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In Vitro–In Vivo Extrapolation Scaling Factors for Intestinal P-glycoprotein and Breast Cancer Resistance Protein: Part II. The Impact of Cross-Laboratory Variations of Intestinal Transporter Relative Expression Factors on Predicted Drug Disposition[S]

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ABSTRACT

Relative expression factors (REFs) are used to scale in vitro transporter kinetic data via in vitro–in vivo extrapolation linked to physiologically based pharmacokinetic (IVIVE-PBPK) models to clinical observations. Primarily two techniques to quantify transporter protein expression are available, immunoblotting and liquid chromatography–tandem mass spectrometry. Literature-collated REFs ranged from 0.4 to 5.1 and 1.1 to 90 for intestinal P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), respectively. The impact of using human jejunum–Caco-2 REFs for P-gp ($REF_{iP\text{-}gp}$) and BCRP (REF_{iBCRP}), generated from the same samples and using different proteomic methodologies from independent laboratories, on PBPK outcomes was assessed. A 5-fold decrease in $REF_{iP\text{-}gp}$ for a single oral dose of digoxin resulted in a 1.19- and 1.31-fold higher plasma area under the curve and C_{max}, respectively. All generated $REF_{iP\text{-}gp}$ values led to simulated digoxin C_{max} values within observed ranges; however, combining kinetic data generated from a different laboratory with the 5-fold lower $REF_{iP\text{-}gp}$ could not recover a digoxin-rifampicin drug-drug interaction, emphasizing the necessity to obtain transporter-specific kinetic estimates and REFs from the same in vitro system. For a theoretical BCRP compound, with absorption taking place primarily in the jejunum, a decrease in the REF_{iBCRP} from 2.22 (University of Manchester) to 1.11 (Bertin Pharma) promoted proximal intestinal absorption while delaying t_{max} 1.44-fold. Laboratory-specific differences in REF may lead to different IVIVE-PBPK outcomes. To understand the mechanisms underlying projected pharmacokinetic liabilities, it is important to assess the potential impact of bias on the generation of REFs on an interindividual basis within a target population.

Introduction

In vitro–in vivo extrapolation linked to physiologically based pharmacokinetic (IVIVE-PBPK) models aim to predict profiles of drug disposition dynamically. This is accomplished by incorporating "drug" data, generated in vitro, and physicochemical knowledge together with "systems" data in a population (Rostami-Hodjegan, 2012). Kinetic data [i.e., maximal flux capacity of the transporter protein (J_{max}) and K_m] describing the active transport processes generated from cell systems can also be included in IVIVE-PBPK models. To scale these data to in vivo, human and in vitro system transporter protein expression or activity data are also required in combination with physiologic, demographic, and genetic information (Rostami-Hodjegan, 2012). To date, intestinal transporter IVIVE scaling factors (Neuhoff et al., 2013a) have been generated based on Western blotting, a relative quantitative technique to quantify transport expression (Troutman and Thakker, 2003b). Yet, absolute transporter protein abundances quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS) have recently been explored for hepatic application in IVIVE-PBPK (Vildhede et al., 2014).

In this study, we provide a systematic analysis of the mRNA and protein expression data available in the literature for generating the relative expression factor (REF), an IVIVE scalar that describes the ratio of in vivo to in vitro systems transporter expression for human jejunum and Caco-2 monolayer P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). We also evaluate the impact of intestinal P-gp and BCRP REFs generated by different laboratories and methodologies on drug absorption in a PBPK model.

Materials and Methods

Literature Review of the Intestinal Expression Data for P-gp and BCRP. Starting with a previously reported meta-analysis that established human intestinal P-gp and BCRP region-specific protein expression (Harwood et al.,

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ABBREVIATIONS: BCRP, breast cancer resistance protein; BPh, Bertin Pharma; DDI, drug-drug interaction; HV, healthy Caucasian volunteer population; IVIVE, in vitro–in vivo extrapolation; LC-MS/MS, liquid chromatography–tandem mass spectrometry; PBPK, physiologically based pharmacokinetic; P-gp, P-glycoprotein; REF, relative expression factor; REF_{iBCRP}, intestinal relative expression factor for breast cancer resistance protein; $REF_{iP\text{-}gp}$, intestinal relative expression factor for P-glycoprotein; TC, theoretical BCRP compound; UoM, University of Manchester.

