**In Vitro Predictability of Drug-Drug Interaction Likelihood of P-Glycoprotein-Mediated Efflux of Dabigatran Etexilate Based on [I]2/IC50 Threshold**

Wataru Kishimoto, Naoki Ishiguro, Eva Ludwig-Schuellinger, Thomas Ebner, and Olaf Schaefer

**Pharmacokinetics and Non-Clinical Safety Department, Nippon Boehringer Ingelheim Co., Ltd., Kobe, Japan (W.K., N.I., and O.S.); Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany (E.L. and T.E.)**

Received July 18, 2013; accepted November 8, 2013

**ABSTRACT**

Dabigatran etexilate, an oral, reversible, competitive, and direct thrombin inhibitor, is an in vitro and in vivo substrate of P-glycoprotein (P-gp). Dabigatran etexilate was proposed as an in vivo probe substrate for intestinal P-gp inhibition in a recent guidance on drug-drug interactions (DDI) from the European Medicines Agency (EMA) and the Food and Drug Administration (FDA). We conducted transcellular transport studies across Caco-2 cell monolayers with dabigatran etexilate in the presence of various P-gp inhibitors to examine how well in vitro IC50 data, in combination with mathematical equations provided by regulatory guidelines, predict DDI likelihood. From a set of potential P-gp inhibitors, clarithromycin, cyclosporin A, itraconazole, ketoconazole, quinidine, and ritonavir inhibited P-gp-mediated transport of dabigatran etexilate over a concentration range that may hypothetically occur in the intestine. IC50 values of P-gp inhibitors for dabigatran etexilate transport were comparable to those of digoxin, a well established in vitro and in vivo P-gp substrate. However, IC50 values varied depending whether they were calculated from efflux ratios or permeability coefficients. Prediction of DDI likelihood of P-gp inhibitors using IC50 values, the hypothetical concentration of P-gp inhibitors, and the cut-off value recommended by both the FDA and EMA were in line with the DDI occurrence in clinical studies with dabigatran etexilate. However, it has to be kept in mind that validity of the cut-off criteria proposed by the FDA and EMA depends on in vitro experimental systems and the IC50-calculations methods that are employed, as IC50 values are substantially influenced by these factors.

**Introduction**

With the progress in the field of drug transporter research, transporter-mediated drug-drug interactions (DDI) are being reported with increasing frequency, thus making inevitable the study of potential interactions during the process of drug development. P-glycoprotein (P-gp) is one of the drug transporters expressed in the gastrointestinal tract and is involved in the efflux of various kinds of drugs into the lumen (Ambudkar et al., 2003; Schinkel and Jonker, 2008). Since local drug concentrations in the intestinal lumen may be high after oral administration and complete dissolution of the drug, intestinal P-gp can be effectively inhibited by drugs, which in turn can result in increased exposure of any coadministered drug that is a substrate of P-gp. It is therefore essential with regard to safety and efficacy of drugs to recognize or predict potential DDIs in the intestine. Recently, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) issued guidances on DDI describing methods for investigating DDIs in vitro and providing decision trees to judge about the necessity to conduct clinical DDI studies (FDA, 2012; EMA, 2012). The EMA and FDA recommended using the ratio of the concentration of a putative inhibitor at DDI site (for gastrointestinal tract, [I]2,maximum oral dose taken at one occasion/250 ml of assumed intestinal fluid volume) to the in vitro inhibition potency, such as IC50 and K_i value.

Dabigatran etexilate (Pradaxa) has been approved in several countries for reducing the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. Dabigatran etexilate is a prodrug and is rapidly hydrolyzed after oral administration to active dabigatran via two short-lived intermediate metabolites (Blech et al., 2008). In vitro studies showed that dabigatran etexilate is a substrate of P-gp, whereas the active drug dabigatran is not a substrate of P-gp (Ishiguro et al., 2013a).

