Retrospective analysis of P-glycoprotein-mediated drug-drug interactions
at the blood-brain barrier in humans

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Abbreviations:
IVIVC, in vitro-in vivo correlation; P-gp, P-glycoprotein; DDI, drug-drug interaction; BBB, blood-brain barrier; $I_{\text{unbound}}$, unbound plasma concentration; PET, positron emission tomography; MDR1, multidrug resistance protein 1; ABC, ATP-binding cassette; UFLC, ultra-fast liquid chromatography; LC/MS/MS, liquid chromatography with triple quad mass spectrometric detection; ER, efflux ratio; MCFR, modified corrected flux ratio; CNS, central nerve system
Abstract

To date, the in vitro-in vivo correlation (IVIVC) of P-glycoprotein (P-gp)-mediated drug-drug interaction (DDI) at the blood-brain barrier (BBB) in rats indicated that the cutoff value to significantly affect the brain penetration of digoxin was [I_unbound/K_i] of 1, where I_unbound is the unbound plasma concentration of P-gp inhibitors. Based on the IVIVC in rats, we speculated that clinically used P-gp inhibitors do not cause DDI at the human BBB, because none of the compounds studied was [I_unbound/K_i]>1 at therapeutic doses. Recently, positron emission tomography studies with P-gp substrates such as [11C]verapamil, [11C]N-desmethyl loperamide, and [11C]loperamide together with potent P-gp inhibitors have indicated that increases in the influx rate constant for brain entry were observed in humans. Therefore, we aimed to retrospectively analyze the results of P-gp-mediated DDIs with in vitro P-gp inhibition assays and to confirm the appropriate cutoff value. In vitro P-gp inhibition assays using verapamil, N-desmethyl loperamide, and loperamide as P-gp probe substrates were performed in human MDR1-expressing LLC-PK1 cells. The efflux ratios decreased in the presence of P-gp inhibitors and the K_i of tariquidar was 10 nmol/L regardless of probe substrates. Taking the in vitro K_i and unbound plasma concentrations in clinical DDI studies together, the criterion [I_unbound/K_i] of 1 was an appropriate cutoff limit to observe significant P-gp-mediated DDI at the BBB in humans. On the other hand, no significant DDI was observed in cases where [I_unbound/K_i] was less than 0.1. This criterion was comparable to the previous IVIVC result in rats.
Introduction

P-glycoprotein (P-gp, multidrug resistance protein 1, MDR1) is a member of the ATP-binding cassette transporters that is encoded by the ABCB1 gene in humans. P-gp is ubiquitously expressed in normal tissues, including the small intestine, liver, kidney, and blood-brain barrier that are involved in drug absorption, elimination and disposition (Tsuji et al., 1992; Tsuji, 2002; Chan et al., 2004). A wide range of structurally diverse compounds including anti-cancer drugs, HIV protease inhibitors, cardioactive drugs, and immunosuppressant agents can be actively transported by P-gp (Eyal et al., 2009). Recently, the clinical relevance of P-gp-mediated DDIs has been summarized by the draft guidance published by the US Food and Drug Administration (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf). Accordingly, it is important to assess whether drug candidates have potential risks of P-gp-mediated DDI in clinical studies.

In the brain capillaries, P-gp is localized at the luminal membrane of the endothelial cells and pumps drugs and xenobiotics out from the brain into the blood. In vivo studies using mdr1a knockout mice indicated that P-gp regulates the brain penetration of several drugs, such as dexamethasone, digoxin, and cyclosporin A (Schinkel et al., 1995). However, current clinical data indicate that there are no consistent examples in which P-gp inhibition is clinically relevant at the human BBB. Besides, there is very limited information available regarding quantitative prediction of the magnitude of P-gp-mediated DDI. We have recently reported the quantitative investigation of the impact of P-gp inhibition on digoxin transport across the rat BBB. The in vitro-in vivo correlation (IVIVC) in rats indicated that the cutoff limit value to significantly affect the brain penetration of digoxin was \[ I_{\text{unbound}}/K_i \] of 1, where \( I_{\text{unbound}} \) is the unbound plasma concentration of P-gp inhibitors. Based on the IVIVC in rats, we speculate that clinically significant DDIs at the human BBB may be limited at conventional therapeutic doses (Sugimoto et al., 2011a).

