increase in clearance for substrates produced by induction of CYP3A4, which is calculated by $R_{induction} - 1$. The alternations in the AUC of substrates produced by induction of CYP3A4 are given by Eq. 6.

$$\frac{AUC^{*}_{oral}}{AUC_{oral}} = \frac{CL_{oral}}{CL^{*}_{oral}} = \frac{CL_{int(H)}}{CL^{*}_{int(H)}} = \frac{1}{1 + CR_{CYP3A4} \cdot IC_{CYP3A4}} \qquad \text{Eq. 6}$$

There is a clear similarity between Eq. 6 and Eq. 7, the latter involves the alternations in the AUC of substrates produced by inhibitory DDIs with CYP3A4.[18]

$$\frac{AUC^{*}_{oral}}{AUC_{oral}} = \frac{1}{1 - CR_{CYP3A4} \cdot IR_{CYP3A4}} \qquad \text{Eq. 7}$$

where IR_{CYP3A4} is a time-averaged apparent inhibition ratio of CYP3A4.

When the nonlinear dose-response of enzyme induction needs to be considered, the following theory would be helpful. In order to estimate *in vivo* enzyme induction

from *in vitro* experimental data, Eq. 8 has been used.[11, 12]

$$R_{induction} = 1 + \frac{E_{max} \cdot I}{EC_{50} + I} \qquad \text{Eq. 8}$$

where E_{max} and EC_{50} are the maximum induction effect and the half maximal effective concentration, respectively, which are determined *in vitro*, and I is the drug concentration at the target site. Under an assumption of linear pharmacokinetics of an inducer drug, Eq. 9 is obtained from Eq. 8.

$$IC_{CYP3A4} = \frac{IC_{CYP3A4(max)} \cdot D}{ED_{50} + D} \qquad \text{Eq. 9}$$

where $IC_{CYP3A4(max)}$, D and ED_{50} are the maximum IC_{CYP3A4}, dose, and half maximal effective dose of induction, respectively. In the present study, however, we could not evaluate $IC_{CYP3A4(max)}$ and ED_{50} values because the dose-response of CYP3A4 induction has not been fully characterized in clinical studies.

Calculation of CR_{CYP3A4} and IC_{CYP3A4} values

The CR_{CYP3A4} values were obtained from the previous report[18] for thirteen substrate drugs, while CR_{CYP3A4} of nine substrate drugs were newly calculated from Eq. 7 based on alternations in AUCs reported in the literature for inhibitory DDIs where itraconazole or ketoconazole was coadministered.[18] The detailed information was listed in Table II. On the other hand, the IC_{CYP3A4} values of CYP3A4 inducers were calculated from Eq. 6 from alternations in the AUC of a standard substrate produced by inductive DDIs. In our analysis, we chose a substrate having as high a level of CR_{CYP3A4} as possible for the standard to minimize the estimation error. From this standpoint, simvastatin was chosen as the standard drug for the calculation of IC_{CYP3A4} of rifampicin, carbamazepine, efavirenz, and bosentan. For pioglitazone and SJW, we used midazolam since the *in vivo* effects of these drug and supplement on simvastatin have not been reported yet. For phenytoin, quetinapine was chosen as the standard drug. We did not use any regression analysis or fitting calculations to estimate CR_{CYP3A4} and IC_{CYP3A4} values. Although overall estimation errors would be minimized by using fitting calculations, we regarded the present standard drug approach is sufficiently precise and more practical. It should be noted that, strictly speaking, all parameters need to be recalculated when new information is added for the fitting approach, because the CR and IR (or IC) values are interdependent.

Statistics

Predicted AUC increases were assessed by the observed values. The average-fold error (AFE) and mean prediction error (MPE) were calculated to indicate relative and absolute precisions, respectively, and root mean square prediction error (RMSE) was calculated to investigate deviations as follows.

$$AFE = 10^{\left|\frac{1}{n}\sum \log\frac{Predicted}{Obesrved}\right|} \quad \text{Eq. 10}$$

$$MPE = \frac{1}{n}\sum(Predicted - Observed) \quad \text{Eq. 11}$$

$$RMSE = \sqrt{\frac{1}{n}\sum(Predicted - Observed)^2} \quad \text{Eq. 12}$$

where n is the number of studies. χ^2-test was performed using the mean square prediction errors.

