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Dabigatran Etexilate and Digoxin: Comparison as Clinical Probe Substrates for Evaluation of P-gp Inhibition

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Dabigatran Etexilate and Digoxin: Comparison as Clinical Probe Substrates for Evaluation of P-gp Inhibition

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P-glycoprotein (P-gp) inhibition is part of routine drug–drug interaction (DDI) investigation in drug development. Selection of P-gp clinical probes depends on selectivity, sensitivity, and comedication relevance. Traditionally, this DDI was assessed clinically using digoxin, primarily because of safety concerns. Digoxin is neither a specific nor sensitive P-gp probe. Dabigatran etexilate (DE) has been proposed as an alternative to study intestinal P-gp inhibition. Comparison of digoxin and DE reveals key aspects of their suitability and limitations as P-gp probes.

P-glycoprotein (P-gp) is a drug transporter recommended by regulatory agencies for clinical evaluation because its inhibition can cause clinically important DDIs. Although P-gp is localized at the apical membrane of multiple tissues (e.g., intestine, kidney, liver, and the blood-brain barrier), inhibition of intestinal P-gp appears to have the most significant impact on DDIs.

Ideally, a clinical probe to study intestinal P-gp inhibition should exhibit high selectivity and sensitivity. Specifically, such a probe should (i) be mainly transported by P-gp, (ii) exhibit primarily P-gp–limited intestinal absorption with low-to-moderate fraction absorbed, (iii) be minimally metabolized, and (iv) have a sufficient clinical safety margin for exposure changes with P-gp inhibitors. Practically, clinical probes need to be commercially available with appropriate analytical assays for quantification.

Digoxin, a narrow therapeutic index drug, is a well-known P-gp substrate. Digoxin DDI studies have been routinely conducted in the development of drugs that are P-gp inhibitors. However, digoxin is not selective for P-gp in vitro and in vivo, and it exhibits low pharmacokinetic (PK) sensitivity to P-gp inhibition with a reasonably high oral bioavailability (60–80%). As such, it is not an ideal probe substrate for studying intestinal P-gp. A digoxin DDI study is clinically relevant for the safe use of digoxin; however, it does not capture the true “worst-case” victim DDI potential caused by intestinal P-gp inhibition that may be used to extrapolate potential DDI effect on other P-gp substrate drugs. Recently, dabigatran etexilate (DE), a prodrug of dabigatran, has been recommended by regulatory agencies as a clinical probe for studying intestinal P-gp inhibition.

The objective of this commentary is to compare the key features, suitability, and limitations of DE and digoxin as clinical probes to study intestinal P-gp inhibition. The information may help determine which probe should be used to study DDIs if a new drug under development is a P-gp inhibitor.

SELECTIVITY OF DE AND DIGOXIN AS P-GP PROBES: COMPARISON OF THEIR ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION AND TRANSPORTER PROFILES

DE is rapidly absorbed and converted to parent dabigatran by carboxylesterase (CES) in the intestine and CES1 in the liver (Figure 1a, New Drug Application (NDA) 022512, Drugs@FDA). Oral bioavailability of dabigatran after DE administration is 3–7%, likely because of low intestinal absorption of DE limited by P-gp efflux. Dabigatran is 80–85% eliminated unchanged in urine primarily via passive glomerular filtration and is not metabolized by cytochrome P450 enzymes; however, dabigatran undergoes glucuronidation in the liver to form pharmacologically active acyl glucuronides (~10% of total dabigatran after absorption).
intravenous administration of DE, which are eliminated in the urine. Dabigatran is not transported by P-gp and, therefore, DE is solely a probe for intestinal P-gp.

In contrast, digoxin exhibits much higher bioavailability (60–80% as Lanoxin tablets), substantially limiting its DDI sensitivity (defined later) as a result of P-gp inhibition. P-gp is involved in digoxin intestinal, urinary, and biliary efflux. After intravenous administration, digoxin is eliminated via renal (50–70%) and hepatobiliary (10–30%) excretion, as well as intestinal secretion (10–20%), with minimal metabolism. Thus, the alteration of systemic exposure of digoxin by P-gp inhibitor drugs may not be solely attributed to P-gp–limited intestinal absorption.

