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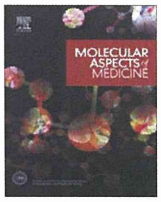

Review

Transporter biology in drug approval: Regulatory aspects ☆

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OAT3 (*SLC22A8*)
Organic cation transporter (OCT)2 (*SLC22A2*)
Multidrug resistance 1 (MDR1, P-glycoprotein (P-gp))
Multidrug and toxin extrusion (MATE)1 (*SLC47A1*)
MATE2-K (*SLC47A2*)

ABSTRACT

Previous *in vitro* and clinical research have indicated that a wide variety of drug transporters as well as metabolic enzymes dominate the pharmacokinetics of drugs and that some drugs modified the expression/function of drug transporters in humans, which lead to the altered pharmacokinetics and subsequent pharmacological/toxicological effects. Thus, regulatory authorities in US and EU have recently emphasized the needs to evaluate the risk of transporter-mediated drug–drug interactions (DDIs) in the (draft) guidance for pharmaceutical industries. The revised guidance includes the key transporters governing pharmacokinetics of drugs and decision trees to determine whether NMEs are substrates or inhibitors of each key transporter and when an *in vivo* clinical study is needed. In the evaluation of the potency of clinical DDIs, estimation of the inhibitor concentration at the target site is essential, but difficult since its direct measurement is almost impossible. Thus, people are now discussing what kind of inhibitor concentration should be used and how much is the appropriate cutoff value of the ratio of plasma AUC in the presence of inhibitor drugs to that in its absence (AUCR) to avoid false-negative predictions and maximize prediction accuracy. This minireview briefly summarizes the current status of the criteria for risk management of transporter-mediated DDIs in the regulatory guidelines, and describes scientific achievements that may affect regulatory decisions. Target transporters include OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*) in the liver, and OAT1 (*SLC22A6*), OAT3 (*SLC22A8*), OCT2 (*SLC22A2*), MATE1 (*SLC47A1*), and MATE2-K (*SLC47A2*) in the kidney, and MDR1 (*ABCB1*) in the intestine.

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1. The need to evaluate the importance of transporters in the pharmacokinetics of drugs during drug development

In the process of drug development, to understand the pharmacokinetic profiles of new molecular entities (NMEs) is one of the critical factors for selecting appropriate drug candidates and considering the proper use of drugs. Since many human drug transporters have been identified and characterized, clinical studies have also directly demonstrated the importance of selected transporters in the regulation of the pharmacokinetics of substrate drugs in humans *in vivo*. Several genetic polymorphisms in transporter genes, which alter the function/expression of transporters *in vitro*, were reported to affect the intestinal absorption and/or systemic clearance of substrate drugs. Some drugs are known to inhibit potently the function of certain transporters and subsequently change the pharmacokinetics of substrates in humans. Drug–drug interactions (DDIs) sometimes lead to the withdrawal of drugs from the market, despite their potential to clinically benefit many patients. For example, cerivastatin, a potent HMG-CoA reductase inhibitor, was voluntarily withdrawn from the market by the manufacturer because a number of patients died from lethal myotoxicity, including rhabdomyolysis, induced by cerivastatin. After thorough inspection of the data, it was found that some of the victims simultaneously took cerivastatin and gemfibrozil, an antihyperlipidemic drug, and the plasma AUC of cerivastatin was reported to be increased 4.4 times by coadministration of gemfibrozil (Backman et al., 2002). We now know that this DDI is mainly caused by the mechanism-based inhibition of CYP2C8-mediated metabolism and inhibition of organic anion transporting polypeptide (OATP)-mediated hepatic uptake of cerivastatin by gemfibrozil glucuronide (Ogilvie et al., 2006; Shitara et al., 2004). Because the substrate specificities of transporters are generally very broad, a functional change in a single transporter affects the pharmacokinetics of a wide variety of structurally unrelated compounds. Transporter inhibitors can also affect the pharmacokinetics of a range of drugs with different classes of pharmacological action. At present, we cannot accurately judge from a compound's chemical structure whether it interacts with transporters, so it is essential to know in the early stage of drug development which transporters can recognize a new drug candidate as a substrate and/or an inhibitor. It must also be noted that drugs that inhibit transporters *in vitro* do not always change the pharmacokinetics of substrate drugs in humans *in vivo*, because many factors modify the influence of coadministration of inhibitors on the total clearance of substrate drugs. Such factors include the ratio of the unbound concentration of an inhibitor at the site of the interacting molecule to its inhibition constant, contribution of a target transporter to the overall membrane transport process of substrate drugs and the rate-determining process (transport vs. metabolism; blood flow rate vs. intrinsic clearance) in the overall clearance of substrate drugs (Maeda and Sugiyama, 2007).