The current study was designed to investigate whether intestinal P-gp-mediated dabigatran etexilate transport is affected by P-gp inhibitors and to evaluate the consistency of in vitro and in vivo regulatory guidelines fits the results found in clinic. For this, transcellular transport assays across the Caco-2 cell monolayers were conducted, and the in vitro IC50 values were determined to assess the DDI likelihood of dabigatran etexilate with P-gp inhibitors and to evaluate the consistency of in vitro assessment with the clinical findings.

**ABBREVIATIONS:** AtoB, apical-to-basal; AUC, area under the curve; BIBR 1087, intermediate metabolite of dabigatran etexilate; BtoA, basal-to-apical; CES, carboxylesterase; DDI, drug-drug interaction(s); DMEM, Dulbecco’s modified Eagle’s medium; EMA, European Medicines Agency; FDA, Food and Drug Administration; HPLC, high-performance liquid chromatography; [I]2, maximum oral dose taken at one occasion/250 ml of assumed intestinal fluid volume; Papp, apparent permeability coefficient; P-gp, P-glycoprotein.
Materials and Methods

Chemicals

[14C]Dabigatran etexilate, dabigatran etexilate and BBIR 1087, ester cleavage of dabigatran etexilate (Blech et al., 2008) and linagliptin were synthesized at Boehringer Ingelheim Pharma GmbH & Co. KG (Biberach, Germany). [3H] Digoxin was obtained from PerkinElmer (Waltham, MA). Clarithromycin was purchased from Wako Pure Chemical Industries (Osaka, Japan). Nelfinavir and ritonavir were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Dulbecco’s modified Eagle’s medium (DMEM) with 3.7 g/l sodium bicarbonate was from Bio Chrom AG (Berlin, Germany). Fetal bovine serum, nonessential amino acids, penicillin-streptomycin, and t-glutamine were from Invitrogen (Carlsbad, CA). Collagen R solution was from Serva (Heidelberg, Germany). All other chemicals were of the highest reagent grade available from commercial sources.

Biologic Materials

Caco-2 cells were obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Pooled human liver microsomes were prepared in-house from human liver tissue. Liver tissue of 10 male and female donors was supplied by Tissue Transformation Biologic Materials (Braunschweig, Germany). Pooled rat liver microsomes were prepared in-house from rat liver tissue of 10 male and female donors and liver microsomes were prepared in-house from human liver tissue. Liver microsomes were of the highest reagent grade available from commercial sources.

Transcellular Transport Assay

Caco-2 cells were seeded at a density of 0.625 × 10^5 cells/filter on the collagen-coated Transwell filter inserts. The inserts were placed on open reservoir plates. The inserts were filled with donor solution containing the radiolabeled compound with or without P-gp inhibitor for 30 minutes, the transport solution supplemented with or without P-gp inhibitor. After preincubation with the chemical inhibitors for 5 minutes. Incubations were initiated by addition of substrate, and reactions were terminated by adding 200 μl of 0.2 M hydrochloric acid and vortex mixing. After centrifugation at 4°C, incubates were directly injected into the high-performance liquid chromatography (HPLC) system. The employed HPLC method showed linearity (r^2 ≥ 0.99) over the range of 500–290,000 dpm (absolute amount injected on column) as assessed by quadruplicate injections of [14C]dabigatran etexilate at various concentrations. Inaccuracy and imprecision of the method were found as ±15% and ±10%, respectively.