DE and digoxin also exhibit different transporter profiles (Figure 1b). In addition to P-gp, digoxin undergoes basolateral uptake by an unidentified sodium-dependent uptake process both in vitro and possibly in vivo; furthermore, sodium-independent organic anion transporting polypeptide 4C1 mediates digoxin uptake into the renal proximal tubule. In addition, digoxin is known as a substrate of organic solute transporter α/β, but their role in digoxin disposition in vivo is unclear (Figure 1b). In contrast, DE is a more specific substrate for P-gp and is not transported by other major efflux transporters expressed in the intestine. Dabigatran is not a substrate of major drug transporters. Transporter mechanisms of dabigatran glucuronides are not known but are expected to have limited impact on dabigatran pharmacokinetics (PK), because glucuronidation is a minor elimination pathway.

**SENSITIVITY OF DE AND DIGOXIN AS P-GP PROBES: COMPARISON OF CLINICAL P-GP INHIBITION STUDIES USING DE AND DIGOXIN AS PROBES**

The sensitivity of a probe drug refers to the fold change in plasma area under the concentration–time curve (AUC) and/or maximum concentration in the presence of an inhibitor relative to control and is an important consideration when selecting clinical DDI probes. Table 1 summarizes in vitro P-gp inhibition data using DE as a probe and clinical DDIs evaluated with both digoxin and DE under similar dosing regimens of the perpetrator drugs. The magnitude of a DDI using digoxin as a probe is generally low. However, these changes are of clinical relevance to digoxin safety, where even a small increase in exposure (e.g., 25–50%) could represent a safety risk (NDA 020405, Drugs@FDA).

Somewhat unexpected, except for cisapride and gliclazide, the magnitude of DDIs using a therapeutic dose of DE is generally comparable to digoxin, despite relatively higher selectivity of DE as a P-gp probe on the basis of available in vitro and absorption, distribution, metabolism, and excretion data (Table 1). DE is a high-affinity P-gp substrate with an apparent Michaelis-Menton constant for transporter affinity (K_m) of ~1 μM. At the therapeutic dose of DE (75–150 mg), maximal theoretical gut concentrations (C_gut = dose/250 ml) of DE are approximately 480–950 μM, which is lower than its aqueous solubility limit of 1.8 mg/ml (2.8 mM), but could be higher than its solubility in neutral and basic conditions, because of its pH-dependent dissolution (NDA 022512, Drugs@FDA). At therapeutic doses of DE, intestinal P-gp is most likely saturated, reducing the effect of P-gp in DE absorption and lowering the DDI magnitude/sensitivity. In contrast, digoxin is a low-affinity P-gp substrate (K_m ~177–220 μM, Figure 1b), and P-gp is unlikely to be saturated at its therapeutic dose (0.25 μg; C_gut = 1.28 μM).

At a subtherapeutic dose, DE is expected to be a more sensitive P-gp probe than digoxin or a therapeutic dose of DE. A clinical microdose study has recently confirmed this hypothesis. When DE was administered at a dose of 375 μg (C_gut = 2.4 μM), rifampin, clarithromycin, and irtraconazole increased plasma AUC of dabigatran 2.4-, 4.2-, and 7.4-fold, respectively, with a comparable increase in maximum concentration. Overall, the magnitude of DDIs observed was at least twofold higher than at the therapeutic dose of DE, which can be explained by a DE microdose not saturating intestinal P-gp.

**CHALLENGES ASSOCIATED WITH DE AS A P-GP PROBE**

The challenges associated with digoxin as a P-gp probe have been discussed previously. We, therefore, focus on DE in this Commentary.