On the other hand, recent studies using noninvasive positron emission tomography (PET) imaging with radiolabeled P-gp substrate such as \(^{11}\text{C}\)verapamil, \(^{11}\text{C}\)N-desmethyl loperamide, and \(^{11}\text{C}\)loperamide revealed that the influx rate constant for brain entry increased in the presence of potent P-gp inhibitors in humans. Verapamil acts only as a P-gp substrate at low concentration and has relatively high lipophilicity to penetrate across the human BBB. The influx rate constant for brain entry of
[11C]Verapamil after intravenous (i.v.) infusion of tariquidar (8 mg/kg) increased 3.3-fold relative to the baseline (Bauer et al., 2012). Tariquidar is a third generation P-gp inhibitor and an effective modulator against resistant MDR human tumor xenografts. However, the Phase III trial of tariquidar with doxorubicin or taxane-containing chemotherapy regimens in non-small cell lung cancer was discontinued due to safety issues (Grandvuinet et al., 2012). N-desmethyl loperamide, which is a major metabolite of loperamide in humans, acts only as a P-gp substrate, although at high concentrations, it acts as both a substrate and an inhibitor (Zoghbi et al., 2008; Kannan et al., 2010). N-desmethyl loperamide is one of the most promising P-gp probe substrates using PET imaging because further demethylated radiometabolites have limited entry into the brain. Kreisl et al. reported that the influx rate constant for the brain entry of [11C]N-desmethyl loperamide after intravenous administration of tariquidar (6 mg/kg) increased 3.7-fold relative to the baseline (Kreisl et al., 2010). Loperamide has no opiate side effects on the central nervous system because P-gp avidly restricts its entry into brain. Passchier et al. reported that the influx rate constant for brain entry of [11C]loperamide was increased 1.3-fold in the presence of quinidine (Passchier et al., 2008). In addition, concurrent administration of quinidine at a dose of 600 mg/man resulted in opiate induced respiratory depression lasted for a couple of hours (Sadeque et al., 2000).

To date, in vitro P-gp inhibition studies in human MDR1 have not been reported for these probe substrates. Moreover, the quantitative investigation for the impact of P-gp inhibition on brain penetration in humans has not been fully examined. Hence, the aims of the present study are to retrospectively evaluate the magnitude of P-gp-mediated DDIs at the human BBB with a use of in vitro P-gp inhibition data and to confirm the appropriate cutoff limit value.
Materials and Methods

Materials

Cyclosporin A, N-desmethyl loperamide and saquinavir were purchased from Toronto Research Chemicals, Inc. (North York, ON, Canada). Loperamide was from MP Biomedicals, LLC (Tokyo, Japan). Lucifer yellow was from Sigma-Aldrich, Co. (St. Louis, MO). Clarithromycin, verapamil, and quinidine were from Wako Pure Chemicals Industries (Osaka, Japan). Tariquidar was from MedKoo Biosciences (Chapel Hill, NC). Ketoconazole was from LKT Labs, Inc. (St. Paul, MN). [3H]Verapamil (80 Ci·mmol⁻¹) was from American Radiolabeled Chemicals, Inc. (Saint Louis, MO). All other chemicals were the highest reagent grade available from commercial sources. HTS Transwell® 96 well permeable support with polyethylene terephthalate membrane, 0.4 μm pore size and 0.143 cm² surface area, was purchased from Corning Life Sciences (Lowell, MA).

Cell culture

The LLC-PK1 cells expressing human MDR1 were cultured as previously described with slight modification (Takeuchi et al., 2006). Briefly, cells were collected by adding 0.05% trypsin/EDTA solution, were suspended in the cell culture media (DMEM with 20% fetal bovine serum, 500 μg/mL G418, and 150 ng/mL colchicine), and then were seeded onto 96-well insert plates (1.125 × 10⁵ cells/well). These plates were incubated in an atmosphere of 5% CO₂-95% air at 37°C. Fresh medium was replaced on the third or fourth day and the transcellular transport study was took place on the seventh day after seeding.

