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DOI:

[10.1002/cpt.2018.104.issue-5](https://doi.org/10.1002/cpt.2018.104.issue-5)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Chu, X., Galetin, A., Zamek-glisczynski, M. J., Zhang, L., & Tweedie, D. J. (2018). Dabigatran Etexilate and Digoxin: Comparison as Clinical Probe Substrates for Evaluation of P-gp Inhibition. *Clinical Pharmacology & Therapeutics*, 104(5), 788-792. <https://doi.org/10.1002/cpt.2018.104.issue-5>

Published in:

Clinical Pharmacology & Therapeutics

Citing this paper

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

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Words: 1763/1760 words

Introduction: 75/75 words

Figures/Tables: 2/2

Conflict of Interest: The authors declared no competing interests for this work.

Funding: No funding was received for this work.

Disclaimer: The contents of this manuscript reflect the views of the authors and should not be construed to represent the FDA's views or policies. No official support or endorsement by the FDA is intended or should be inferred. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the FDA.

Abbreviations: absorption, distribution, metabolism, and excretion (ADME), area under the concentration-time curve (AUC), carboxylesterase (CES), drug-drug interaction (DDI), dabigatran etexilate (DE), maximum concentration (C_{max}), maximal theoretical gut concentration (C_{gut}), organic solute transporter (OST), P-glycoprotein (P-gp), Uridine 5'-diphosphoglucuronosyltransferase (UGT)

1 **Introduction (75/75 words)**

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

P-glycoprotein (P-gp) is a drug transporter recommended by regulatory agencies for clinical evaluation as its inhibition can cause clinically important drug-drug interactions (DDIs) (1). 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 (1).

Ideally, a clinical probe to study intestinal P-gp inhibition should exhibit high selectivity and sensitivity. Specifically such a probe should 1) be mainly transported by P-gp, and 2) exhibit primarily P-gp-limited intestinal absorption with low-to-moderate fraction absorbed, 3) be minimally metabolized, and 4) have 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 (1, 2). 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 exhibits low pharmacokinetic (PK) sensitivity to P-gp inhibition with a reasonably high oral bioavailability (60-80%) (2). As such, it is not an ideal probe substrate for studying intestinal P-gp. Digoxin DDI study is clinically relevant for the safe use of digoxin, however, it does not capture the true “worst-case” victim DDI potential due to intestinal P-gp inhibition that may be used to predict 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 (3). 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 (ADME) and transporter profiles

DE is rapidly absorbed and converted to parent dabigatran by carboxylesterases CES2 (in the intestine) and CES1 (in the liver) (Figure 1A, NDA 022512, Drugs@FDA). Oral bioavailability

of dabigatran following DE administration is 3-7%, likely due to low intestinal absorption of DE limited by P-gp efflux. Dabigatran is 80-85% eliminated unchanged in urine primarily via passive glomerular filtration, is not metabolized by cytochrome P450 enzymes, but undergoes glucuronidation in the liver to form pharmacologically active acyl glucuronides (~10% of total dabigatran following 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 below) as a result of P-gp inhibition. P-gp is involved in digoxin intestinal, urinary, and biliary efflux (2). Following intravenous administration, digoxin is eliminated via renal (50-70%), 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 (OATP)4C1 mediates digoxin uptake into the renal proximal tubule (2). Additionally, digoxin is known as a substrate of OST α / OST 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 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 PK, as 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 AUC and/or C_{max} 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 (3). 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 cobicistat and glecaprevir/pibrentasvir, 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 based on available *in vitro* and ADME data (Table 1). DE is a high affinity P-gp substrate with an apparent K_m of $\sim 1 \mu\text{M}$ (4). At the therapeutic dose of DE (75-150 mg), maximal theoretical gut concentrations ($C_{\text{gut}} = \text{dose}/250 \text{ ml}$) of DE are approximately 480-950 μM , which is below its aqueous solubility limit of 1.8 mg/ml (2.8 mM), but could be above its solubility in neutral and basic conditions, due to 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 ($K_m \sim 177\text{-}220 \mu\text{M}$, Figure 1B), and P-gp is unlikely to be saturated at its therapeutic dose (0.25 mg; $C_{\text{gut}} = 1.28 \mu\text{M}$).

At a sub-therapeutic 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 (5). When DE was administered at a dose of 375 μg ($C_{\text{gut}} = 2.4 \mu\text{M}$), rifampin, clarithromycin, and itraconazole increased plasma AUC of dabigatran 2.4-, 4.2-, and 7.4-fold, respectively, with comparable increase in C_{max} . Overall, the magnitude of DDIs observed was at least 2-fold higher than at the therapeutic dose of DE, which can be explained by a DE microdose not saturating intestinal P-gp (5).