**Interindividual PK variability**

Dabigatran PK exhibits relatively high interindividual variability, which fluctuated across different studies and dosing regimens (the coefficient of variation of AUC ranged from 30–60%). Such variability might be attributed to interindividual differences in P-gp and CES activities. However, the impact of genetic polymorphisms of CES and P-gp on dabigatran PK in healthy subjects is not fully confirmed, and the effects of multiple coexisting genetic covariates in the same subject are not known. Furthermore, a pH-dependent dissolution of the mesylate salt of DE may also contribute to variability of intestinal absorption. PK variability of DE needs to be considered when designing clinical DDI studies. Therefore, a crossover study design and appropriate numbers of subjects are critical for DE P-gp DDI studies.

**CES inhibition by perpetrator drugs**

DE is hydrolyzed by CES to dabigatran (NDA 022512, Drugs@FDA). Clinical DDI data with DE may be confounded if the perpetrator drugs also inhibit intestinal and/or hepatic CES. For correct interpretation of clinical DE DDIs, preassessment of the inhibitory effects of a perpetrator drug on CES activity needs to be considered.

**Stability of DE in vitro**

DE is not stable in cell-based assays because of endogenous CES-catalyzed hydrolysis. Studies in Caco-2 cells indicated that DE is stable if dosed in the basal, but not the apical, compartment of transwells, where DE has direct access to CES. Similar results have been observed in P-gp–transfected MDCKII and LLC-PK1 cells (Chu et al., unpublished data). Therefore, in vitro P-gp half maximal inhibitory concentration IC_{50} values for DE can be determined by monolayer flux only in the basal-to-apical direction or as bidirectional transport in cells pretreated with esterase inhibitors.

IC_{50} values for several P-gp inhibitors measured with DE for basal-to-apical transport predicted the risk for DDIs with no false negatives (Table 1). Further evaluations are needed to validate the assay conditions, explore other assay systems (e.g., vesicles), and understand potential interlaboratory variability and its translational impact.
Figure 1. BCRP, breast cancer resistance protein; CES, carboxylesterase; DDI, drug–drug interaction; DE, dabigatran etexilate; \( K_m \), Michealis-Menton constant for transporter affinity; MRP, multidrug resistance protein; OATP, organic anion transporting polypeptide; OAT, organic anion transporter; OCT, organic cation transporter; OST, organic solute transporter; P-\( \text{gp} \), P-glycoprotein; UGT, uridine 5′-diphosphoglucuronosyl transferase. In vivo disposition of DE and dabigatran in human (a) and the comparison of their in vitro transporter profiles with digoxin (b). DE absorption, distribution, metabolism, and excretion data are obtained from NDA 022512 (Drugs@FDA). DE, dabigatran, and digoxin in vitro transporter data are obtained from the University of Washington DDI database (https://www.druginteractioninfo.org) and Reference.\(^{10}\) aUGT2B15 is more prominently expressed in the liver and is the major contributor to the glucuronidation of dabigatran; UGT1A9 and UGT2B7 have minor contribution. The formation of dabigatran acyl glucuronides in the gut is low, and their possible interplay with P-\( \text{gp} \)-mediated DE efflux is, therefore, less likely.\(^{9}\) It is not known whether DE is a substrate of intestinal uptake transporters. Because DE has moderate to high passive permeability and DE can be converted to dabigatran by intestinal CES2, the involvement of intestinal uptake transporters on absorption of DE could be limited. Given that DE is almost completely converted to dabigatran presystemically, hepatic and renal transporters are less likely involved in the disposition of DE.\(^{9}\) Transporter phenotyping data for dabigatran glucuronides have not been reported.
worst-case scenario for P-gp inhibition

On balance, a DE microdose evaluates the attributed to pH-dependent dissolution.

pibrentasvir
Glecaprevir/Cobicistat 150 mg, q.d., for Itraconazole 200-mg capsule, q.d., for 4–5 days. Amiodarone 600 mg, s.d. or b.i.d., for 8 days. Itraconazole 200–800 mg/day for 10 days. Ketoconazole 400 mg, s.d., or 200 mg, q.d., for 5 days. Verapamil 120 mg (immediate release), s.d. or b.i.d., 1 hour before or concomitantly with DE

P-gp is envisaged that a qualified physiological basis for DDIs with P-gp, quinidine exposure in the gut under these two study conditions can be different. hBoth glecaprevir and pibrentasvir are for P-gp.