2013), a new search for the relevant published data quantifying P-gp and BCRP mRNA or protein expression in human jejunum and filter-grown Caco-2 monolayers using PubMed (http://www.ncbi.nlm.nih.gov/pubmed) was undertaken. The following keyword combinations were used in PubMed: human; jejunum; Caco-2; P-gp; MDR1; BCRP; ABCB1; ABCG2; mRNA; protein; expression; absolute; abundance; proteomics. Graphical data were extracted, where required, by GetData Graph Digitizer (http://getdata-graph-digitizer.com).

Generation of the Relative Expression Factors. A REF for P-gp or BCRP was generated, where P-gp or BCRP mRNA/protein expression for human jejunum and Caco-2 monolayers was available using the same technique within a laboratory, including reference genes. These were compared with REFs generated from two different LC-MS/MS workflows (two independent laboratories; matching samples) for P-gp and BCRP in human jejunum and 21-day-cultivated Caco-2 monolayers. Methodological details and individual values are provided in the companion study (Harwood et al., 2016).

Incorporating Intestinal REFs into IVIVE-PBPK Models. The impact of the laboratory-specific intestinal relative expression factor for P-glycoprotein ($REF_{iP\text{-}gp}$) and intestinal relative expression factor for breast cancer resistance protein (REF_{iBCRP}) in virtual healthy Caucasian volunteers (HVs) was assessed in a PBPK model (version 14.1; Simcyp, a Certara company, Sheffield, UK) containing the regional distribution of intestinal P-gp and BCRP and their population variability (Harwood et al., 2013). P-gp and BCRP transport in the model is driven by the unbound intracellular enterocyte concentration and is multiplied by REF and the regional-specific transporter expression to yield effective permeability (Yang et al., 2007; Neuhoff et al., 2013a).

The Impact of P-gp and BCRP REF in IVIVE-PBPK. The impact of $REF_{iP\text{-}gp}$ values generated by the LC-MS/MS [University of Manchester (UoM), Manchester, UK; Harwood et al., 2016) compared with the immunoblotting approach (Troutman and Thakker, 2003b) on digoxin C_{max} was investigated using identical digoxin parameter inputs as the previously reported digoxin IVIVE-PBPK model (Neuhoff et al., 2013a). Caco-2–derived J_{max} and K_m data (Troutman and Thakker, 2003a) were applied to intestinal and hepatic P-gp, assuming P-gp activity in vitro in healthy individuals corresponds to that in vivo, and that J_{max} is related to P-gp protein expression. Simulations were run with a single oral digoxin dose of 0.5 mg in 100 HV individuals to evaluate if kinetic data for digoxin generated in a Caco-2 system from another laboratory (Troutman and Thakker, 2003a) to the $REF_{iP\text{-}gp}$ from the UoM Caco-2 system, could capture the observed digoxin-rifampicin drug-drug interaction (DDI) via induction of intestinal P-gp (Greiner et al., 1999), thus verifying the correct contribution of the active transport built into the digoxin PBPK model.

The impact of laboratory-specific differences for REF_{iBCRP} on pharmacokinetic parameter predictions was evaluated using the Simcyp simulator. A permeable theoretical BCRP compound (TC); see (Supplemental Materials, Technical Note with limited gut metabolism and a specific BCRP activity was administered orally (10 mg in solution) to 100 HV individuals, with the default region-specific BCRP expression within the PBPK model, as published by Harwood et al. (2013).