P-gp inhibition assay for [3H]verapamil, N-desmethyl loperamide, and loperamide transport

The P-gp inhibition assay for [3H]verapamil, N-desmethyl loperamide, and loperamide transport across LLC-PK1 cells expressing human MDR1 was performed according to the previously described method (Sugimoto et al., 2011b). Samples from the receiver side (25 μL of apical side or 100 μL of basolateral side) were taken after 2h incubation. The radioactivity of [3H]verapamil was measured using a liquid scintillation counter (LSC-6100, Hitachi Aloka Medical, Ltd., Tokyo, Japan) after the addition of 2 mL of scintillation cocktail A (Wako Pure Chemicals, Osaka, Japan). The concentrations of N-desmethyl loperamide and loperamide were determined with tandem mass spectrometry (MS/MS) analysis (API4000,
AB SCIEX, Foster City, CA) equipped with an ultra-fast liquid chromatography (UFLC) apparatus (Shimadzu, Kyoto, Japan). Samples from in vitro P-gp inhibition assay were mixed with an equal volume of acetonitrile including 100 ng/mL of alprenolol (internal standard) and centrifuged at 4°C, 5084 × g for 5 min (Sorvall Legend RT, Thermo Fisher Scientific, Yokohama, Japan). The supernatant was diluted with an equal volume of 0.1% formic acid solution. The sample was mixed, centrifuged at 4°C, 5084 × g for 5 minutes, and the supernatant was analyzed by MS/MS analysis (API4000) equipped with UFLC. The analytical column used was a Shim-pack XR-ODS C18 column (20 × 2.0 mm, 5 μm) from Shimadzu Co. Ltd. The total run time, flow rate, and column temperature were 2.6 min, 0.5 ml/min, and 50°C, respectively. Mobile phases A and B consisted of 10 mmol/L ammonium formate (pH 3) and acetonitrile, respectively. The initial concentration of mobile phase B was 10%, and this condition was maintained for 0.2 min, followed by a linear increase of B to 95% over 0.2 min, and held for 1 min. The initial concentration was then reinstated and held for 1.2 min for re-equilibration. Detailed MS conditions are shown in Table 1.

The permeability coefficient of probe substrates (P_app) was calculated according to equation 1 and the efflux ratio (ER) was calculated using equation 2.

\[
P_{\text{app}} = \frac{\text{Amount}}{\text{Area} \times C_0 \times \text{Time}} \quad (1)
\]

\[
\text{Efflux ratio} = \frac{P_{\text{app,BtoA}}}{P_{\text{app,AtoB}}} \quad (2)
\]

where Amount is the amount of transported substrates per well of the cell monolayer, Area is the surface area of the cell monolayer (0.143 cm² in a 96-well plate), C_0 is initial concentration of substrates in the donor side, Time is the incubation time, P_{\text{app,AtoB}} is the apical-to-basal passive permeability-surface area product, and P_{\text{app,BtoA}} is the basal-to-apical passive permeability-surface area product. In vitro K_i values were calculated based on a Dixon plot using the parameter shown in equation 3.

\[
[MCFR -1] \times [\text{initial concentration of probe substrates}] \quad (3)
\]

where MCFR is modified corrected flux ratio, which was defined as the flux ratio in MDR1-expressing cells divided by the corresponding ratio in functionally MDR1-inhibited cells using a potent P-gp inhibitor.
The $[\text{MCFR -1} \times \text{initial concentration of probe substrates}]$ (4)

**Determination of the plasma protein binding**

The unbound fraction in human plasma for tariquidar (final concentration: 1 $\mu$mol/L) was determined using the 96-well equilibrium dialysis apparatus (HTDialysis, LLC, Gales Ferry, CT) according to the previously described method (Sugimoto et al., 2011b). The unbound fraction was calculated by the ratio of the concentrations in the receiver and donor sides of the dialysate. The analytical column used was Shim-pack XR-ODS, C18 column (20 mm × 2.0 mm, 5 $\mu$m) from Shimadzu Co. Ltd. (Kyoto, Japan). The total run time, flow rate and column temperature were 2.6 min, 0.5 mL/min, and 50 ºC, respectively. The mobile phases A and B consisted of 10 mmol/L ammonium formate and acetonitrile. The initial concentration of mobile phase B was 10% and the condition was maintained for 0.2 min followed by linear increase of B to 95% in the next 0.2 min, held for 1 min. The condition was then returned initial concentration and held for 1.2 min for re-equilibration. Detailed MS conditions are shown in Table 1.
Results and Discussion