HPLC with Radioactivity Detection

In vitro incubation samples were analyzed by a validated reversed phase HPLC with on-line radiodetection for quantification of [14C]dabigatran etexilate and [14C]BBIR 1087. An HPLC system was composed of an autosampler HTC PAL (Chromtech GmbH, Idstein, Germany) and HPLC pump PU-980, ternary gradient unit LC-980-02. 3-line-degaser DG-980-50, and UV/VIS detector UV-975 (Jasco, Gross-Umstadt, Germany). Chromatography was performed for total run time of 33 minutes on LiChroCART Purospher RP 18 e 5-μm 125-2 analytical column with LiChroCART Purospher RP 18 e 5-μm 10-2 guard column (Merck, Darmstadt, Germany). Mobile phases were 0.05 M formic acid adjusted to pH 4.0 with ammonia solution (A) and acetonitrile (B) using a programmed gradient [0 minutes: 10% (B), 21 minutes: 60% (B), 23 minutes: 80% (B), 24 minutes: 80% (B), 25 minutes: 10% (B)) at a flow rate of 0.4 ml/minute. Analytes were on-line quantified on a flow scintillation analyzer TR 525 with addition of Ultima-Flo M (3:1) (PerkinElmer).

Data Evaluation

Apparent Permeability Coefficient. The apparent permeability coefficient (Papp) is described by the following equation:

\[ P_{app} = \frac{1}{A \times C_0} \times \frac{V_R \times \Delta C_R}{\Delta t} \]

where \( P_{app} \) is the apparent permeability, \( C_0 \) is the concentration of the monolayer at time 0, \( V_R \) is the volume in the receiver compartment, \( \Delta C_R \) is the change in substance concentration over time in the receiver compartment, \( \Delta t \) is the time difference between the two concentrations.

Efflux Ratio. The efflux ratio is defined by the following equation:

\[ \text{Efflux ratio} = \frac{P_{app BtoA}}{P_{app AtoB}} \]

where \( P_{app BtoA} \) and \( P_{app AtoB} \) represent the apparent permeability of the test compound from basal to apical and from apical to basal, respectively.

Half-Maximal Inhibitor Concentration. The apparent IC50 value was calculated by means of nonlinear least squares regression using the XLfit (IDBS, London, UK) according Eqs. (3a) for \( P_{app AtoB} \) and (3b) for \( P_{app BtoA} \) and efflux ratio.

\[ T = T_0 \times \frac{(T_{max} - T_0) \times \frac{1}{h}}{T_{max}} \]

(3a)

\[ T = T_0 \times \frac{(T_{max} - T_0) \times \frac{1}{h}}{T_{max}} \]

(3b)

where \( T \) is the observed Papp or efflux ratio, \( T_{max} \) is the Papp or efflux ratio at \( I = \infty \), \( T_0 \) is the Papp or efflux ratio at \( I = 0 \), \( I \) is the concentration of inhibitor applied, and \( IC_{50} \) is the inhibitor concentration for 50% inhibition and \( h \) is the slope factor.
Results

In Vitro IC₅₀ Determination on Digoxin Transport. The in vitro inhibitory effect of various P-gp inhibitors on P-gp-mediated [³H]digoxin (1 μM) transport was determined across the Caco-2 cell monolayers (Fig. 1). Concentration ranges employed and IC₅₀ values obtained of six P-gp inhibitors and linagliptin, which was used as a negative control as it does not inhibit P-gp, are summarized in Table 1. Cyclosporin A was found to be the most potent inhibitor with IC₅₀ values of less than 1 μM, followed by itraconazole and ketoconazole. These in vitro IC₅₀ values determined from three different parameters, efflux ratio, PappAtoB values and PappBtoA values, were comparable to those published before (references in Table 1), although

Fig. 1. Inhibitory effect of P-gp inhibitors on the AtoB and BtoA Papp values and efflux ratios of digoxin. [³H]Digoxin (1 μM) was incubated with Caco-2 cells in the absence or presence of clarithromycin (A), cyclosporin A (B), itraconazole (C), ketoconazole (D), linagliptin (E), quinidine (F), and ritonavir (G). Mean ± S.D. Papp values and efflux ratios from n = 3 filters were presented. Open and closed points indicate the observed PappBtoA and PappAtoB values, respectively (upper), and closed points indicate the observed efflux ratio (lower). Solid line indicates the fitting curve.
and nelfinavir were found less potent, with IC50 values between 1