potentially reduce PK variability,5 likely to provide a more sensitive readout of transporter saturation compared with digoxin or DE therapeutic dose. In addition, a DE microdose can minimize safety concerns of a therapeutic dose of this oral anticoagulant agent in healthy subjects. Furthermore, a DE microdose can potentially reduce PK variability,5 likely attributed to pH-dependent dissolution. On balance, a DE microdose evaluates the worst-case scenario for P-gp inhibition and, as such, represents the most sensitive clinical effect. However, the magnitude of the resulting DDIs should not be directly extrapolated to a therapeutic dose of DE or other P-gp substrates as an indicator of safety concerns or dose adjustment. It is envisaged that a qualified physiological based PK model for DE can bridge this gap to support P-gp clinical DDI study design in the future. The feasibility of conducting a DE microdose study can be limited by the sensitivity of analytical assays. Compared with digoxin, in vitro and in vivo data using DE as a P-gp probe are still limited and additional data are needed to verify in vitro to in vivo translation. In addition, DE will not be suitable to study P-gp inhibition in other tissues when a potential P-gp inhibitor is a non-oral drug. Despite a lack of P-gp selectivity and sensitivity, digoxin DDI evaluation may still be warranted to assess the risk of perpetrator drugs on digoxin safety because of its narrow therapeutic index. To interrogate renal P-gp DDIs with digoxin, renal clearance would also need to be measured in the DDI study.

In summary, a DE microdose is a more selective and sensitive P-gp probe than digoxin to study inhibition of intestinal P-gp, with CES inhibition as a potential confounding factor. Clinical digoxin DDI studies with P-gp inhibitors should primarily be conducted to ensure safe co-medication. Additional in vitro and clinical studies with DE would greatly advance our understanding of its suitability as a P-gp probe, and investigators are encouraged to share these data.

**ACKNOWLEDGMENT**
The authors would like to acknowledge Dr. Xinning Yang for valuable comments on the manuscript.

**CONSIDERATIONS IN SELECTION OF A P-GP CLINICAL PROBE FOR DDI ASSESSMENT**

In clinical DDI studies, P-gp probes should be selected on the basis of the specific DDI questions to be addressed. DE may be a more selective probe than digoxin for the assessment of P-gp–mediated DDIs in the intestine. DE at a subtherapeutic dose (e.g., microdose) is likely to provide a more sensitive readout for intestinal P-gp DDIs because of lack of transporter saturation compared with digoxin or DE therapeutic dose. In addition, a DE microdose can minimize safety concerns of a therapeutic dose of this oral anticoagulant agent in healthy subjects. Furthermore, a DE microdose can potentially reduce PK variability,5 likely attributed to pH-dependent dissolution. On balance, a DE microdose evaluates the worst-case scenario for P-gp inhibition and, as such, represents the most sensitive clinical effect. However, the magnitude of the resulting DDIs should not be directly extrapolated to a therapeutic dose of DE or other P-gp substrates as an indicator of safety concerns or dose adjustment. It is envisaged that a qualified physiological based PK model for DE can bridge this gap to support P-gp clinical DDI study design in the future. The feasibility of conducting a DE microdose study can be limited by the sensitivity of analytical assays. Compared with digoxin, in vitro and in vivo data using DE as a P-gp probe are still limited and additional data are needed to verify in vitro to in vivo translation. In addition, DE will not be suitable to study P-gp inhibition in other tissues when a potential P-gp inhibitor is a non-oral drug. Despite a lack of P-gp selectivity and sensitivity, digoxin DDI evaluation may still be warranted to assess the risk of perpetrator drugs on digoxin safety because of its narrow therapeutic index. To interrogate renal P-gp DDIs with digoxin, renal clearance would also need to be measured in the DDI study.

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The authors would like to acknowledge Dr. Xinning Yang for valuable comments on the manuscript.