Under such circumstances, the US Food and Drug Administration (FDA) launched the International Transporter Consortium (ITC), which consists of scientists in the field of drug transporter science from the US, EU and Asia, and from industry, academia and the FDA. The ITC is intended to facilitate intensive discussion of the key transporters related to therapeutic and adverse drug responses, and to develop *in vitro* and *in vivo* tools and techniques to evaluate transporter function. It has also developed a decision tree for each key transporter, to judge whether a new molecular entity (NME) is a substrate or inhibitor of a certain transporter at relevant clinical concentrations and whether a clinical DDI study is recommended in the development of NMEs. This achievement was published as a review article in Nature Reviews Drug Discovery, and is recognized as the "FDA transporter white paper" (Giacomini et al., 2010). The US FDA recently released a revised draft guidance titled "Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations", which basically follows the contents of the white paper with some modifications (FDA, 2012). In April 2010, the European Medicines Agency (EMA) released a revised guideline titled "Guideline on the Investigation of Drug Interactions", which added to the discussion of transporter-mediated DDIs and it was finalized in June 2012. (EMA, 2012).

2. Key transporters as determinants of the pharmacokinetics of drugs

Fig. 1 illustrates the tissue distribution and membrane localization of major drug transporters in the "FDA transporter white paper" (Giacomini et al., 2010). The US FDA draft guidance on DDIs indicates that OATP1B1, OATP1B3, organic anion transporter 1 (OAT1), OAT3 and organic cation transporter 2 (OCT2) are key uptake transporters and that P-gp (P-glycoprotein) and BCRP (breast cancer resistance protein) are key efflux transporters. In addition, the EMA guideline suggests that DDIs mediated by OCT1, MATEs (multidrug and toxin extrusions) and BSEP (bile salt export pump) should also be considered. Both documents note that transporters might be added to or removed from the list of key transporters based on future advances in transporter science.

OATP1B1 and OATP1B3, encoded by the *SLCO1B1* and *SLCO1B3* genes, respectively, are exclusively expressed on the basal side of hepatocytes and are responsible for the hepatic uptake of several clinically important anionic drugs including HMG-CoA reductase inhibitors (statins) and angiotensin II type 1 receptor antagonists (sartans) (Fahrmayr et al., 2010). OAT1 and OAT3, encoded by the *SLC22A6* and *SLC22A8* genes, respectively, are mainly expressed on the basal side of renal tubular epithelial cells and are involved in the renal secretion of several anionic drugs (Rizwan and Burckhardt, 2007). OAT1 accepts hydrophilic compounds with relatively low molecular weight such as nucleotide analog antiviral drugs (adefovir, tenofovir,