To investigate the inhibitory potency of P-gp inhibitors, which were used in the clinical DDI studies, on [3H]verapamil, N-desmethyl loperamide and loperamide transport, a transcellular transport study in human MDR1-expressing cells was performed. *In vitro* $K_i$ values were ascertained from the intersection of the Dixon plot described in Figure 1 and summarized in Table 2. Tariquidar showed the most potent P-gp inhibitory effect ($K_i$, 0.01 μmol/L) among the P-gp inhibitors tested followed by cyclosporin A ($K_i$, 0.45 μmol/L) and quinidine ($K_i$, 0.82 μmol/L). Recent reports suggest that P-gp may contain multiple drug binding sites rather than a single site of broad substrate specificity by using radioligand binding techniques and the X-ray structure of P-gp (Martin et al., 2000; Aller et al., 2009). However, the substrate dependency of P-gp inhibition has not fully understood and further investigation should be required to clarify the issue by using a number of substrates that bind to different binding sites of P-gp. Thus, it is important to design the *in vitro* P-gp inhibition assay using the combination of substrates and inhibitors which are used in the clinical trials.

The relationship between the endpoint of the central nerve system (CNS) in P-gp-mediated clinical DDI studies and unbound plasma concentrations of P-gp inhibitors ([I unbound]) divided by *in vitro* $K_i$ values was retrospectively investigated to set the appropriate criterion to observe significant DDI in humans (Table 3). The plasma concentrations of the concomitant drugs in humans were obtained or assumed based on the previous report (Jamis-Dow et al., 1997; Stewart et al., 2000; Kharasch et al., 2004; Brunton et al., 2005). The [I unbound/$K_i$] values of tariquidar after intravenous administration were 1.28 and 1.17, where 3.3 and 3.7-fold increases of influx rate constant for brain entry of [11C]verapamil and [11C]N-desmethyl loperamide were observed, respectively. The [I unbound/$K_i$] values of quinidine (600 mg/man) after oral administration was 1.17, where the respiratory depressant effects of loperamide were observed. It should be noted that the dosage of quinidine in this study was relatively higher than the conventional therapeutic dosage (Brunton et al., 2005). Accordingly, the relationship of IVIVC indicated that significant DDI at the BBB occurred in the presence of the P-gp inhibitors with [I unbound/$K_i$] > 1 in humans. In the presence of the P-gp inhibitors with 0.1 < [I unbound/$K_i$] ≤ 1, P-gp-mediated DDIs may or may not be observed. The [I unbound/$K_i$] values of tariquidar after intravenous infusion for 30 min at a dose of 2 mg/kg was 0.48, where slight but notable increase of influx rate constant for brain entry was observed. The [I unbound/$K_i$] of
cyclosporin A after intravenous infusion for 1 h at a dose of 2.5 mg/kg/h was 0.44, where the \( \text{AUC}_{\text{brain}} / \text{AUC}_{\text{blood}} \) of verapamil increased by 88\% (Sasonko et al., 2005). However, in the clinical study, the precise quantitation of P-gp function in the presence of a P-gp inhibitor by measuring total \([^{11}\text{C}]\text{radioactivity} \) may be impaired because the metabolites of \([^{11}\text{C}]\text{verapamil} \) also have the properties of P-gp substrates (Lubberink et al., 2007). In addition, the peak plasma concentration achieved in the clinical study (2.80 \( \mu \text{mol/L} \)) was more than twice as much as the reported one at the therapeutic concentration (1.11 \( \mu \text{mol/L} \)) (Brunton et al., 2005). The \([ \frac{I_{\text{unbound}}}{K_i} ] \) values of tariquidar after intravenous infusion for 30 min at a dose of 6 mg/kg was 0.87, where the moderate DDI compared with higher dosage was observed (data not shown). However, loperamide’s opioid brain effect in humans was not observed after intravenous administration of 150 mg tariquidar even though the \([ \frac{I_{\text{unbound}}}{K_i} ] \) was 0.41 (Kurnik et al., 2008). The unbound plasma concentrations of tariquidar in this study may be inadequate to significantly increase the brain penetration of loperamide to induce opioid induced toxicity. Further investigations may be required to investigate the changes of the influx rate constant of loperamide in the presence of tariquidar. In the presence of the P-gp inhibitors with \([ \frac{I_{\text{unbound}}}{K_i} ] < 0.1 \), no clinically relevant DDI was reported. Hence, the criterion \([ \frac{I_{\text{unbound}}}{K_i} ] = 1 \) was a clear cutoff limit to observe significant DDI at the BBB in humans. On the other hand, no significant DDI was observed in cases where \([ \frac{I_{\text{unbound}}}{K_i} ] \) was less than 0.1. This finding was consistent with our previous report using rats as an experimental model for P-gp-mediated DDIs (Sugimoto et al., 2011a). In addition, at the conventional dose of clinically used P-gp inhibitors, P-gp-mediated DDI at the BBB in humans may be limited. The further clinical data accumulation will be necessary to define the precise cutoff limit to avert the P-gp-mediated DDIs.