AUC) using digoxin as probe drug. Since six of these drugs were

Concentration ranges employed and IC50 values were summarized in

[14C]dabigatran etexilate and liquid scintillation counting detection in

calculated from PappBtoA, because the IC50 assay was conducted using

Ritonavir (Ishiguro et al., 2013a). However, this study did not use a CES inhibitor; instead, the impact of various drugs on CES-mediated hydrolysis of
dabigatran etexilate to the intermediate metabolite BIBR 1087 was

stable only in the BtoA transport assay, whereas profound hydrolysis

was observed in the AtoB transport assay in the presence and absence of

CES inhibitor (Ishiguro et al., 2013a).

In Fig. 2, the PappBtoA Values of dabigatran etexilate in the presence of

10 drugs were plotted together with the IC50 fitting curves. Concentration ranges employed and IC50 values were summarized in Table 2. Cyclosporin A, itraconazole, and tacrolimus showed the strongest inhibition with IC50 values less than 1 μM. Ketoconazole and nelfinavir were found less potent, with IC50 values between 1 μM and 10 μM. IC50 values of clarithromycin, quinidine, and ritonavir were greater than 10 μM. Digoxin showed weak inhibition with IC50 values higher than 100 μM. Less than 50% inhibition was observed for amiodarone up to its solubility limit.

Assessment of DDI Likelihood. The FDA and EMA released the guidelines to evaluate the risk of DDI via drug transporters between known inhibitors and potentially coadministered drugs with new medical entities (EMA, 2012; FDA, 2012), and they recommended using the ratio of the concentration of a putative inhibitor at site of the DDI (e.g., [I]2, maximum oral dose taken at one occasion/250 ml of assumed intestinal fluid volume) to the in vitro inhibition potency, such as IC50 and Ki value. In Table 1, the [I]2/IC50 ratios of seven drugs tested in this study are shown together with the magnitude of area-under-the-curve (AUC) increase in clinical interaction studies (AUC/AUC) using digoxin as probe drug. Since six of these drugs were known clinical P-gp inhibitors and the [I]2/IC50 ratios determined from parameters, either PappAtoB, PappBtoA, or efflux ratio, exceeded the cutoff value of 10 that was set as threshold in the guidelines. These assessments using in-house in vitro IC50 data were consistent with findings of clinical interaction studies using digoxin as a P-gp probe drug in a way that AUC/AUC for these six drugs was >1.25. On the other hand, the [I]2/IC50 ratio of linagliptin was less than 10 and this assessment was in agreement with the absence of P-gp-mediated clinical DDI between digoxin and linagliptin (Friedrich et al., 2011).

The DDI likelihood for putative P-gp inhibitors on the pharmacokinetics of dabigatran etexilate was assessed in the same way (Table 2). The [I]2/IC50 ratios of almost all drugs assessed were higher than 10. Exact [I]2/IC50 ratio of amiodarone could not be determined due to its low solubility and/or low in vitro inhibitory potential. Among the drugs assessed, only digoxin had the [I]2/IC50 ratio less than 10, which was in line with the 1.1-fold AUC increase observed clinically after coadministration of dabigatran etexilate with digoxin (Stangier et al., 2011). Although the [I]2/IC50 ratio of clarithromycin was higher than 10, AUC/AUC was <1.25 (Pradaxa package insert, 2010). Ketoconazole and quinidine had high [I]2/IC50 ratios (higher than 10) and these predictions were confirmed in clinic (Pradaxa package insert, 2010).

Discussion

The evaluation of potential DDIs of new medical entities with marketed, coadministered drugs is of importance during drug development. The DDI-likelihood assessment based on the ratio of expected local concentrations of the inhibitor at the DDI site and its in vitro inhibitory potency, such as Ki or IC50, therefore, in vitro IC50 value is one of the important parameters to assess the DDI likelihood. Nevertheless, the IC50 values on P-gp activity have been determined by many different equations, data parameters, and assay systems, and thereby it is well known that interlaboratory variability of IC50 values can be substantial. To clarify the causes of large interlaboratory variability, in vitro IC50 values in this study were evaluated by several aspects.