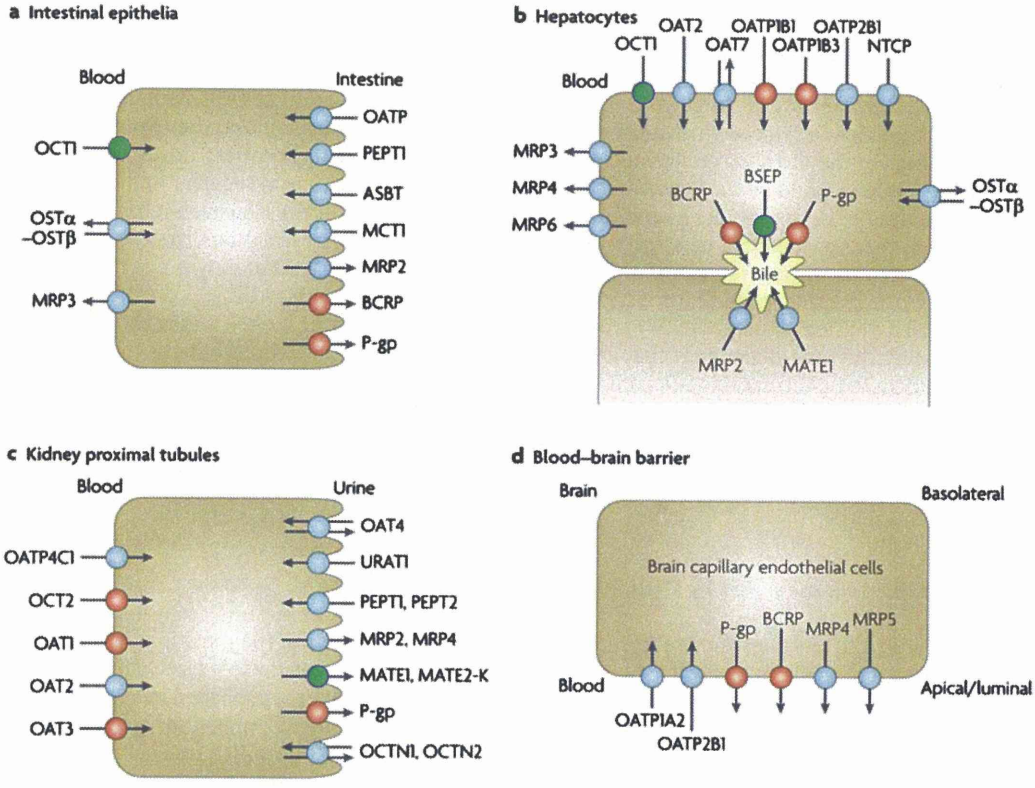

Fig. 1. Schematic diagram of major drug transporters in various tissues (cited from (Giacomini et al., 2010) with slight modification) Major drug transporters in the intestine (a), liver (b), kidney (c), and blood–brain barrier (d) are shown. Arrows represent the direction of substrate transport for each transporter. Orange circles: key drug transporters mentioned in both US FDA draft guidance and EMA guideline on DDIs. Green circles: key drug transporters mentioned only in EMA guideline on DDIs.

etc.), while OAT3 accepts hydrophobic compounds with high molecular weight such as pravastatin and several antibiotics (new quinolones, cephalosporins, etc.) and has a substrate specificity similar to that of the OATP family transporters. OCT1 and OCT2, encoded by the *SLC22A1* and *SLC22A2* genes, are involved in the hepatic and renal uptake, respectively, of relatively small organic cations such as biguanides (metformin, phenformin, etc.) (Koepsell et al., 2007).

P-gp and BCRP are expressed on the apical membrane of various tissues including intestine, blood–brain barrier, liver and kidney, and are involved in the restriction of the intestinal absorption of drugs and of drug penetration to brain, as well as in the biliary and urinary excretion of drugs. Both can accept various types of structurally diverse compounds as substrates. The *in vivo* significance of these transporters has been demonstrated by the use of transporter knockout mice. Several clinical reports indicated that some genetic polymorphisms of the *ABCB1* gene encoding P-gp alter its expression/function and the subsequent pharmacokinetics of P-gp substrates, although reproducible results have not often been obtained (Cascorbi, 2011). On the other hand, previous reports indicated that the Q141K polymorphism in BCRP (encoded by the *ABCG2* gene) increased the plasma concentration of several substrates such as rosuvastatin and topotecan, and multiple *in vitro* studies confirmed a decrease in expression/function of BCRP in the presence of the Q141K mutation, although the detailed mechanisms reported differed between papers (Meyer zu Schwabedissen and Kroemer, 2011).

BSEP, encoded by *ABCB11*, is responsible for the biliary excretion of bile acids. Previous reports indicated that several drugs and their metabolites potently inhibit BSEP function *in vivo* and that, as a result, the bile acid level in plasma and liver is increased, the so-called "drug-induced cholestasis".

3. Basics of the quantitative prediction of drug–drug interactions

In general, to estimate quantitatively the inhibition potency of inhibitor drugs, assuming that inhibitor drugs simply inhibit the transporters in a competitive or noncompetitive manner and substrate concentration is far below the K_m value (Michaelis constant) for the target transporter, the ratio of the intrinsic clearance of substrate drugs (CL_{int}) in the presence of inhibitor drugs to that in their absence is used, as described by the following equation:

$$\frac{CL_{int}(+\text{inhibitor})}{CL_{int}(-\text{inhibitor})} = \frac{1}{1+\frac{I_u}{K_i}} \left(= \frac{1}{R}\right) \quad (1)$$