Even though the cutoff value for P-gp-mediated DDI at the BBB was found to be identical between rats and humans, the magnitude of DDI in humans is relatively lower than that in rodents (Lee et al., 2010). One of the possible reasons is the difference in the inhibition pattern between human MDR1 and rat Mdr1a. The relationship between plasma tariquidar concentration and the influx rate constant of \([^{11}\text{C}]\text{verapamil} \) indicated that Hill coefficients in humans were higher than that in rats suggesting that there was a different cooperative binding site to human MDR1 and rat Mdr1a (Bauer et al., 2012). The accumulation of \textit{in vitro} P-gp inhibition data will be necessary for a further understanding of the inhibition pattern between human MDR1 and rat Mdr1a. Another possible reason for the species difference is the influence of cerebral blood
flow. The cerebral blood flow rate in rats under isoflurane anesthesia (1.11 ± 0.43 mL/g/min) was approximately 3-fold higher than that in humans (0.348 ± 0.023 mL/g/min) (Wei et al., 1993; Kreisl et al., 2010). When the function of P-gp is completely inhibited, the influx rate constant from blood to brain would approach to the cerebral blood flow. In fact, the influx rate constant of [11C]verapamil increased from 0.16 (baseline) to 1.16 mL/g/min in rats after bolus administration of 30 mg/kg tariquidar (Bauer et al., 2012). Therefore, the species difference of cerebral blood flow may at least partially be attributed to the different influence of P-gp inhibition on influx rate constant from blood to brain. The other possible factor for the species difference is the expression level of P-gp at the BBB between rats and humans or their functional activities. Although the P-gp expression levels at the BBB in rodents is 2.3-fold higher than that in humans (Uchida et al., 2011), Syvänen et al. suggested that the intrinsic P-gp activity at the BBB in humans may be less than that in rats (Syvanen et al., 2009). Further studies regarding species difference of intrinsic P-gp function between humans and rats will be required to address these issues.

In conclusion, this retrospective study indicated that significant DDI at the BBB in humans may occur in the presence of P-gp inhibitor with [I\textsubscript{unbound}/K\textsubscript{i}] > 1. On the contrary, no significant DDI would be observed in cases where [I\textsubscript{unbound}/K\textsubscript{i}] was less than 0.1. This criterion was comparable to the previous IVIVC results in rats. This investigation will facilitate the understandings of the appropriate cutoff limit to avert the risk of P-gp-mediated DDIs in human BBB.
Authorship Contributions

Participated in research design: Sugimoto, Hirabayashi, Amano, Moriwaki

Conducted experiments: Sugimoto

Performed data analysis: Sugimoto

Wrote or contributed to the writing of the manuscript: Sugimoto, Hirabayashi, Amano, Moriwaki
DMD #49577