First, the impact of the use of different parameters, e.g., PappAtoB, PappBtoA, and efflux ratio from same data set, on IC50 values was assessed. As shown in Table 1, the IC50 values determined from PappAtoB or PappBtoA were approximately three-times larger on average than those determined from efflux ratios. This difference may be derived from the complexities of transcellular transport assay. The transport across Caco-2 cell monolayers is determined by a total of five different permeability coefficients, viz., the influx and efflux.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. Range Used</th>
<th>IC50 Value</th>
<th>[I]2/IC50 Ratio</th>
<th>AUC/AUC Ratio of Digoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μM</td>
<td>μM</td>
<td>μM</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>1–100</td>
<td>7.0</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>0.1–12</td>
<td>0.29</td>
<td>0.72</td>
<td>0.54</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.1–10</td>
<td>0.46</td>
<td>1.1</td>
<td>0.83</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.3–30</td>
<td>0.42</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Linagliptin</td>
<td>10–300</td>
<td>9.4</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>Quinidine</td>
<td>0.03–10</td>
<td>0.60</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>1–100</td>
<td>1.5</td>
<td>3.6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ebert et al., 2007; Cook et al. 2010 (efflux ratio and net efflux ratio); <sup>b</sup>Choo et al., 2000 (net efflux ratio); Cook et al. 2010 (efflux ratio and net efflux ratio); <sup>c</sup>Faadell et al., 2008 (PappBtoA); Cook et al. 2010 (efflux ratio and net efflux ratio); <sup>d</sup>Linagliptin, 2010; <sup>e</sup>Ishiguro et al., 2013b (PappBtoA); Choo et al., 2000 (net efflux ratio); Fenner et al., 2009 (net efflux ratio); <sup>f</sup>Gurley et al., 2006; 2008; <sup>g</sup> Jalava et al., 1997; <sup>h</sup>Friedrich et al., 2011; <sup>i</sup>Pedersen et al., 1983; Ramens, 1985; <sup>j</sup>Peazer et al., 2004; Kirby et al., 2012.
transport across the apical and basal membranes in addition to P-gp-mediated efflux. As shown in Fig. 3, an efflux ratio is described as a ratio of a Papp for the P-gp-mediated efflux transport to a Papp for efflux transport across the apical membrane when an assumption that PS₁ × PS₃ equals PS₂ × PS₄ is applied (Mizuno et al., 2003). At 50% of P-gp inhibition, when PS₃/P₄ is half of control condition, the ratio of efflux ratio minus one at 50% inhibited condition to that at control condition is exactly half, indicating that the IC₅₀ values determined from an efflux ratio represents 50% inhibition of P-gp-mediated efflux accurately. On the other hand, the ratio of CLBtoA, which is determined by subtracting intrinsic passive permeability from the PappBtoA at 50% P-gp inhibition, is higher than half when looking at an equation (Fig. 3), suggesting that the IC₅₀ values determined from PappBtoA are theoretically higher than intrinsic IC₅₀ values. The efflux ratio-based curve declines faster than Papp AtoB-based curve since additional decline in the efflux ratio-based curve occurs as the PappAtoB increases in the denominator. Since the IC₅₀ values were different among parameters employed for IC₅₀ calculation, a threshold value according to the data parameter employed is necessary for accurate assessment of DDI likelihood, although the draft FDA guidance recommends the use of efflux ratios.