The *R*-value determines the inhibition potency of inhibitor drugs for drug transporters/enzymes and consists of 2 important parameters, I_u and K_i. I_u represents the protein-unbound concentration of inhibitor drugs at the site of interaction and K_i represents the inhibition constant of inhibitor drugs. The K_i value can be obtained from an *in vitro* inhibition assay using transporter expression systems. However, the precise I_u value used for the prediction of DDIs depends on the target site. For example, when we consider DDIs occurring in the small intestine, the I_u value should be the theoretical concentration of unbound inhibitor drugs in the intestinal tract. However, when we consider DDIs occurring during the efflux process in the liver or kidney, the I_u value should be the concentration of unbound inhibitor drugs in the liver or kidney, or the concentration in the plasma if inhibitor drugs are taken up into the tissues by passive diffusion. However, it is difficult to accurately estimate the I_u value in humans as it cannot be directly measured. In addition, in real situations, the I_u value always changes over time. If we consider that the time-dependent inhibitory effect of inhibitor drugs accurately predicts the ratio of plasma AUC of substrate drugs in the presence of inhibitor drugs to that in their absence (AUCR; $AUC_{+inhibitor}/AUC_{control}$), that is, if we use a physiologically based pharmacokinetic (PBPK) model (referred to as a "dynamic model" in the US FDA draft guidance), then we must also consider the time-dependent change in plasma and tissue concentrations of both substrate and inhibitor drugs. However, as the detailed pharmacokinetic parameters of NMEs have not been identified in humans, it is difficult to apply a dynamic model approach to the prediction of DDIs, especially in the early stage of drug development. Instead, the I_u value is assumed to be set as a constant. This approach is referred to as a "static model" in the US FDA draft guidance, which recommends the use of a static model with a theoretical maximum concentration at the target site at the first stage of DDI prediction to avoid false-negative predictions. The key factor for DDI prediction with a static model is how to estimate the I_u value.

4. Investigation of the DDI risk mediated by P-gp in the small intestine

As discussed above, it is difficult to estimate the concentration of inhibitor drugs in the intestinal tract and there is considerable debate about what I_u values and cutoff values of I_u/K_i are appropriate to avoid false-negative predictions when predicting the risk of intestinal P-gp-mediated DDIs. In the previous US FDA draft guidance published in September 2006, if $[I_1]/IC_{50}$ (or K_i) is larger than 0.1 ($[I_1]$ = mean steady-state maximum plasma total [bound + unbound] concentration following administration of the highest proposed clinical dose), a clinical DDI study with a P-gp substrate such as digoxin is recommended. Zhang et al. identified from the literature 9 P-gp inhibitor drugs that can increase the plasma AUC of orally administered digoxin by more than 25% (AUCR $\geqslant$1.25), and checked whether the DDIs could be correctly predicted using that criterion (Zhang et al., 2008). Their results showed that false-negative predictions were obtained for 6 of the 9 drugs. As P-gp is expressed in the small intestine as well as in the liver and kidney, and the drug concentration in the small intestine is thought to be very high compared with that in the plasma, most P-gp-mediated DDIs occur in the intestine. Therefore, the current US FDA draft guidance proposes new criteria: $[I_1]/IC_{50}$ (or K_i) $\geqslant$0.1 or $[I_2]/IC_{50}$ (or K_i) $\geqslant$10 ($[I_2]$ = dose/250 mL (= a glass of water with which a drug is orally administered). Zhang et al. indicated that using the new criteria, the incidence of false-negative predictions was decreased to 2 of 9 drugs (talinolol and captopril) (Zhang et al., 2008). These remaining false negatives may be occurring because of additional mechanisms involved in these DDIs. False-positive predictions were not observed for 5 drugs that did not significantly increase the plasma AUC of digoxin (AUCR <1.25). Therefore, the cutoff value for $[I_2]/IC_{50}$ was set to 10 in the draft guidance. Since then, several researchers have validated the criteria in different sets of clinical DDI studies. Fenner et al. analyzed clinical studies of DDIs between digoxin and 19 coadministered drugs, and suggested that $[I_1]/IC_{50}$ >0.1 is predictive of positive DDIs and $[I_2]/IC_{50}$ <10 is predictive of negative DDIs with a relatively low

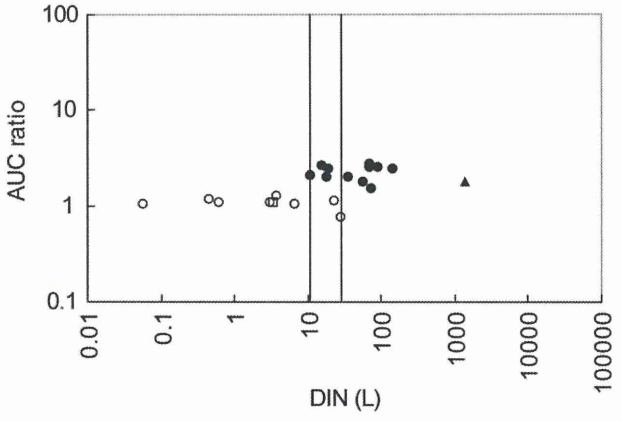

Fig. 2. Relationship between the drug interaction number (DIN) and the increase in the plasma AUC ratio of P-gp substrates co-administered with an inhibitor. (cited from (Tachibana et al., 2009)) Open circles: AUC ratio <1.25 and $D_{o,i}$ < 7.7 or unknown Open squares: AUC ratio <1.25 and $D_{o,i}$ $\geqslant$7.7 Closed circles: 1.25$\leqslant$ AUC ratio $\leqslant 1/F_aF_g$ Closed triangles: $1/F_aF_g$ < AUC ratio. $D_{o,i}$ represents the dose number for an inhibitor defined as dose divided by the 250 mL (= a glass of water with which a drug is orally administered) and solubility.