[Results]

In the present study, we surveyed 42 *in vivo* inductive DDI studies published in 37 articles (table I). In typical induction studies, the inducers were administered consecutively for several days. The IC_{CYP3A4} values were estimated for seven inducers of CYP3A4 from Eq. 6 using known CR_{CYP3A4} values of substrates and alterations in AUCs observed in 10 clinical DDI studies of CYP3A4 induction. These 10 clinical DDI studies to determine IC_{CYP3A4} values are referred to as the estimation set and are shown in Table I. In the calculation of IC_{CYP3A4}, the algebraic mean of the reduction in the AUCs was used when more than one article was available for the same interaction set. In these cases, however, a significant deviation was sometimes observed in the AUC reductions between or among reports even if the doses were the same, as shown in Fig. 1.

In order to validate the suitability of this method, the extent of reduction in AUCs was predicted for additional clinical studies which are independent of the estimation set. These 32 clinical DDI studies to validate the method are referred to as the validation set in Table I. This prediction was performed by substituting the values of CR_{CYP3A4} and IC_{CYP3A4} in Eq. 6. For this purpose, we extended the survey conducted in our previous report[18] to determine the CR_{CYP3A4} values. Table II lists the CR_{CYP3A4} values for 22 substrate drugs.

The predicted alternations in AUC were plotted against the observed values (Fig. 2). It was demonstrated that we can 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 were less than 20% of the AUCs of the respective substrate drugs before administration of inducers. The AFE values were from 0.76 to 1.17 for each inducer, and 1.07 for the validation set (Table III). The MPE values were within -0.1~0.1 for all inducers. These data suggested overall small average deviations between the predictions and the observations even although we did not conducted any fitting calculations. The individual deviation for each substrate drug was also small as represented by an acceptable RMSE value of 0.09 for the validation set. No significant difference was detected for any of the inducers between the prediction and the observation by χ^2-test ($p > 0.1$).

The relationships between the CR_{CYP3A4} values and the reductions in the AUCs of

several substrates were plotted for each inducer (Fig. 3). The lines represent the calculated AUC changes by Eq. 6 from IC_{CYP3A4} and CR_{CYP3A4}. The open and closed symbols represent the dataset shown in Figs. 1 and 2, respectively. As indicated in Eq. 9, IC_{CYP3A4} values are theoretically dose-dependent. However, higher doses of an inducer were not always associated with a more marked reduction in the AUC of substrates as shown in Fig. 3. For example, although 900 mg of SJW reduced the AUC of ciclosporin to 68% of the control, 600 mg of SJW also reduced the AUC of ciclosporin to 55% of the control. Such deviation may be due to fluctuation in the *in vivo* experiments. The present analysis indicated that the deviation in the inducer dose may not markedly affect the results, as long as the dose selected is within the therapeutic range. The reductions in AUCs for 154 matches of DDIs between 22 substrate drugs and 7 inducers were systemically predicted (Fig. 4). It was found that a marked reduction in AUCs is anticipated when substrate drugs with a high CR_{CYP3A4} were administered with a potent inducer with a high IC_{CYP3A4} (Fig. 4).

Among the compounds analyzed in the present study, rifampicin was found to be the most potent inducer and the AUCs of coadministered drugs have often reduced to less than 1/10. As shown in table III, The IC_{CYP3A4} of rifampicin was calculated to be 7.7, followed by phenytoin and carbamazepine with values of approximately 4.7 and 3.0, respectively. Rifampicin may reduce AUCs of coadministered CYP3A4 substrate drugs to less than half if the CR_{CYP3A4} of the substrate drugs is greater than 0.13 (Fig. 3a). Phenytoin and carbamazepine may also reduce the AUCs of substrate drugs to less than half if the CR_{CYP3A4} of the substrate drugs is greater than 0.21 and 0.33, respectively (Fig. 3b,c). The IC_{CYP3A4} of all the other CYP3A4 inducers analyzed in the present study were approximately 1 or less, which suggests that the AUCs of coadministered drugs will not be decreased to less than approximately 1/2 even in the case of a substrate drug metabolized solely by CYP3A4 (Fig. 3d-g).