Results and Discussion

$REF_{iP\text{-}gp}$ and REF_{iBCRP} Generation from Different Laboratories. According to our literature analysis, human jejunum mRNA and protein expression was identified in 19 studies for P-gp and nine studies for BCRP [(Supplemental Materials; (Supplemental Table 1)]. Expression data for Caco-2 P-gp and BCRP from the same laboratory using the same protocol to generate an REF_{iPgp} or REF_{iBCRP} were found for five and four studies, respectively (Table 1). For P-gp, relative mRNA expression analysis (reverse-transcription polymerase chain reaction) enabled the generation of $REF_{iP\text{-}gp}$ from two laboratories in three studies (Taipalensuu et al., 2001; Seithel et al., 2006; Hilgendorf et al., 2007), as the data from Seithel et al. (2006) and Hilgendorf et al. (2007) used Caco-2 monolayers cultivated in the same laboratory for 23 and 16 days, respectively. An $REF_{iP\text{-}gp}$ from two independent laboratories that used Western blotting (Troutman and Thakker, 2003b; von Richter et al., 2009) is available, but not for LC-MS/MS quantification for either P-gp or BCRP, as the Caco-2 cell abundances reported by Oswald et al. (2013) were from plastic, not filter-grown cells (Dr. Stefan Oswald, personal communication). The $REF_{iP\text{-}gp}$ based on mRNA expression ranged 7.1-fold, and for LC-MS/MS quantification, 2.6-fold. The REF_{iBCRP} of 90 (Taipalensuu et al., 2001) may result from low BCRP levels or variability in the housekeeping gene used (Seithel et al., 2006). The $REF_{iP\text{-}gp}$ and REF_{iBCRP} generated from P-gp and BCRP quantification by LC-MS/MS from two different laboratories, UoM and Bertin Pharma (BPh), for the same samples are provided in Table 1 (Harwood et al., 2016). The $REF_{iP\text{-}gp}$ (Troutman and Thakker, 2003b) from independent samples quantified by Western blotting was 5-fold higher than the $REF_{iP\text{-}gp}$ generated by the UoM (UoM-$REF_{iP\text{-}gp}$), whereas the UoM-REF_{iBCRP} was approximately 2-fold higher than BPh (LC-MS/MS) and Altana AG (Western blot; Wesel, Germany) (von Richter et al., 2009).

Assessing the Sensitivity of $REF_{iP\text{-}gp}$ in IVIVE-PBPK. Both the UoM-$REF_{iP\text{-}gp}$ of 0.4 (Harwood et al., 2016) and the $REF_{iP\text{-}gp}$ of 2

TABLE 1

Generation of $REF_{iP\text{-}gp}$ and REF_{iBCRP} from human jejunum and Caco-2 from relative mRNA expression and protein abundance (relative and absolute) data available in the literature for P-gp (MDR1) and BCRP

Transporter and Method	Reference Standard Gene/Protein/Peptide	Jejunum Abundance Mean (S.D., Sample *n*)	Caco-2 Abundance Mean (S.D., Sample *n*)	REF	Source
P-gp (MDR1)					
RT-PCR	Villin	7.90 (± 1.4, *n* = 13)	11.0 (*n* = 1)	0.7	Taipalensuu et al., 2001[a]
RT-PCR	Cyclophilin-A	0.63 (± 0.23, *n* = 4)	0.13 (*n* = 1)	5.0	Seithel et al., 2006[b]
RT-PCR	Cyclophilin-A	1.1 (± 0.67, *n*=5)	0.21 (± 0.04, *n*=3 - 6)	5.1	Hilgendorf et al., 2007[b]
Western blot	Not run in blot	2.12 (*n* = 1)	1.04 (± 0.16, *n* = 3)	2.0	Troutman and Thakker, 2003b[c]
Western blot	Not run in blot	1.00 (*n* = 5, pooled)	1.29 (*n* = 2)	0.8	Von Richter et al., 2009[c]
LC-MS/MS	AGAVAEEVLAAIR	1.89 (± 1.07, *n* = 3)	4.67 (± 0.47, *n* = 3)	0.4	Harwood et al., 2016[d]
LC-MS/MS	FYDPLAGK	0.77 (± 0.35, *n* = 3)	2.08 (± 0.19, *n* = 3)	0.4	Harwood et al., 2016[e]
BCRP					
RT-PCR	Villin	2.7 (± 1.4, *n* = 13)	0.03 (*n* = 1)	90	Taipalensuu et al., 2001[a]
RT-PCR	Cyclophilin-A	0.38 (± 0.08, *n* = 4)	0.07 (*n* = 1)	5.5	Seithel et al., 2006[b]
RT-PCR	Cyclophilin-A	2.36 (± 0.29, *n* = 5)	0.36 (*n* = 3 - 6)	6.6	Hilgendorf et al., 2007[b]
Western blot	Not run in blot	1.00 (*n* = 5, pooled)	0.84 (*n* = 2)	1.2	Von Richter et al., 2009[c]
LC-MS/MS	VIQELGLDK	(2.56 ± 0.82, *n* = 3)	(1.16 ± 0.04, *n* = 3)	2.2	Harwood et al., 2016[d]
LC-MS/MS	SSLLDVLAAR	(2.06 ± 1.11, *n* = 3)	(1.86 ± 0.14, *n* = 3)	1.1	Harwood et al., 2016[e]