Second, the impact of the use of different probe substrate on IC₅₀ values was assessed. As well as digoxin, dabigatran etexilate is one of the in vivo P-gp probe substrates recommended in the DDI guidances of the EMA and FDA for evaluating P-gp-mediated DDI (EMA, 2012; FDA, 2012). Although the impact of the use of different probe substrates should be evaluated by comparing mathematically accurate IC₅₀ values determined from efflux ratios, only the IC₅₀ values determined from PappBtoA were compared between digoxin and dabigatran etexilate due to hydrolysis of dabigatran etexilate when used for Caco-2 transport assays in the AtoB direction. Since the IC₅₀ values determined from the same data parameter (PappBtoA) were compared, there is no need to take the different data parameter-derived differences in the IC₅₀ values into consideration (Fig. 4A). The IC₅₀ values of five drugs, clarithromycin, cyclosporin A, itraconazole, ketoconazole, and ritonavir were similar and within threefold correlation range. IC₅₀ value of quinidine was approximately 18-times higher when dabigatran etexilate was used as a P-gp probe substrate than when digoxin was used. Shift of IC₅₀ values of quinidine by changing in vitro P-gp probe substrate from digoxin to other probe substrates was previously reported (Ayesh et al., 1996). Therefore, P-gp seems to have different binding sites for digoxin and dabigatran etexilate, which may explain the more than 10-fold differences in IC₅₀ values, although an exact mechanism needs to be elucidated.

Rivaroxaban, a factor Xa inhibitor used for anticoagulant therapy, is a P-gp substrate and its in vivo pharmacokinetics is affected by P-gp inhibitors (Gnoth et al., 2011; Mueck et al., 2013). IC₅₀ values of P-gp inhibitors on rivaroxaban transcellular transport were determined from MDR1-overexpressing LLC-PK1 cells (Gnoth et al., 2011). To assess the impact of different cell systems and substrates on IC₅₀ under conditions where no parameter effect is anticipated, the IC₅₀ values of those P-gp inhibitors calculated from efflux ratios of rivaroxaban transport were plotted over those of digoxin transport based on efflux ratio in this study (Fig. 4B). The IC₅₀ values on rivaroxaban transport obtained from MDR1-overexpressing LLC-PK1 cells were higher than those on digoxin transport obtained from Caco-2 cells, except for itraconazole, which deviated from the upper border of 3-fold correlation. Kₘ values vary among cell types and correlate with P-gp protein expression levels (Shirasaka Y et al., 2008). Therefore, IC₅₀ values may also vary with assay systems and P-gp expression. Furthermore, it was reported that different IC₅₀ values were given from different laboratories for the same P-gp inhibitor (Bentz et al., 2013). Hence, the cell systems

Fig. 2. Inhibitory effect of P-gp inhibitors on the PappBtoA Values of dabigatran etexilate. [¹⁴C]Dabigatran etexilate (1 μM) was incubated with Caco-2 cells in the absence or presence of amiodarone (A), clarithromycin (B), cyclosporin A (C), digoxin (D), itraconazole (E), ketoconazole (F), nelfinavir (G), quinidine (H), ritonavir (I), and tacrolimus (J). Mean ± S.D. Papp values from n = 3 filters were presented. Closed points and solid line indicate the observed PappBtoA and the fitting curve, respectively.
for the determination of in vitro \( IC_{50} \) values should be taken into consideration also when the DDI likelihood is assessed. Since the \( IC_{50} \) values of itraconazole determined from the assay using dabigatran etexilate as substrate and from the assay using digoxin as substrate are in a similar range, it is reasonable to argue that itraconazole may affect rivaroxaban transcellular transport across cell monolayer of P-gp-expressing LLC-PK1 cells differently, leading to far lower \( IC_{50} \) values compared with those obtained using digoxin as substrate. As the reason behind the low \( IC_{50} \) value of itraconazole when rivaroxaban is used as substrate is unclear, further studies to elucidate the mechanism would be needed to address this phenomenon.