Challenges associated with DE as a P-gp probe

The challenges associated with digoxin as a P-gp probe have been discussed previously (1, 2). We therefore focus on DE in this Commentary.

Inter-individual PK variability

Dabigatran PK exhibits relatively high inter-individual variability, which fluctuated across different studies and dosing regimens (the coefficient of variation of AUC ranged 30-60%) (6). Such variability might be attributed to inter-individual differences in P-gp and CES activities (7). 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 cross-over study design and appropriate numbers of subjects are critical for DE P-gp DDI studies.

Carboxylesterase inhibition by perpetrator drugs

DE is hydrolyzed by CES to dabigatran (8, 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, pre-assessment 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 due to 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 CES1 (3, 9). Similar results have been observed in P-gp-transfected MDCKII and LLC-PK1 cells (Chu et al. unpublished data). Therefore, *in vitro* P-gp IC₅₀ values for DE can be determined by monolayer flux only in the basal-to-apical direction or as bi-directional transport in cells pretreated with esterase inhibitors (9). IC₅₀ values for several P-gp inhibitors measured with DE for basal-to-apical transport predicted the risk for DDIs with no false negatives (3) (Table 1). Further evaluations are needed to validate the assay conditions, explore other assay systems (e.g., vesicles) and understand potential inter-lab variability and its translational impact.

Considerations in selection of a P-gp clinical probe for DDI assessment

In clinical DDI studies, P-gp probes should be selected based on 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 sub-therapeutic dose (e.g., microdose) is likely to provide a more sensitive readout for intestinal P-gp DDIs due to lack of transporter saturation compared to digoxin or DE therapeutic dose. In addition, a DE microdose can minimize safety concerns of 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 PBPK 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 to 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 due to 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.

1 **Acknowledgement**

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

4

Figure Legend:

Figure 1. *In vivo* disposition of dabigatran etexilate (DE) and dabigatran in human (A) and the comparison of their *in vitro* transporter profiles with digoxin (B)

DE ADME 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.

^a: UGT2B15 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 acylglucuronides in the gut is low and their possible interplay with P-gp-mediated DE efflux is therefore less likely.

^b: It is not known whether DE is a substrate of intestinal uptake transporters. Since 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 pre-systemically, hepatic and renal transporters are less likely involved in the disposition of DE.

^c: Transporter phenotyping data for dabigatran glucuronides have not been reported.

Abbreviations: OST α , organic solute transporter- α ; OST β , organic solute transporter- β .

References

- (1) Agarwal S, Arya V, Zhang L. Review of P-gp inhibition data in recently approved new drug applications: utility of the proposed $[I(1)]/IC(50)$ and $[I(2)]/IC(50)$ criteria in the P-gp decision tree. *Journal of clinical pharmacology* 2013 Feb;53(2):228-33.
- (2) Lee CA, Kalvass JC, Galetin A, Zamek-Gliszczyński MJ. ITC commentary on the prediction of digoxin clinical drug-drug interactions from in vitro transporter assays. *Clinical pharmacology and therapeutics* 2014 Sep;96(3):298-301.
- (3) Kishimoto W, Ishiguro N, Ludwig-Schwellinger E, Ebner T, Schaefer O. In vitro predictability of drug-drug interaction likelihood of P-glycoprotein-mediated efflux of dabigatran etexilate based on $[I]_2/IC_{50}$ threshold. *Drug metabolism and disposition: the biological fate of chemicals* 2014 Feb;42(2):257-63.
- (4) Zhao Y, Hu ZY. Physiologically based pharmacokinetic modelling and in vivo $[I]/K(i)$ accurately predict P-glycoprotein-mediated drug-drug interactions with dabigatran etexilate. *British journal of pharmacology* 2014 Feb;171(4):1043-53.
- (5) Prueksaritanont T, Tatosian DA, Chu X, Railkar R, Evers R, Chavez-Eng C, et al. Validation of a microdose probe drug cocktail for clinical drug interaction assessments for drug transporters and CYP3A. *Clinical pharmacology and therapeutics* 2017 Apr;101(4):519-30.
- (6) Ollier E, Hodin S, Basset T, Accassat S, Bertoletti L, Mismetti P, et al. In vitro and in vivo evaluation of drug-drug interaction between dabigatran and proton pump inhibitors. *Fundamental & clinical pharmacology* 2015 Dec;29(6):604-14.
- (7) Pare G, Eriksson N, Lehr T, Connolly S, Eikelboom J, Ezekowitz MD, et al. Genetic determinants of dabigatran plasma levels and their relation to bleeding. *Circulation* 2013 Apr 2;127(13):1404-12.
- (8) Laizure SC, Parker RB, Herring VL, Hu ZY. Identification of carboxylesterase-dependent dabigatran etexilate hydrolysis. *Drug metabolism and disposition: the biological fate of chemicals* 2014 Feb;42(2):201-6.
- (9) Ishiguro N, Kishimoto W, Volz A, Ludwig-Schwellinger E, Ebner T, Schaefer O. Impact of endogenous esterase activity on in vitro p-glycoprotein profiling of dabigatran etexilate in Caco-2 monolayers. *Drug metabolism and disposition: the biological fate of chemicals* 2014 Feb;42(2):250-6.
- (10) Hodin S, Basset T, Jacqueroix E, Delezay O, Clotagatide A, Perek N, et al. In Vitro Comparison of the Role of P-Glycoprotein and Breast Cancer Resistance Protein on Direct Oral Anticoagulants Disposition. *European journal of drug metabolism and pharmacokinetics* 2017 Sep 11.