RT-PCR, reverse-transcription polymerase chain reaction.
[a]Units for expression of mRNA are number of transcripts per microgram of total RNA.
[b]Units for expression given as relative units of target to reference gene ($2^{-\Delta Ct}$).
[c]Values for expression given as signal intensity as measured by densitometry image analysis.
[d]Units for abundance given as fmol protein/μg total membrane protein; abundance determined at UoM. The same samples were quantified.
[e]Units for abundance given as fmol protein/μg total membrane protein; abundance determined at BPh. The same samples were quantified.

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(Troutman and Thakker, 2003b) led to digoxin C_{max} values within observed ranges after a single oral dose of 0.5 mg of digoxin (Fig. 1A), implying both $REF_{iP\text{-}gp}$ reflect realistic contributions of P-gp in estimating observed C_{max} values when using the J_{max} and K_m for P-gp reported by Troutman and Thakker (2003a). Using the UoM-$REF_{iP\text{-}gp}$ of 0.4 compared with the $REF_{iP\text{-}gp}$ of 2 led to a modest 1.31- and 1.19-fold lower mean C_{max} and area under the curve, respectively, in 100 HV individuals (Fig. 1B). A previous study showed that the observed digoxin-rifampicin DDI, which was attributed to a 3.5-fold increase in intestinal P-gp expression (Greiner et al., 1999), could be recovered using an IVIVE-PBPK strategy, in which the $REF_{iP\text{-}gp}$ of 2.0 was increased 3.5-fold to 7 after (Fig. 1C) induction (Neuhoff et al., 2013b). A 3.5-fold increase in the UoM-$REF_{iP\text{-}gp}$ of 0.4 gave an $REF_{iP\text{-}gp}$ of 1.4, leading to a simulated underprediction in the observed DDI (Fig. 1D). This indicates that the lower UoM-$REF_{iP\text{-}gp}$ (that is not derived from the same Caco-2 system in which the apparent kinetic data were generated) is not sufficient to recover the contribution of P-gp induction by rifampicin to digoxin plasma concentration in HVs. The inability to recover the observed DDI when using the UoM-$REF_{iP\text{-}gp}$ may result from lower P-gp expression and hence lower activity in the UoM Caco-2 systems. This can be due to laboratory differences in methods of expression quantification, Caco-2 cell cultivation, and the variability in jejunum expression. To recover the activity shortfall when using the UoM-$REF_{iP\text{-}gp}$, a 4.3-fold increase in J_{max} (1874 pmol/min/cm^2) was required to recover the observed DDI (Fig. 1E) after using the Nelder-Mead minimization method and weighted least-squares algorithm in the simulators parameter estimation module (Jamei et al., 2014).

Assessing the Sensitivity of REF_{iBCRP} in IVIVE-PBPK. The sensitivity of the region-specific fraction of dose absorbed and enterocyte concentrations to REF_{iBCRP} generated by BPh (1.11) and the UoM (2.22) (Table 1; Harwood et al., 2016) was assessed for the BCRP test compound TC. Figure 2 shows the free segmental enterocyte concentration for TC, used as the driving force for apical efflux transporters. As expected, the lower BPh-REF_{iBCRP} leads to higher TC enterocyte concentrations in proximal regions than the higher UoM-REF_{iBCRP}, whereas an increasing importance of intestinal BCRP UoM-(REF_{iBCRP}) results in higher TC absorption and higher enterocyte concentration in the distal intestine due to the efflux activity promoting TC retention in the gut lumen and transit to the colon, a region with 7.7-fold lower BCRP levels (Harwood et al., 2013). The higher REF_{iBCRP} has a limited impact on lowering C_{max} and area under the curve (1.22- and 1.03-fold, respectively), but increases t_{max} 1.44-fold to 2.8 hours and is in line with clinical observations, where an inhibition of intestinal BCRP leads to a decrease in t_{max} (Schneck et al., 2004). Alongside differences in BCRP expression, interindividual variability in system parameters, such as the small intestinal transit time (range 0.5–10 hours; Yu et al., 1996), also contributes to region-specific fraction of dose absorbed [Supplemental Fig. 1; (Supplemental Materials)]. Acidic BCRP substrates, such as rosuvastatin, are expected to possess higher enterocyte concentrations, as limited metabolism, low

