In Vitro Predictability of Drug-Drug Interaction Likelihood of P-Glycoprotein-Mediated Efflux of Dabigatran Etexilate Based on \([I]_2/IC_{50}\) Threshold

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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 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 IC_{50} data, in combination with mathematical equations provided by regulatory guidance, predict DDI likelihood. From a set of potential P-gp inhibitors, clarithromycin, cytochrome P450 3A4, and ritonavir inhibited P-gp-mediated transport of dabigatran etexilate over a concentration range that may hypothetically occur in the intestine. IC_{50} 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, IC_{50} values varied depending whether they were calculated from efflux ratios or permeability coefficients. Prediction of DDI likelihood of P-gp inhibitors using IC_{50} 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 IC_{50}-calculation methods that are employed, as IC_{50} 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, 2003). 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 return can result in increased exposure of any concomitantly administered 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 guidelines 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 IC_{50} 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 (Bleich 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 whether the DDI likelihood assessment for intestinal P-gp inhibition by in vitro data following 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 IC_{50} 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.

This study was supported by Boehringer Ingelheim.

The authors were fully responsible for all content and editorial decisions. They were involved at all stages of manuscript development and have approved the final version.

dx.doi.org/10.1124/dmd.113.053769.

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

\[^{14}C\]Dibagranet etxilate, dabigranet etxilate and BB1B 1087, ester cleavage of dibagranet etxilate (Bleck et al., 2008) and linaglipitin were synthesized at Böhringer Ingelheim Pharma GmbH & Co. KG (Biberach, Germany). [\(^{3}H\)] digoxin was obtained from PerkinElmer (Waltham, MA). Amiodarone, cyclosporin A, digoxin, iraconazole, ketocanozone, quinidine, and tacrolimus were purchased from Sigma-Aldrich (St. Louis, MO). 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 BioChem AG (Berlin, Germany). Fetal bovine serum, nonessential amino acids, penicillin-streptomycin, and l-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, Inc. (New York, NY). Human liver microsomes were prepared in house from human liver tissue. Liver tissue of 10 male and female donors was supplied by Tissue Transformation, Inc. (New York, NY). 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, Inc. (New York, NY).

Transcellular Transport Assay

The efflux ratio of a test compound, I, is defined by the following equation:

\[ \text{Efflux ratio} = \frac{\text{Papp}_{0}}{\text{Papp}_{0}} \]

where Papp is the apparent permeability coefficient, Papp\(_{0}\) is the observed Papp for the test compound, and Papp\(_{0}\) is the observed Papp for an untreated control.

Effect of Transport Inhibitors

The effect of transport inhibitors on the efflux ratio of a test compound, I, can be calculated using the following equation:

\[ \text{Efflux ratio} = \frac{\text{Papp}_{0}}{\text{Papp}_{0}} \]

where Papp is the apparent permeability coefficient, Papp\(_{0}\) is the observed Papp for the test compound, and Papp\(_{0}\) is the observed Papp for an untreated control.

HPLC with Radioactivity Detection

In vitro incubation samples were analyzed by a validated reversed phase HPLC with on-line radiodetection for quantification of \[^{14}C\]dibagranet etxilate and \[^{14}C\]BB1B 1087. An HPLC system was composed of an autosampler HTCL P4 (Chromtech GmbH, Idstein, Germany) and HPLC pump PU-980, ternary gradient unit LC-9800-02, 3-line-degasser 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 18e 5-\(\mu\)m 125-2 analytical column with LiChroCart Purospher RP 18e 5-\(\mu\)m 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: 100% (B), 21 minutes: 80% (B), 23 minutes: 60% (B), 24 minutes: 10% (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:

\[ \text{Papp} = \frac{1}{V_r \times \Delta C_r / \Delta t} \]

where Papp is the apparent permeability, Vr is the volume of the receiver compartment, \(\Delta C_r / \Delta t\) is the initial slope factor.

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

\[ \text{Efflux ratio} = \frac{\text{Papp}_{0}}{\text{Papp}_{0}} \]

where Papp\(_{0}\) and Papp\(_{0}\) represent the apparent permeability of the test compound from basal to apical and from apical to basal, respectively.