[Discussion]

In the present study, we have successfully demonstrated that the alternations in AUCs produced by enzyme induction of CYP3A4 for any drug-drug combination can be predicted by evaluating the CR_{CYP3A4} values for substrate drugs and the IC_{CYP3A4} values for inducer drugs. Nevertheless, it needs to be kept in mind that the accuracy is for the average values and that marked inter- and intra-subject differences are observed routinely in drug blood concentrations. Cares should be taken when this method is applied to predictions of individual pharmacokinetics.

It has been reported that the role of the intestinal CYP3A4 in drug metabolism was comparable to hepatic CYP3A4 for some substrate drugs even although the expression level of the former is only 1/100 of the latter.[71, 72] For an example of inductive DDI, bioavailability of ciclosporin with concomitant rifampicin administration was reported to be markedly less than that predicted by hepatic enzyme induction.[42] Theoretically, intestinal CYP3A4 plays an important role in the first-pass effect but does not alter total drug clearance after a drug reaches the systemic circulation.[73] In contrast, alterations in the expression levels of hepatic CYP3A4 regulate the oral clearance by affecting the first-pass effect as well as affecting the total clearance. Furthermore, it has been well-established that MDR1 acts as an

efficient absorption barrier in conjunction with CYP3A4 in the intestine.[73-75] Strictly speaking, the contributions to drug clearance in the liver and the intestine, and also by CYP3A4 and MDR1, need to be accounted separately for the accurate prediction of DDIs. However, it is practically impossible to apply the precise theory to the very wide range of drugs available commercially, since these factors have been evaluated separately only for a few drugs.[73] Therefore, we adopted the simplified theory in the present study assuming that these factors may closely correlate with each other and can be combined. Indeed, similarities have been reported in the substrate specificities of CYP3A4 and MDR1.[73] In addition, CYP3A4 and MDR1 are co-induced via the PXR/FXR mechanism.[76] The present simplified method successfully predicted alternations in AUCs by CYP3A4 induction without exceptions, suggesting the appropriateness of our hypothesis.

The IC_{CYP3A4} values should be compared with the degree of *in vivo* induction of CYP3A4 and MDR1. The measurement of human hepatic CYP3A4 content by biopsy revealed that the induction ratio was approximately 3- to 5-fold after administration of rifampicin.[77, 78] In the same manner, rifampicin also induced CYP3A4 and MDR1 in the intestine by approximately 3.3- to 4.4-fold[79, 80] and 3.5- to 4.2- fold,[79, 81, 82] resepectively, under *in vivo* experimental conditions. The synergistic effect of the induction of both CYP3A4 and MDR1 in both the intestine and liver may be responsible for the induction ratio of 7.7 (Table III) estimated in the present study from the IC_{CYP3A4} of rifampicin.

Inductions are also frequently observed for metabolizing enzymes and transporters other than CYP3A4. It has been established that PXR/RXR are involved in the inductions of CYP2C8, 2C9, and 2C19[19] along with CYP3A4 and MDR1. Consequently, if these enzymes are also involved in the elimination of the standard substrates which were used in the present study for the calculation of the IC_{CYP3A4} of an inducer, the value of IC_{CYP3A4} could possibly be overestimated. In contrast, if the substrate drug undergoes metabolism by inducible CYPs other than CYP3A4, the alternations in AUC would be underestimated even if the IC_{CYP3A4} was evaluated accurately. In some cases, these over- and under-estimations can be canceled out. One of such examples may be amitriptyline which has a low CR_{CYP3A4} value of 0.25 and undergoes extensive metabolism by CYP2C19.[68] Although the present prediction for alternations in the AUC of amitriptyline by coadministration of SJW agreed with the observed ratio of 0.78, a contribution of CYP2C19 may be significant to some degree because CYP2C19 is also inducible *via* PXR-mediated process.