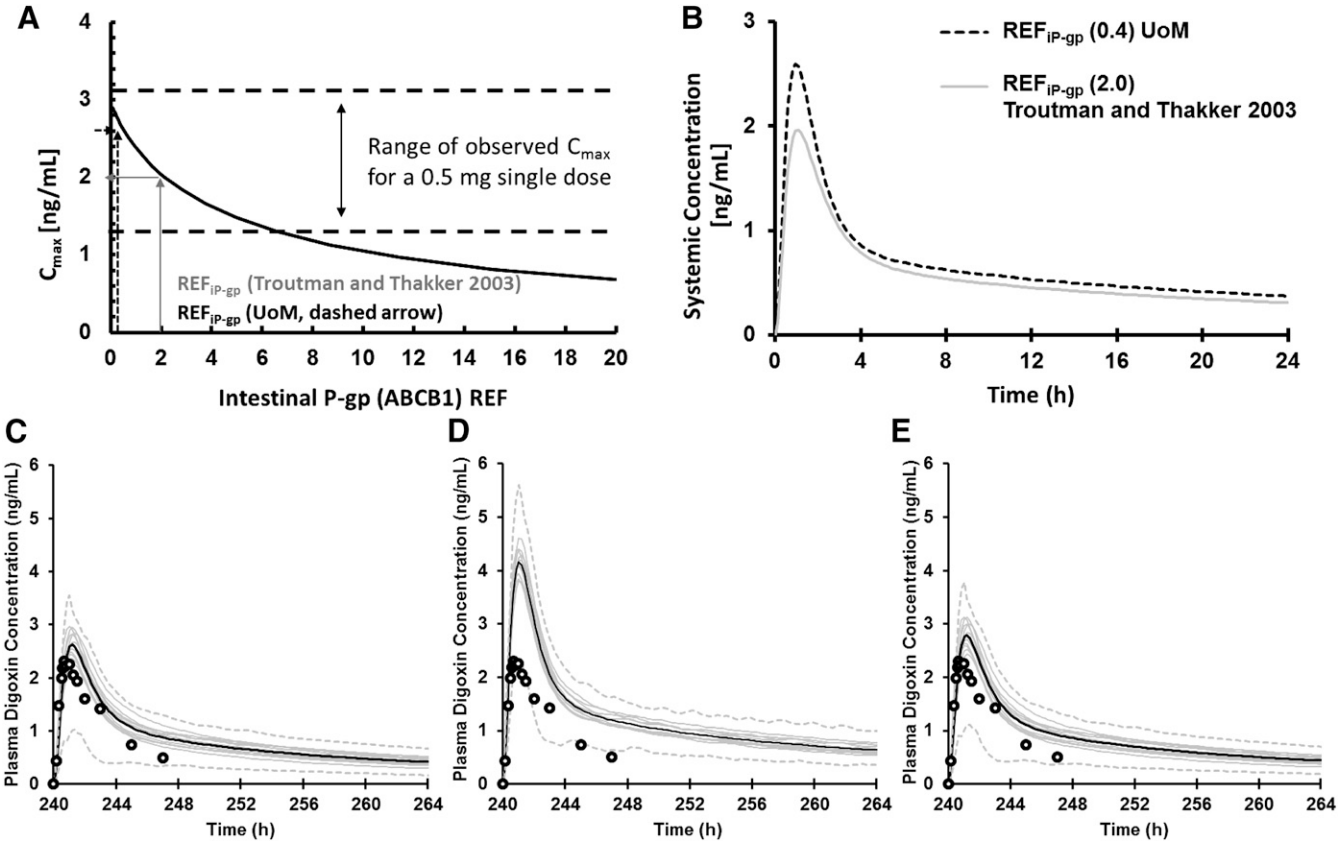

Fig. 1. (A) Sensitivity of C_{max} to $REF_{iP\text{-}gp}$ for digoxin (single oral dose, 0.5 mg) in 100 HV individuals. The ranges of observed C_{max} values are given between the dashed lines, and the $REF_{iP\text{-}gp}$ of 2.0 from Troutman and Thakker (2003b) (gray arrows) and the UoM-$REF_{iP\text{-}gp}$ (Harwood et al., 2016) (dashed arrows) are shown. (B) The mean digoxin plasma concentration when using the $REF_{iP\text{-}gp}$ of 2.0 (Troutman and Thakker, 2003b) and the UoM $REF_{iP\text{-}gp}$ of 0.4 in 100 HVs. The observed and predicted plasma concentrations for digoxin (single oral dose, 1 mg) in 80 HV individuals after the dosing of rifampicin (600 mg, 11 doses, once daily) using