Half-Maximal Inhibitor Concentration. The apparent IC\(_{50}\) value was calculated by means of nonlinear least squares regression using the XLfit (IDBS, London, UK) according Eqs. (3a) for Papp\(_{0}\) and (3b) for Papp\(_{0}\) and efflux ratio.

\[ T_0 = \frac{T_{max} - T_0}{T_{max} - T_0} \]

where T is the observed Papp or efflux ratio, T\(_{max}\) is the Papp or efflux ratio at I = infinity, 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.](image-url)
and nelfinavir were found less potent, with IC\textsubscript{50} values between 1

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

under-the-curve (AUC) increase in clinical interaction studies (AUC\textsubscript{i}/

of 10 drugs were plotted together with the IC\textsubscript{50} fitting curves. The IC\textsubscript{50} values were

calculated from Papp\textsubscript{BtoA}, because the IC\textsubscript{50} assay was conducted using

\textsuperscript{\textsuperscript{14}C}dabigatran etexilate and liquid scintillation counting detection in

clarithromycin, digoxin, itraconazole, ketoconazole, quinidine, and

1087 from dabigatran etexilate was not inhibited by amiodarone,

determined using human liver microsomes. The formation of BIBR

dabigatran etexilate to the intermediate metabolite BIBR 1087 was

instead, the impact of various drugs on CES-mediated hydrolysis of

(Ishiguro et al., 2013a). However, this study did not use a CES inhibitor;

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

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

In Fig. 2, the Papp\textsubscript{BtoA} Values of dabigatran etexilate in the presence of

10 drugs were plotted together with the IC\textsubscript{50} fitting curves. Concentration ranges employed and IC\textsubscript{50} values were summarized in Table 2. Cyclosporin A, itraconazole, and tacrolimus showed the strongest inhibition with IC\textsubscript{50} values less than 1 \(\mu\text{M}\). Ketoconazole and nelfinavir were found less potent, with IC\textsubscript{50} values between 1 \(\mu\text{M}\) and 10 \(\mu\text{M}\). IC\textsubscript{50} values of clarithromycin, quinidine, and ritonavir were greater than 10 \(\mu\text{M}\). Digoxin showed weak inhibition with IC\textsubscript{50} values higher than 100 \(\mu\text{M}\). 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., \(\text{[I]}_2\), maximum oral dose taken at one occasion/250 ml of

assumed intestinal fluid volume) to the in vitro inhibition potency, such as IC\textsubscript{50} and \(K_i\) value. In Table 1, the \(\text{[I]}_2/\text{IC}_{50}\) 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 \(\text{[I]}_2/\text{IC}_{50}\) ratios determined from parameters, either Papp\textsubscript{AtoB}, Papp\textsubscript{BtoA} or efflux ratio, exceeded the cut-off value of 10 that was set as threshold in the guidances. These assessments using in-house in vitro IC\textsubscript{50} 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 \(\text{[I]}_2/\text{IC}_{50}\) 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 \(\text{[I]}_2/\text{IC}_{50}\) ratios of almost all drugs assessed were higher than 10. Exact \(\text{[I]}_2/\text{IC}_{50}\) 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 \(\text{[I]}_2/\text{IC}_{50}\) 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 \(\text{[I]}_2/\text{IC}_{50}\) ratio of clarithromycin was higher than 10, AUC/AUC was <1.25 (Pradaxa package insert, 2010). Ketoconazole and quinidine had high \(\text{[I]}_2/\text{IC}_{50}\) 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 \(K_i\) or IC\textsubscript{50}. Therefore, in vitro IC\textsubscript{50} value is one of the important parameters to assess the DDI likelihood. Nevertheless, the IC\textsubscript{50} 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 IC\textsubscript{50} values can be substantial. To clarify the causes of large interlaboratory variability, in vitro IC\textsubscript{50} values in this study were evaluated by several aspects.