Table 1 Examples of P-gp-related Clinical DDIs Using DE and Digoxin as Probe Substrates

Drugs	Dose regimen		IC ₅₀ (μ M) ^a	I ₂ /IC ₅₀ for DE	% Change of Dabigatran AUC ^{b, c}	% Change of Digoxin AUC ^c	References
	DE as probe drug	Digoxin as probe drug					
Clarithromycin	500 mg, BID for 4-5 days	500 mg, BID for 7 days	28	36	49-114, 302 ^e	35.1-68.2	3, 5, NDA 022512 (Drugs@FDA)
Verapamil	120 mg (immediate release) SD or BID 1 h before or concomitantly with DE	80mg TID for 10 days following BID for 4 days	NR	NR	39.3-142.5	51	NDA 022512 (Drugs@FDA)
Amiodarone	600 mg SD	400-800 mg/day for 1 week	>10	<470	58	64-68	3
Itraconazole	200 mg capsule QD for 5 days	200 mg QD for 5 days	0.41	1100	117 ^d , 592 ^e	68	3, 5
Ketoconazole	400 mg SD or 400 mg QD for 8 days		3.4	880	138-153	NR ^f	3, NDA 022512 (Drugs@FDA)
Quinidine ^g	200 mg every 2 h (1000 mg in total)	600 mg BID for 8 days	33	150	53	166	3, NDA 022512 (Drugs@FDA)

Dronedarone	400 mg BID	400 mg BID for 10 days	NR	NR	130	133	NDA 022512 (Drugs@FDA)
Cobicistat	150 mg QD for 22 days	150 mg for 10 days	NR	NR	110-137	8.8	
Glecaprevir/Pibrentasvir ^h	Glecaprevir 300 mg/ Pibrentasvir 120 mg QD	Glecaprevir 400 mg/ Pibrentasvir 120 mg QD	NR	NR	138	48	NDA 209394 (Drugs@FDA)

AUC, area under the concentration-time curve; DE, dabigatran etexilate; DDI, drug-drug interaction; IC₅₀, half maximal inhibitory concentration; I₂, concentration of inhibitor in the gastrointestinal tract based on dose divided by a volume of 250 mL; NR, not reported.

^a In vitro P-gp IC₅₀ data are obtained from Caco-2 cells using DE as substrate; ^b 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. ^c Clinical DDI data are obtained from the University of Washington DDI database (<https://www.druginteractioninfo.org>) or specified in the references; Unpublished data: itraconazole dosing regimen 200 mg capsules QD for 5 days; ^e DE dose 375 µg SD PO; digoxin DDIs at microdose have not been reported ^f Clinical DDI data are only available at ketoconazole dose of 200 mg QD for 4 days. ^g 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. ^h Both glecaprevir and pibrentasvir are in vitro inhibitors for P-gp.

A.

Dabigatran etexilate (DE)

B.

Digoxin	DE
Substrate for P-gp (K_m 177-220 μM), OATP4C1 (K_m 7.8 μM), OST α/β , and sodium-dependent uptake transporter(s)	Substrate for P-gp (K_m 1 μM)
Not a substrate for OATP1A2, OATP1B1, OATP1B3, and OATP2B1	Not a substrate for BCRP and MRP2 ^b
P-gp may contribute to intestinal absorption, renal and hepatic elimination	P-gp-mediated DDIs are restricted to intestinal absorption of DE
	Parent drug dabigatran is not a substrate for P-gp, MRP2, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, and likely a marginal substrate for OCT2 ^c