an $REF_{iP\text{-}gp}$ of 2 (Troutman and Thakker, 2003b) (C), $REF_{iP\text{-}gp}$ of 0.4 (Harwood et al., 2016) (D), and $REF_{iP\text{-}gp}$ of 0.4 (E) after optimizing the J_{max} of P-gp by parameter estimation. The thin gray lines represent mean values for 10 individual virtual trials of eight individuals, males aged 21–37 years; the thick black lines are the overall means of the virtual population (n = 80); and the dashed gray lines are the 95th and 5th percentiles of the confidence interval. Open circles mark the observed digoxin concentrations when coadministered with multiple doses of rifampicin (Greiner et al., 1999).

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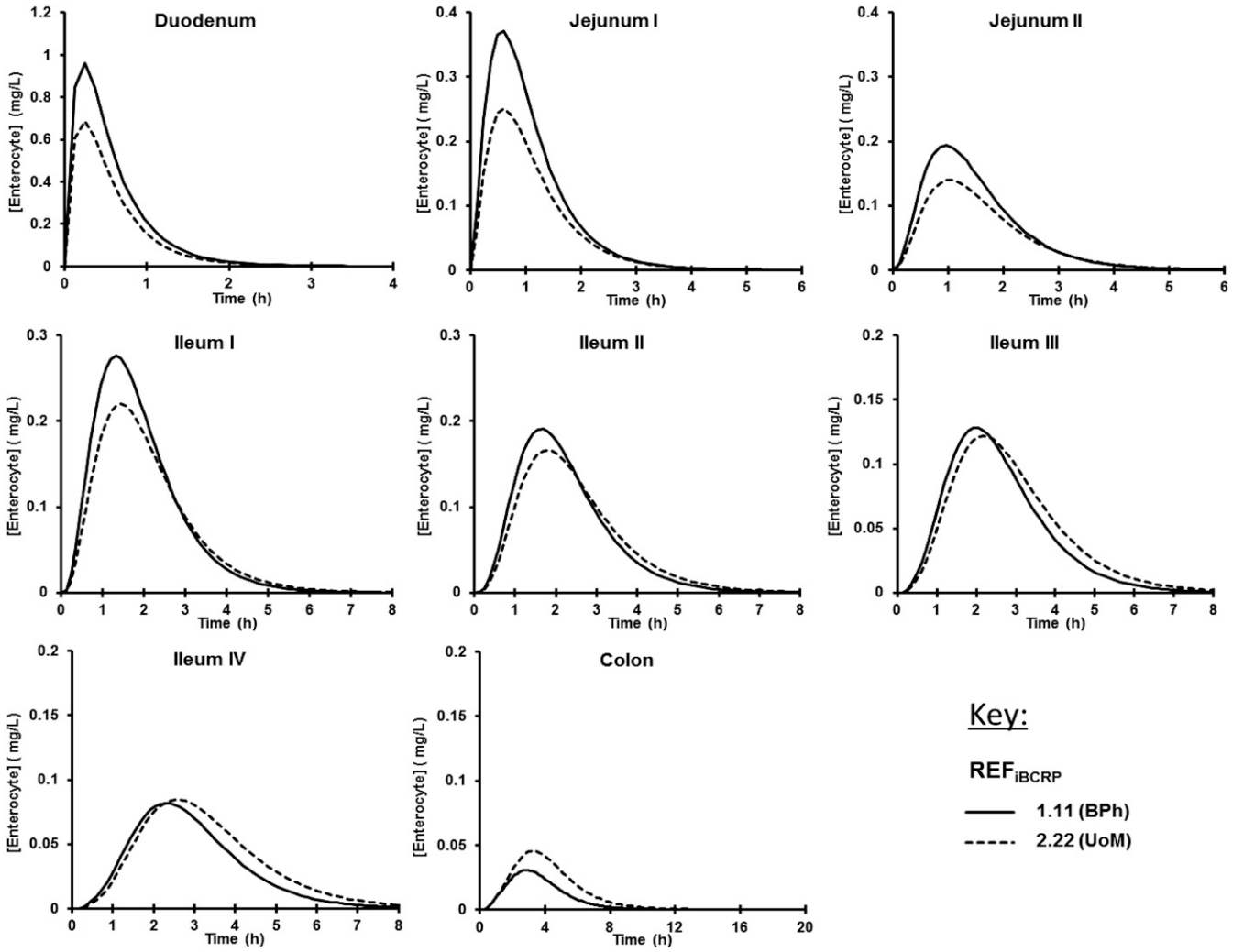