First, the impact of the use of different parameters, e.g., Papp\textsubscript{AtoB}, Papp\textsubscript{BtoA} and efflux ratio from same data set, on IC\textsubscript{50} values was assessed. As shown in Table 1, the IC\textsubscript{50} values determined from Papp\textsubscript{AtoB} or Papp\textsubscript{BtoA} 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>IC\textsubscript{50} Value</th>
<th>([\text{I]}<em>2/\text{IC}</em>{50}) Ratio</th>
<th>AUC/AUC Ratio of Digoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu\text{M})</td>
<td>(\mu\text{M})</td>
<td>(\mu\text{M})</td>
<td>(\mu\text{M})</td>
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<td>Clarithromycin</td>
<td>1–100</td>
<td>7.0</td>
<td>13</td>
<td>17</td>
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<td>Cyclosporin A</td>
<td>0.1–12</td>
<td>0.29</td>
<td>0.72</td>
<td>0.54</td>
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<td>Itraconazole</td>
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<td>0.46</td>
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<td>0.42</td>
<td>1.2</td>
<td>1.2</td>
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<tr>
<td>Linagliptin</td>
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<td>Quinidine</td>
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<td>0.60</td>
<td>3.0</td>
<td>1.9</td>
</tr>
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<td>Ritonavir</td>
<td>1–100</td>
<td>1.5</td>
<td>3.6</td>
<td>4.5</td>
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\(\text{[H]}\textsuperscript{3}\text{H}\)Digoxin was incubated at 1 \(\mu\text{M}\). Drugs added as putative P-gp inhibitor were present in both donor and receiver compartments. The IC\textsubscript{50} values from publication were determined from efflux ratio-based parameters except for Papp\textsubscript{BtoA}-based parameters (indicated by asterisk).
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 PS$_1$ equals PS$_3$ is applied (Mizuno et al., 2003). At 50% P-gp inhibition, when PS$_{P-gp}$ 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 IC$_{50}$ 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 Papp$_{BtoA}$ at 50% P-gp inhibition, is higher than half when looking at an equation (Fig. 3), suggesting that the IC$_{50}$ values determined from Papp$_{BtoA}$ are theoretically higher than intrinsic IC$_{50}$ 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 Papp$_{AtoB}$ increases in the denominator. Since the IC$_{50}$ values were different among parameters employed for IC$_{50}$ 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 IC$_{50}$ 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 IC$_{50}$ values determined from efflux ratios, only the IC$_{50}$ values determined from Papp$_{BtoA}$ 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 IC$_{50}$ values determined from the same data parameter (Papp$_{BtoA}$) were compared, there is no need to take the different data parameter-derived differences in the IC$_{50}$ values into consideration (Fig. 4A). The IC$_{50}$ values of five drugs, clarithromycin, cyclosporin A, itraconazole, ketoconazole, and ritonavir were similar and within threefold correlation range. IC$_{50}$ 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 IC$_{50}$ 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 IC$_{50}$ 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). IC$_{50}$ 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 IC$_{50}$ under conditions where no parameter effect is anticipated, the IC$_{50}$ 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 IC$_{50}$ 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, IC$_{50}$ values may also vary with assay systems and P-gp expression. Furthermore, it was reported that different IC$_{50}$ 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 Papp$_{BtoA}$ values of dabigatran etexilate. $[^{14}$C]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. Pap p values from $n$ = 3 filters were presented. Closed points and solid line indicate the observed Papp$_{BtoA}$ and the fitting curve, respectively.](image-url)
for the determination of in vitro IC50 values should be taken into consideration also when the DDI likelihood is assessed. Since the IC50 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 IC50 values compared with those obtained using digoxin as substrate. As the reason behind the low IC50 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 IC50 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 corrected because the 1.2-fold AUC change of dabigatran by clarithromycin is 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/in vivo 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 IC50 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 IC50 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

<table>
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<tr>
<th>Drug</th>
<th>Conc. Range Used</th>
<th>IC50 Value</th>
<th>[I]2/IC50 for Dabigatan Etexilate</th>
<th>AUC/AUC of Dabigatan</th>
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<td>Tacrolimus</td>
<td>0.1–6</td>
<td>0.66</td>
<td>74</td>
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</tbody>
</table>

a Pradaxa package insert, 2010; b Stangier et al., 2011.
tools and method for data analysis, as well as consistency of in vitro assessment to the clinical findings regarding DDI occurrence.

Acknowledgments
The authors thank Asami Saito, Naoko Ohtsu, Ikumi Washio, Etsuka Fujimoto, and Masahito Takatani for excellent technical assistance in conducting the in vitro experiments. [14C]Dabigatran etexilate was kindly provided by Ralf Kiesling, and Masahito Takatani for excellent technical assistance in conducting the in vitro assessment to the clinical findings regarding DDI occurrence.

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.

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