frequency of false-negative predictions (2 of 9 drugs) (Fenner et al., 2009). Agarwal et al. also evaluated the validity of the criteria using 11 P-gp inhibitor drugs selected from recently approved (2003–2010) new drug applications and showed that the current criteria are effective at avoiding both false-negative (0 of 4 drugs) and false-positive (2 of 7 drugs) predictions (Agarwal et al., in press).

Tachibana et al. sought to devise an appropriate criterion to evaluate the risk of P-gp-mediated DDIs using a different indicator, DIN (drug interaction number) (Tachibana et al., 2009). The DIN is calculated by dividing the dose by the K_i value. We predicted that the dose/K_i value would be an alternative indicator to the conventional $[I]/K_i$ value, assuming that the "apparent" intestinal volume is constant. Analysis of 21 DDI studies with 3 P-gp-selective substrates showed that inhibitor drugs with a DIN <10.8 L have a low risk of P-gp-mediated DDIs and those with a DIN >27.9 L have a high risk (Fig. 2). This criterion is calculated to be equivalent to values of $[I_2]/K_i$ <43.2 and $[I_2]/K_i$ >112, representing a low and high risk of DDIs, respectively, suggesting that our criterion is a little less strict than that in the US FDA draft guidance. Recently, this concept has also been applied to the evaluation of nonlinear intestinal absorption of drugs by saturation of P-gp in the small intestine (Tachibana et al., 2012).

Cook et al. sought to determine statistically the most appropriate cutoff values of $[I_1]/IC_{50}$ and $[I_2]/IC_{50}$ using ROC (receiver operating characteristic) curve analysis of the same clinical data that Fenner et al. used (Cook et al., 2010). They weighted false negatives 2–5 times more than false positives and found that $[I_1]/IC_{50} \geqslant 0.1$ or $[I_2]/IC_{50} \geqslant 5$ were the best criteria to minimize both false-negative and false-positive predictions. Sugimoto et al. analyzed clinical studies of DDIs between digoxin and 25 coadministered drugs, using IC_{50} values obtained from the efflux ratio ($PS_{b\text{-}to\text{-}a}/PS_{a\text{-}to\text{-}b}$) in MDR1-expressing LLC-PK1 cells to optimize the cutoff value of $[I_2]/IC_{50}$, and proposed that 30 is the optimal cutoff value to predict clinically relevant DDIs(Sugimoto et al., 2011).

In contrast, the EMA guideline recommends a clinical DDI study with a P-gp-sensitive probe substrate (e.g., dabigatran etexilate, fexofenadine, digoxin) if $[I_2]/K_i$ is more than 10. This criterion is partly consistent with the US draft guideline.

Both (draft) guidelines recommend use of the same criteria for BCRP as for P-gp, although the clinical DDI data reported to date are insufficient.

5. Investigation of the DDI risk mediated by OATP transporters in the liver

To consider the potency of inhibitor drugs on hepatic uptake based on Eq. (1), the I_u value should be the protein-unbound concentration at the inlet to the liver. As drugs in the portal vein come both from blood circulation and from the intestinal lumen after their oral administration, the inhibitor concentration at the inlet to the liver sometimes exceeds that in the plasma. To predict simply the risk of OATP-mediated DDIs in the liver with minimal false-negative predictions, a static model using the theoretical maximum inhibitor concentration at the inlet to the liver is useful. Ito et al. reported that the maximum I_u value at the inlet to the liver of inhibitor drugs ($I_{u,in,max}$) can be calculated from the following equation(Ito et al., 1998):

$$I_{u,in,max} = f_u \cdot \left(I_{max} + \frac{k_a \cdot F_a F_g \cdot D}{Q_h} \right) \quad (2)$$