Fig. 2. Simulated enterocyte concentration profiles for all intestinal segments of the model for the BCRP compound TC (10 mg oral, single dose, in solution) in 100 HVs using the BPh REF_{iBCRP} (1.11) or UoM-REF_{iBCRP} (2.22) with a mean small intestinal transit time of 3.34 hours, a passive apparent permeability of 115.2×10^{-6} cm/s, and an intrinsic clearance for BCRP of 17 μl/min/cm^2.

passive permeation, and apical uptake transporters operate (Li et al., 2012; Jamei et al., 2014). Therefore, increased BCRP expression alters t_{max} and regional absorption, while not limiting overall absorption and bioavailability. This is dissimilar to the cooperation of P-gp and CYP3A4 activities that facilitate a drug's repeated exposure to intestinal CYP3A4, increasing overall gut metabolism and reducing bioavailability (Wacher et al., 1998). To our knowledge, the current study is the first highlighting this difference of the colocalized transporters P-gp and BCRP.

Combining IVIVE scalars and activity data generated from different laboratories for ATP-dependent transporters may not lead to successful IVIVE, whereas we postulate that laboratory-specific differences in REF may impact the mechanistic understanding of projected pharmacokinetic liabilities (efficacy/toxicity). This is due to in vitro activity, reproducibility of in vitro assays, culture conditions, and proteomic workflows. As discussed previously, direct translation of protein expression to activity may not always occur; therefore, accounting for deviations in this linear relationship via activity-abundance scalars will be required (Harwood et al., 2013). Ideally, scaling factors should be defined on a laboratory-specific basis against a common reference and combined with activity data from the same system. However, it is improbable within an industrial setting that groups will possess a bank of human intestinal tissues by which to obtain the in vivo abundance for in-house intestinal REF generation. It is therefore advocated that commercially available pooled human intestinal microsomes (constituting $\geq$20 intestines) are used to generate an REF using the same proteomic methods as those used for quantifying in vitro system abundances used for determining activity. Alternatively, a link between human liver microsomes, intestinal microsomes, and Caco-2 cells can be approached.

Authorship Contributions

Participated in research design: Harwood, Neuhoff, Warhurst, Rostami-Hodjegan.

Conducted experiments: Harwood, Achour.

Contributed new reagents or analytic tools: Russell, Carlson.

Performed data analysis: Harwood, Achour, Neuhoff.

Wrote or contributed to the writing of the manuscript: Harwood, Achour, Neuhoff, Warhurst, Rostami-Hodjegan.

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SUPPLEMENTAL INFORMATION

Table S1. Publications reporting P-gp (MDR1) and BCRP mRNA and protein expression in human jejunum obtained from literature analysis.

Protein (Gene)	mRNA/ Protein	Technique	Included for REF analysis (Yes (Y)/No (N))	Reason for Inclusion or Exclusion	Source
P-gp (MDR1)	mRNA	Slot Blot	N	**Exclusion:** No literature data available for Caco-2 MDR1 mRNA expression using the Slot Blot technique from this laboratory	(Fojo et al., 1987)
P-gp (MDR1) & BCRP	mRNA	RT-PCR	Y	**Inclusion:** Data available using the same techniques from the same laboratory for both human jejunum and Caco-2 cell monolayers	(Taipalensuu et al., 2001)
P-gp (MDR1)	mRNA/ Protein	RT-PCR & Western Blot	N	**Exclusion:** Data available for Caco-2 cells and jejunum MDR1 mRNA expression using this RT-PCR technique from this laboratory (Goto et al., 2003), however the Caco-2 cells were grown on plastic and not filter systems, therefore are not relevant for transporter assays and IVIVE	(Hashida et al., 2001)
P-gp (MDR1)	Protein	Western Blot	Y	**Inclusion:** Data available using the same techniques from the same laboratory for both human jejunum and Caco-2 cell monolayers	(Troutman and Thakker, 2003)
P-gp (MDR1)	Protein	Western Blot	N	**Exclusion:** Data available for Caco-2 and jejunum MDR1 mRNA expression using this RT-PCR technique from this laboratory, however the Caco-2 cells were a non-standard phenotype having been cultured with 1α,25(OH_2-vitamin D3) to induce CYP3A4 potentially influencing P-gp expression	(Mouly and Paine, 2003)
P-gp (MDR1)	mRNA	RT-PCR	N	**Exclusion:** Data available for Caco-2 cells and jejunum MDR1 mRNA expression using this RT-PCR technique	(Masuda et al., 2005)