Some very important clinical DDIs like those with oral contraceptives do not result exclusively from alternations in CYP activities.[83] Cumulative pieces of evidences suggest that ethinyl estradiol is metabolized by CYP3A4, but it was not included in the present study because a clinical DDI study capable to estimate CR_{CYP3A4} have not been reported. For the determination of CR_{CYP3A4}, coadministration of a selective CYP3A4 inhibitor, such as ketoconazole or itraconazole, is required. Nevertheless, its AUC was reported to be increased by 25 to 30% by coadministration with fluconazole,[84, 85] a potent inhibitor of both CYP3A4 and CYP2C9.[86] Accordingly, the CR value of ethinyl estradiol for CYP3A4 and CYP2C9 in total was assumed to be approximately 0.2 which was roughly in accordance with an observation

that its AUC decreased approximately by half by coadministration with rifampicin (Fig. 3a).[87] However, we should refrain from predicting pharmacokinetics of ethinyl estradiol only from a viewpoint of CYP activities since it has been well established that other inducible metabolizing pathways, particularly sulfation and glucuronidation contribute greatly to its clearance.[88, 89]

Furthermore, it should be noted that rifampicin, a representative CYP3A4 inducer, also inhibits OATPs which are transporters responsible for the uptake of a broad range of organic anions, such as atorvastatin and fexofenadine.[90-92] Accordingly, the involvements of inhibition of OATPs would need to be considered carefully for DDIs involving rifampicin. Attentions should be paid to consider actions of any alternations in drug clearance triggered by enzyme induction. In the future, it would be advantageous to separate each contribution by metabolizing enzyme/transporter for substrate drugs, and also to separate each alternation of clearance pathways for inducer drugs.

For prediction of any pharmacokinetic alterations, particular care needs to be taken when multiple factors are simultaneously involved. Some of CYP3A4 inducers may also inhibit CYP3A4, as is frequently found in the case of drugs used for the treatment of HIV infection, such as ritonavir, atazanavir, indinavir, and saquinavir.[93-96] In some cases, the inhibition is accounted for by mechanism-based inhibition,[93, 96] which may lead to the nonlinear and/or time-dependent pharmacokinetics of these kinds of inducers. Based on these considerations, the analysis of the effect of these inducers was not included in the present study. For such analysis, construction of much precise *in vivo* / *in vitro* pharmacokinetic model is required in which the time profiles of the disposition of these inducers can be taken into account.

Although *in vitro* experiments using primary cultured hepatocytes or several cultured cell lines have been used to examine the induction of compounds,[11, 97-99] the current method for the prediction of inductive DDIs based on *in vivo* information will be more accurate and broadly applicable to a variety of drugs. Indeed, the present method may also be used during the clinical drug development. For drug candidates which may induce CYP3A4, the following procedure may be used for the quantitative prediction of their ability to induce the enzyme.

- A clinical study will be conducted for an evaluation of inductive DDIs where inductive potential of CYP3A4 has been suggested by *in vitro* experiments and/or in experimental animals. The pharmacokinetics of a standard CYP3A4 substrate with a known CR_{CYP3A4} (such as midazolam and simvastatin) will be investigated before and after consecutive dosing of a drug candidate typically for 1 to 2 weeks.
- From such *in vivo* studies, the IC_{CYP3A4} value for this compound will be calculated. If clinically important DDIs are anticipated with other any CYP3A4 substrates with the known CR_{CYP3A4} values, clinical studies will also be performed, and the results should be compared with the predicted values.

For drug candidates which are metabolized by CYP3A4 in *in vitro* experiments, the following procedure may be used for the quantitative prediction of alterations in the AUCs produced by coadministered inducers.

- CR_{CYP3A4} value of such a drug candidate can be determined *in vivo* by performing inhibitory and/or inductive DDI studies. In the case of the inhibitory DDI studies,

a standard inhibitor of CYP3A4, typically itraconazole or ketoconazole, will be coadministered to examine the increase in AUC. For the inductive DDI studies, a standard inducer of CYP3A4, typically rifampicin, will be coadministered to examine the decrease in AUC.

- From these results, CR_{CYP3A4} values can be calculated. If clinically important DDIs are anticipated with any other inducers with known IC_{CYP3A4} values, clinical studies will be performed, and the results should be compared with the predicted values.

By applying and extending these methods, it will be possible to predict almost all clinical significant pharmacokinetic DDIs *via* metabolism by CYP3A4 in a quantitative manner in the future.

[Conclusion]

We have constructed general framework for the prediction of oral drug interactions caused by CYP3A4 induction from *in vivo* information. The accuracy and robustness of the method have been demonstrated satisfactorily. By using this method, the susceptibilities of a CYP3A4 substrate to both inhibitory and inductive DDIs can be estimated by conducting a single inhibitory DDI study during the early stages of clinical development.

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