where f_u, I_{max}, k_a, F_aF_g, D and Q_h represent the protein-unbound fraction of inhibitor drugs in the blood, observed maximum inhibitor drug concentration in blood, the absorption rate constant of inhibitor drugs, intestinal availability of inhibitor drugs, dose of inhibitor drugs and hepatic blood flow rate, respectively. If k_a and F are unknown, maximum values can be used ($k_a = 0.1\ min^{-1}$, $F = 1$) to avoid false-negative predictions. This equation has already been applied in Japanese DDI guidelines to predict the risk of CYP-mediated DDIs (MHLW, 2001). Two papers (Hirano et al., 2006; Matsushima et al., 2008) from our group calculated the *R*-values of several *in vitro* inhibitor drugs for OATP1B1 and OATP1B3. Their results indicate that rifamycin SV, clarithromycin and indinavir are clinically relevant OATP1B1 inhibitors and that cyclosporin A and rifampicin are potent OATP1B1 and OATP1B3 inhibitors in the clinical situation, because the *R*-values of these drugs exceed 2. The published clinical DDI data indicate that clinical doses of these drugs do actually decrease the hepatic (nonrenal) clearance in humans of OATP substrates such as statins. Thus, the decision tree for OATP inhibitor drugs that appears in the "FDA transporter white paper" tells us that clinical DDI studies are recommended if the *R*-values calculated from $I_{u,in,max}$ of new drugs exceed 2. However, because the FDA draft guidance defines positive DDI cases as those with an AUCR value of more than 1.25, the cutoff *R*-value is also set at 1.25. In contrast, the EMA guideline recommends the use of clinical DDI studies if $I_{u,in,max}$ divided by the K_i value is more than 0.04. This criterion is stricter than that in US draft guidance ($[I_{u,in,max}]/Ki > 0.25$).

Yoshida et al., by comparing the predicted AUCR values with clinically observed AUCRs, recently demonstrated the type of assumptions that improve the prediction accuracy and minimize false-negative predictions when the risk of clinical DDIs involving hepatic OATP substrates is evaluated based on a static model(Yoshida et al., 2012). They defined positive DDIs when the AUCR value was more than 1.25, which is the same criterion used in the US FDA draft guidance. For the inhibition of efflux transporters (P-gp, BCRP) and metabolic enzyme (CYP3A4) in the small intestine, the number of false-negative predictions was decreased when the intestinal availability (F_aF_g) was assumed to increase to a maximum of 1 if the DIN (dose/K_i value) of inhibitor drugs exceeded the threshold values mentioned above. Using Eq. (1), the false-negative predictions were minimized when the I_u value was set at the maximum I_u value at the inlet to the liver ($I_{u,in,max}$) calculated from Eq. (2), compared with the maximum protein-unbound plasma concentration ($I_{u,max}$) and total plasma concentration (I_{max}) of inhibitor drugs. Moreover, the number of false-negative predictions was the lowest (11 of 58 examples) when the maximum

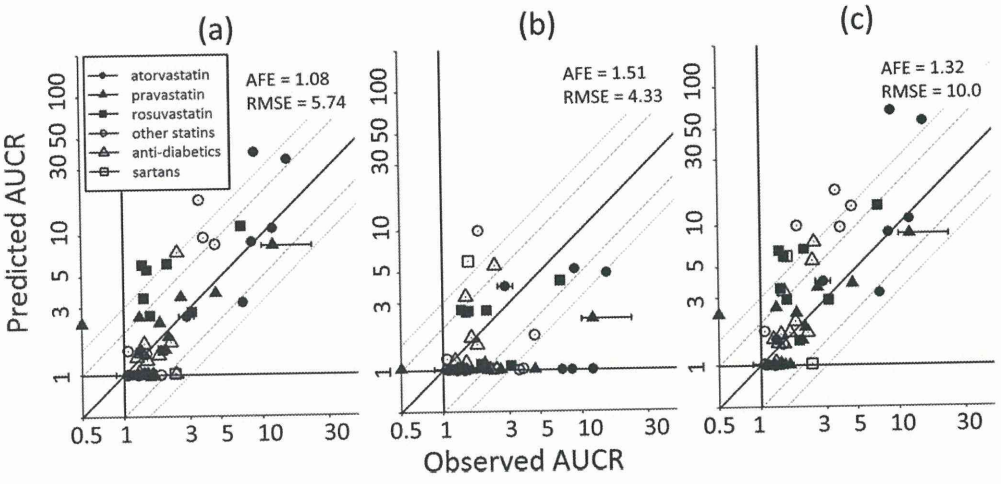

Fig. 3. Predicted and observed AUCRs in each DDI study, assuming that the hepatic clearance of substrate drugs is affected by (a) inhibition of only the basolateral uptake process, (b) inhibition of only the apical efflux/metabolic process, or (c) inhibition of both uptake and apical efflux/metabolic process. (cited from (Yoshida et al., in press)). Each point and horizontal bar represents the median, maximum, and minimum values of the observed AUCRs for the same combination of drugs. The solid, dashed, and dotted lines represent the line of unity, the 50–200% range, and the 33–300% range of the observed AUCRs, respectively. AFE, average fold error; RMSE, root mean squared error.