Our assessments of the DDI likelihood of dabigatran etexilate based on the proposed equations and the cut-off value of 10 by the FDA and EMA and in vitro \( IC_{50} \) values obtained (Tables 1 and 2) yielded three true-positive, one true-negative, and one false-positive predictions. The latter relates to the prediction for clarithromycin that was formally not correct because the 1.2-fold AUC change of dabigatran by clarithromycin observed in clinics was within bioequivalence criteria [Pradaxa package insert, 2010]. In this case, however, solubility limitations of clarithromycin may have influenced the outcome of the clinical interaction study. In general, the prediction of DDI likelihood of digoxin with several P-gp inhibitors correlates well to AUC changes of digoxin (four true-positive, one true-negative predictions). The cut-off value recommended by the FDA and EMA seems to fit to our in vitro Caco-2 cells system when dabigatran etexilate and digoxin are used as in vitro P-gp probe substrates of P-gp. However, it has to be kept in mind that clinical data showing no DDI via intestinal P-gp are very limited, and therefore more clinical DDI studies to confirm validity of the cut-off value are highly desirable for a more accurate DDI assessment.

In conclusion, clear in vitro/in vivo correlation between in vitro \( IC_{50} \) values using dabigatran etexilate as in vitro P-gp substrate and clinical AUC change of dabigatran suggests that dabigatran etexilate is one of the suitable in vitro and in vivo P-gp probe substrates. In addition, it was found that many factors, such as the differences in data parameters employed, in vitro probe substrate used, and cell system used produce substantial differences for determining \( IC_{50} \) values, although this conclusion was lead out from six P-gp inhibitors on the transport of digoxin and dabigatran etexilate as in vitro P-gp probe substrate. For accurate assessment of DDI likelihood, it is suggested from this study that the threshold should be set depending on the in vitro experimental results.

### Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. Range Used</th>
<th>( IC_{50} ) Value</th>
<th>( [I]^2/IC_{50} ) for Dabigatran Eteixlate</th>
<th>AUC/AUC of Dabigatran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>1–10</td>
<td>&gt;10</td>
<td>&lt;470</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.3–30</td>
<td>28</td>
<td>36</td>
<td>1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>0.1–12</td>
<td>0.69</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>1–100</td>
<td>125</td>
<td>0.0080</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.3–10</td>
<td>0.41</td>
<td>1100</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.1–6</td>
<td>3.4</td>
<td>880</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>0.3–10</td>
<td>3.6</td>
<td>2100</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>1–60</td>
<td>33</td>
<td>150</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>0.3–30</td>
<td>13</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>0.1–6</td>
<td>0.66</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Pradaxa package insert, 2010; <sup>b</sup> Stangier et al., 2011.


Fig. 4. Ratios of efflux ratio- and Papp\textsubscript{BtoA}-based data parameters at 50% inhibition of P-gp to those at control condition, \(P_{50}\) and \(P_{90}\), represent the permeability–surface area products for the influx and non-Pgp-mediated efflux across the apical membrane of the cell monolayers, respectively. \(P_{50}\) and \(P_{90}\) represent the permeability–surface area products for the efflux and influx across the basolateral membrane of the cell monolayers, respectively. \(P_{50}\text{Pgp}\) represents the permeability–surface area products for P-gp-mediated efflux across the apical membrane. \(A\) represents the area of the filter and \(P_{50}\text{Pgp}\) represents the intrinsic passive permeability coefficient.

Acknowledgments

The authors thank Asami Saito, Naoko Ohtsu, Ikumi Washio, Etsuko Fujimoto, and Masahito Takatani for excellent technical assistance in conducting the in vitro experiments. [1|C]Dabigatran etexilate was kindly provided by Ralf Kiesel, head of the isotope chemistry laboratory, Boehringer Ingelheim, Germany.

Authorship Contributions

Participated in research design: Kishimoto, Ishiguro, Ludwig-Schwellinger, Ebner.
Conducted experiments: Kishimoto, Ludwig-Schwellinger.
Performed data analysis: Kishimoto, Ludwig-Schwellinger.
Wrote or contributed to the writing of the manuscript: Kishimoto, Ishiguro, Ebner, Schaefer.