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11C-loperamide and its N-desmethyl radiometabolite are avid substrates for brain
Legends for Figures

Fig. 1. Dixon plots for the inhibitory effect of tariquidar (A, 0.003, 0.01, 0.03 μmol/L), cyclosporine A (B, 0.1, 0.3, 1 μmol/L), clarithromycin (C, 20, 60, 200 μmol/L) on [³H]verapamil efflux ratio, tariquidar (D, 0.001, 0.003, 0.01 μmol/L) on N-desmethyl loperamide efflux ratio, and tariquidar (E, 0.001, 0.003, 0.01 μmol/L), quinidine (F, 0.1, 0.3, 1 μmol/L), ketoconazole (G, 0.3, 1, 3 μmol/L), saquinavir (H, 20, 60, 200 μmol/L) on loperamide efflux ratio in human MDR1-expressing cells. The transport study was conducted at 37°C for 2h. Each result represents the mean value (n = 4).
## Tables

### Table 1. Analytical condition in LC/MS/MS analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ionization mode</th>
<th>Detection mode</th>
<th>m/z</th>
<th>Source temp. (°C)</th>
<th>CE (eV)</th>
<th>DP (V)</th>
<th>EP (V)</th>
<th>CXP (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-desmethyl loperamide</td>
<td>Electrospray ionization (Positive ion mode)</td>
<td>Multiple reaction monitoring (MRM)</td>
<td>463.3/252.4</td>
<td>33</td>
<td>6</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Loperamide</td>
<td></td>
<td></td>
<td>477.2/265.8</td>
<td>550</td>
<td>39</td>
<td>81</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Tariquidar</td>
<td></td>
<td></td>
<td>647.4/335.3</td>
<td></td>
<td>49</td>
<td>11</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Alprenolol (I.S.)</td>
<td></td>
<td></td>
<td>250.3/116.3</td>
<td></td>
<td>71</td>
<td>25</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

I.S., internal standard; CE, collision energy; DP, declustering potential; EP, entrance potential; CXP, collision cell exit potential
Table 2.
$K_i$ values for verapamil, N-desmethyl loperamide and loperamide transport via human MDR1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>$K_i$ (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>Tariquidar</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Cyclosporin A</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Clarithromycin</td>
<td>434</td>
</tr>
<tr>
<td>N-desmethyl loperamide</td>
<td>Tariquidar</td>
<td>0.010</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Tariquidar</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Quinidine</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Ketoconazole</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Saquinavir</td>
<td>148</td>
</tr>
</tbody>
</table>

Mean (n=4)
Table 3.

The summary of clinical trials and the estimated \([I_{\text{unbound}}]/K_i\) of P-gp inhibitors in humans

<table>
<thead>
<tr>
<th>Dose and route of administration (substrates)</th>
<th>Inhibitor</th>
<th>Dose and route of administration (inhibitors)</th>
<th>Central nerve system (CNS) endpoint</th>
<th>Inhibitor peak plasma concentration (μmol/L)</th>
<th>Unbound fraction of inhibitor in human plasma</th>
<th>[(I_{\text{unbound}}]/K_i]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>379 ± 9 MBq of (R)-[11C]verapamil i.v. administration</td>
<td>Tariquidar</td>
<td>8 mg/kg i.v. infusion for 30 min</td>
<td>3.3-fold increase of the influx rate constant</td>
<td>2.03</td>
<td>0.0063 ± 0.0021</td>
<td>1.28</td>
<td>(Bauer et al., 2012)</td>
</tr>
<tr>
<td>384 ± 13 MBq of (R)-[11C]verapamil i.v. administration</td>
<td>Tariquidar</td>
<td>2 mg/kg i.v. infusion for 30 min</td>
<td>1.49-fold increase of the influx rate constant</td>
<td>0.76 ± 0.26</td>
<td>0.0063 ± 0.0021</td>
<td>0.48</td>
<td>(Wagner et al., 2009)</td>
</tr>
<tr>
<td>7.4 MBq/kg of [11C]verapamil i.v. administration</td>
<td>Cyclosporin A</td>
<td>2.5 mg/kg/h i.v. infusion for 1 hour</td>
<td>1.88-fold increase of the AUC&lt;sub&gt;brain&lt;/sub&gt;/AUC&lt;sub&gt;blood&lt;/sub&gt; ratio</td>
<td>2.80 ± 0.40</td>
<td>0.070 ± 0.020</td>
<td>0.44</td>
<td>(Sasongko et al., 2005)</td>
</tr>
<tr>
<td>7.4 MBq/kg of [11C]verapamil i.v. administration</td>
<td>Cyclosporin A</td>
<td>2.5 mg/kg/h i.v. infusion for 1 hour</td>
<td>1.8-fold increase of the influx rate constant</td>
<td>2.80 ± 0.40</td>
<td>0.070 ± 0.020</td>
<td>0.44</td>
<td>(Eyal et al., 2010)</td>
</tr>
<tr>
<td>745 ± 16 MBq of (R)-[11C]verapamil i.v. administration</td>
<td>Clarithromycin</td>
<td>400 mg/day oral administration (b.i.d. for 2 days)</td>
<td>None</td>
<td>2.00</td>
<td>0.44 ± 0.04</td>
<td>&lt;0.01</td>
<td>(Arakawa et al., 2010)</td>
</tr>
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</table>
Table 3. (continued)