inhibitory effects of both uptake and efflux/metabolism were simply calculated by multiplying the *R*-value for uptake transporters by that for efflux transporters and/or metabolic enzymes. However, in this scenario, the number of false-positive predictions increased and the prediction accuracy decreased (69/83% for predictions within 2-/3-fold of observed AUCR values) (Fig. 3). In contrast, when considering only the maximum inhibitory effects on the uptake process, the number of false-negative predictions was a little higher (16 of 58), but the prediction accuracy was also higher (76/90% for predictions within 2-/3-fold of observed AUCR values) compared with the scenario where maximum inhibitory effects on both uptake and efflux/metabolism were considered (Fig. 3). As discussed above, this may indicate that hepatic uptake is the rate-determining process for the overall hepatic clearance of most OATP substrates.

6. Impact of MATEs (multidrug and toxin extrusions) on DDIs in the kidney

Clinical cases of DDIs mediated by renal transporters have also been reported. For example, in the US FDA draft guidance, in the clinical situation, probenecid increased the plasma AUCs of OAT1 and OAT3 substrate drugs by a significant inhibition of OAT1 and OAT3, while cimetidine increased the plasma AUCs of OCT2 substrates such as dofetilide, pindolol and metformin by the inhibition of OCT2 (FDA, 2012). When considering the inhibition of renal transporters based on the Eq. (1), the I_u value should be the protein-unbound concentration of inhibitor drugs ($I_{u,max}$). The US FDA draft guidance recommends clinical DDI studies of new drugs if $I_{u,max}/IC_{50}$ is more than 0.1, while the EMA guideline recommends clinical DDI studies if $I_{u,max}/K_i$ is more than 0.02. Although there are many drugs that inhibit OAT1 and OAT3 *in vitro*, few drugs, with the exception of some cephem antibiotics and probenecid, are reported to interact with OAT1 and/or OAT3 in the clinical situation, because the clinical plasma-unbound concentration of these drugs is generally lower than their K_i values for OAT1 and OAT3 (Shitara et al., 2005). In the case of probenecid, when 500 mg of probenecid is orally administered, $I_{u,max}$ reaches 13.4 μM. Its reported

Table 1
Cimetidine inhibition constants for the renal organic cation transporters (cited from (Ito et al., 2012)).

	Inhibition Constant (K_i)				
	TEA	Metformin	MPP^+	ASP	MIBG
	μM				
hOCT1	155 ± 27	104 ± 23	223 ± 34	186 ± 13	101 ± 13
hOCT2	144 ± 25	124 ± 20	146 ± 32	104 ± 10	95 ± 16
mOct1	99 ± 13	54 ± 15	55 ± 6	67 ± 13	94 ± 18
mOct2	122 ± 24	61 ± 13	143 ± 32	71 ± 10	84 ± 14
hMATE1	1.1 ± 0.3	3.8 ± 0.8	2.7 ± 0.5	2.9 ± 0.3	1.7 ± 0.3
hMATE2-K	2.1 ± 0.5	6.9 ± 2.3	4.0 ± 0.8	2.5 ± 0.3	2.7 ± 0.3
mMate1	1.4 ± 0.4	3.6 ± 0.7	3.0 ± 0.8	2.6 ± 0.4	2.0 ± 0.5

Each parameter represents the mean value ± computer-calculated S.D.
TEA: tetraethylammonium, MPP^+: 1-methyl-4-phenylpyridinium, ASP: [4-(4-(dimethylamino)styryl)-*N*-methylpyridinium, MIBG: *m*-iodobenzylguanidine.
h: human, m: mouse.