**Table 1** Examples of P-gp–related clinical DDIs using DE and digoxin as probe substrates

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose regimen</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>I&lt;sub&gt;L&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt; for DE</th>
<th>% Change of dabigatran AUC&lt;sup&gt;b, c&lt;/sup&gt;</th>
<th>% Change of digoxin AUC&lt;sup&gt;c&lt;/sup&gt;</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>500 mg, b.i.d., for 4–5 days</td>
<td>28</td>
<td>36</td>
<td>49–114, 302&lt;sup&gt;e&lt;/sup&gt;</td>
<td>35.1–68.2</td>
<td>3, 5, NDA 022512 (Drugs@FDA)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>120 mg (immediate release), s.d. or b.i.d., 1 hour before or concomitantly with DE</td>
<td>NR</td>
<td>NR</td>
<td>39.3–142.5</td>
<td>51</td>
<td>NDA 022512 (Drugs@FDA)</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>600 mg, s.d.</td>
<td>&gt;10</td>
<td>&lt;470</td>
<td>58</td>
<td>64–68</td>
<td>3</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>200-mg capsule, q.d., for 5 days</td>
<td>0.41</td>
<td>1,100</td>
<td>117&lt;sup&gt;d&lt;/sup&gt;, 592&lt;sup&gt;e&lt;/sup&gt;</td>
<td>68</td>
<td>3, 5</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>400 mg, s.d. or 400 mg, q.d., for 8 days</td>
<td>3.4</td>
<td>880</td>
<td>138–153</td>
<td>NR&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3, NDA 022512 (Drugs@FDA)</td>
</tr>
<tr>
<td>Quinidine&lt;sup&gt;g&lt;/sup&gt;</td>
<td>200 mg every 2 hours (1,000 mg in total)</td>
<td>33</td>
<td>150</td>
<td>53</td>
<td>166</td>
<td>3, NDA 022512 (Drugs@FDA)</td>
</tr>
<tr>
<td>Dronedarone</td>
<td>400 mg, b.i.d.</td>
<td>NR</td>
<td>NR</td>
<td>130</td>
<td>133</td>
<td>NDA 022512 (Drugs@FDA)</td>
</tr>
<tr>
<td>Cobicistat</td>
<td>150 mg, q.d., for 22 days</td>
<td>NR</td>
<td>NR</td>
<td>110–137</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Glecacrevir/pibrentasvir&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Glecacrevir, 300 mg/ pibrentasvir, 120 mg, q.d.</td>
<td>Glecacrevir, 400 mg/ pibrentasvir, 120 mg, q.d.</td>
<td>Glecacrevir, 400 mg/ pibrentasvir, 120 mg, q.d.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC, area under the concentration–time curve; DE, dabigatran etexilate; DDI, drug–drug interaction; IC<sub>50</sub>, half maximal inhibitory concentration; I<sub>L</sub>, concentration of inhibitor in the gastrointestinal tract based on dose divided by a volume of 250 ml; NR, not reported; P-gp, P-glycoprotein.

<sup>a</sup> In vitro P-gp IC<sub>50</sub> data are obtained from Caco-2 cells using DE as substrate. <sup>b</sup> DE clinical dose range, 75–300 mg; total dabigatran (dabigatran plus its glucuronide) was measured in clinical DDI studies with amiodarone, ketoconazole, quinidine, verapamil, and dronedarone. <sup>c</sup> Clinical DDI data are obtained from the University of Washington DDI database (https://www.druginteractioninfo.org) or specified in the references. <sup>d</sup> Unpublished data: Itraconazole dosing regimen, 200-mg capsules, q.d., for 5 days. <sup>e</sup> DE dose, 375 μg, s.d., single dose; digoxin DDIs at microdose have not been reported. <sup>f</sup> Clinical DDI data are only available at ketoconazole dose of 200 mg, q.d., for 4 days. <sup>g</sup> Quinidine was administered in different dose regimen in these clinical DDI studies. Given that the gut is the major site for DDIs with P-gp, quinidine exposure in the gut under these two study conditions can be different. <sup>h</sup> Both glecaprevir and pibrentasvir are in vitro inhibitors for P-gp.
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The authors declared no competing interests for this work.

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