<table>
<thead>
<tr>
<th>Probe substrate: N-desmethyl loperamide</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Dose and route of administration</strong> (substrates)</td>
<td><strong>Inhibitor</strong></td>
<td><strong>Dose and route of administration</strong> (inhibitors)</td>
<td><strong>Central nerve system (CNS) endpoint</strong></td>
<td><strong>Inhibitor peak plasma concentration (μmol/L)</strong></td>
<td><strong>Unbound fraction of inhibitor in human plasma ([I,unbound]/Ki)</strong></td>
</tr>
<tr>
<td>621-722 MBq of [11C]N-desmethyl loperamide, i.v. administration</td>
<td>Tariquidar</td>
<td>6 mg/kg i.v. administration</td>
<td>3.7-fold increase of the influx rate constant</td>
<td>1.86</td>
<td>0.0063 ± 0.0021</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probe substrate: loperamide</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>343 ± 31 MBq of [11C]loperamide i.v. administration</strong></td>
<td>Quinidine</td>
<td>600 mg Oral administration</td>
<td>1.33-fold increase of the influx rate constant</td>
<td>6.00</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td><strong>16 mg loperamide Single oral administration</strong></td>
<td>Quinidine</td>
<td>600 mg Oral administration</td>
<td>Respiratory depression</td>
<td>6.00</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td><strong>32 mg loperamide Single oral administration</strong></td>
<td>Tariquidar</td>
<td>150 mg/500 ml i.v. infusion for 30 min</td>
<td>None</td>
<td>0.64</td>
<td>0.0063 ± 0.0021</td>
</tr>
<tr>
<td><strong>16 mg loperamide Single oral administration</strong></td>
<td>Ketoconazole</td>
<td>400 mg Oral administration</td>
<td>None</td>
<td>9.17</td>
<td>0.0071 ± 0.0017</td>
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<tr>
<td><strong>16 mg loperamide Single oral administration</strong></td>
<td>Saquinavir</td>
<td>600 mg Oral administration</td>
<td>None</td>
<td>1.41</td>
<td>0.014 ± 0.004</td>
</tr>
</tbody>
</table>
Figure 1

(A) 1/(ER - 1) x conc.

(B) 1/(ER - 1) x conc.

(C) 1/(ER - 1) x conc.

(D) 1/(ER - 1) x conc.

(E) 1/(ER - 1) x conc.

(F) 1/(ER - 1) x conc.

(G) 1/(ER - 1) x conc.

(H) 1/(ER - 1) x conc.

Tariquidar (μmol/L)

Cyclosporin A (μmol/L)

Clarithromycin (μmol/L)

Tariquidar (μmol/L)

Tariquidar (μmol/L)

Quinidin (μmol/L)

Ketoconazole (μmol/L)

Saquinavir (μmol/L)