K_i values in *in vitro* inhibition studies are 4.4–12 μM for human OAT1 and 4.4–30 μM for human OAT3. Thus, the *R*-values of probenecid for OAT1 and OAT3 are calculated to be 2.1–4.0 and 1.4–4.0, respectively, indicating that a static model correctly predicted the clinical DDIs. However, Ito et al. questioned the inhibition of OCT2 by clinical doses of cimetidine, and calculated the *R*-values of cimetidine for OCT2 (Ito et al., 2012). They performed an *in vitro* assay to measure the inhibition by cimetidine of the OCT2-mediated uptake of several substrates, and estimated that the K_i values of cimetidine were within the range of 95–146 μM (Table 1). However, the clinically reported plasma-unbound concentration of cimetidine was 3.6–7.8 μM and the *R*-value of cimetidine for OCT2 was calculated to be 1.02–1.08, suggesting that *in vivo* inhibition of OCT2 by clinical doses of cimetidine is unlikely. As an alternative, Ito et al. proposed MATEs (multidrug and toxin extrusions) as a major target of clinical DDIs with cimetidine (Ito et al., 2012). The MATE family transporters MATE1 (encoded by the *SLC47A1* gene) and MATE2-K (encoded by *SLC47A2*) are expressed on the apical side of renal proximal tubular cells and work as efflux transporters with an exchange of H^+ (Terada and Inui, 2008). As MATEs can recognize several types of cationic drugs as substrates, they are thought to be involved in the renal secretion of organic cations in combination with OCT2-mediated renal uptake. The *in vivo* significance of MATEs has also been demonstrated by decreased renal clearance of metformin and cephalexin in *Slc47a1*-knockout mice (Tsuda et al., 2009a; Watanabe et al., 2010) and decreased renal clearance of metformin in healthy human subjects after coadministration with pyrimethamine, a potent MATE inhibitor(Kusuhara et al., 2011). Ito et al. also showed that the K_i value of cimetidine for MATEs was within the range of 1.1–6.9 μM (Table 1) (Ito et al., 2012). Thus, assuming that the unbound concentration of cimetidine in plasma is equal to that in kidney, the *R*-value of cimetidine for MATEs is calculated to be 1.5–8.1, suggesting a significant contribution of MATEs to clinical DDIs with cimetidine. This is supported by previous reports demonstrating the weak inhibition of OCT2-mediated uptake and the potent inhibition of MATE-mediated transport by cimetidine in models using kidney slices, transporter expression systems and OCT2/MATE1 double-transfected cells (Matsushima et al., 2009; Tsuda et al., 2009b). Therefore, we propose that, clinically, cimetidine is not an OCT2 inhibitor but a MATE inhibitor, and that MATEs should be added to the guidelines as a target transporter for *in vivo* clinical DDIs. Cimetidine has been reported to decrease renal secretion and clearance of many cationic drugs (Ito et al., 2012), but whether these DDIs can also be explained by the potent inhibition of MATEs by cimetidine is still under investigation.

7. Conclusions and future directions

In this review, we have given a brief overview of the recently released US FDA draft guidance and the EMA guideline for the prediction of transporter-mediated DDIs, and have discussed the validity of their criteria in the light of pharmacokinetic theory. It is very difficult to set a threshold for the *R*-value: if the threshold is set more strictly, false-negative predictions are likely to be avoided but false-positive predictions are increased, which could lead to an increase in the number of unnecessary clinical DDI studies. The same difficulty arises in setting the maximum I_u value. These parameters may have to be adjusted depending on the safety margin of NMEs and should be validated using previous DDI studies, although the number of such cases is limited.

We have not considered certain other points such as the active uptake of inhibitor drugs in the tissues (Grime et al., 2008), substrate-dependent inhibitory effects (Noe et al., 2007) or sustainable inhibition of transporters (Shitara et al., 2009) by certain inhibitor drugs. For the quantitative prediction of AUCR values, a dynamic model should be used because theoretical AUCRs predicted from a static model always overestimate the observed values. As part of a regulatory review at the FDA, Zhao et al. described a number of examples of the use of PBPK models in efficient decision making, including the accurate prediction of DDIs so as to avoid unnecessary clinical DDI studies (Zhao et al., 2011), and Huang and Rowland discussed the use of simulation with PBPK modeling to address regulatory questions in clinical pharmacology reviews (Huang and Rowland, 2012). To correctly manage a dynamic/PBPK model, it is essential to set all the parameters in the model appropriately with the aid of *in vitro* experiments, because multiple sets of parameters sometimes fit with the clinically observed time profile of the plasma concentration of drugs. However, at present, a general procedure to construct a realistic PBPK model has not been well established and validated. Such a procedure must be developed to avoid the improper use of dynamic models.

We also have to discuss DDIs that occur in the tissues without changes in the plasma concentration of victim drugs (Giacomini and Sugiyama, 2011). If the efflux transporters are inhibited by inhibitor drugs and organ clearance is limited by the uptake process, or the distribution volume of the target organ is very small compared with that of whole body, the drug concentration in plasma is not changed while that in the target tissue is significantly increased. If the target tissue for DDIs is also important for the pharmacological/toxicological effects of drugs, this type of DDI must be carefully evaluated, because it cannot be detected by the change in plasma concentration of drugs. To overcome this problem, an imaging technique such as PET (positron emission tomography) is useful to noninvasively and directly measure the tissue concentration of drugs in the target organ (Takashima et al., 2012), and a PBPK model greatly assists in evaluation of the time profile of the tissue concentration of drugs. Advances in such basic technologies and validation of DDI prediction methods are required to further improve regulatory guidelines.

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