				from this laboratory (Goto et al., 2003), however the Caco-2 cells were grown on plastic and not filter systems, therefore are not relevant for transporter assays and IVIVE	
P-gp (MDR1)	mRNA	RT-PCR	N	**Exclusion:** Data available for Caco-2 cells and jejunum MDR1 mRNA expression using this RT-PCR technique from this laboratory (Goto et al., 2003), however the Caco-2 cells were grown on plastic and not filter systems, therefore are not relevant for transporter assays and IVIVE	(Terada et al., 2005)
P-gp (MDR1) & BCRP	mRNA	RT-PCR	N	**Exclusion:** Data available for Caco-2 and jejunum mRNA MDR1 expression from the same laboratory, however the Caco-2 expression data (Taipalensuu et al., 2001) are not in the same units (transcripts /µg total RNA) as for the jejunum study by Englund et al. (2006) (relative expression to villin)	(Englund et al., 2006)
P-gp (MDR1) & BCRP	mRNA	RT-PCR	Y	**Inclusion:** Data available using the same techniques from the same laboratory for both human jejunum and Caco-2 cell monolayers (23d cultivated)	(Seithel et al., 2006)
P-gp (MDR1)	mRNA	RT-PCR	N	**Exclusion:** Data available for Caco-2 and jejunum mRNA MDR1 expression from the same laboratory, however the Caco-2 expression data (Taipalensuu et al., 2001) are not in the same units (transcripts /µg total RNA) as for the study by Berggren et al. (2007) (percentage of integrated optical density for villin)	(Berggren et al., 2007)
P-gp (MDR1)	mRNA	RT-PCR	N	**Exclusion:** No literature data available for Caco-2 MDR1 mRNA expression using the RT-PCR technique from these laboratories	(Canaparo et al., 2007)
P-gp (MDR1) & BCRP	mRNA	RT-PCR	Y	**Inclusion:** Data available using the same techniques from the same laboratory for both human jejunum and Caco-2 cell monolayers (16d cultivated). Note, it is	(Hilgendorf et al., 2007)

				understood that 4 of the same jejunum samples reported in Seithel et al. (2006) are reported in the 5 samples Hilgendorf et al. (2007)	
P-gp (MDR1) & BCRP	Protein	Western Blot	Y	**Inclusion:** Data available using the same techniques from the same laboratory for both human jejunum and Caco-2 cell monolayers	(von Richter et al., 2009)
P-gp (MDR1) & BCRP	Protein	Western Blot	N	**Exclusion:** No data available for Caco-2 MDR1 mRNA expression using the Western blotting technique from these laboratories	(Bruyere et al., 2010)
P-gp (MDR1)	mRNA	RT-PCR	N	**Exclusion:** No literature data available for Caco-2 MDR1 mRNA expression using the RT-PCR technique from this laboratory	(Ulvestad et al., 2013)
P-gp (MDR1) & BCRP	Protein	QTAP	N	**Exclusion:** The Caco-2 abundance data for this study were quantified from Caco-2 cells grown on plastic and not filter systems, therefore are not relevant for transporter assays and IVIVE	(Oswald et al., 2013)
P-gp (MDR1) & BCRP	Protein	QTAP	N	**Exclusion:** Caco-2 cells P-gp abundances are available from the same laboratory (Oswald et al., 2013) as for this study, however these were quantified from Caco-2 cells grown on plastic and not filter systems, therefore are not relevant for transporter assays and IVIVE	(Gröer et al., 2013)
P-gp (MDR1) & BCRP	Protein	QTAP	N	**Exclusion:** Caco-2 cells P-gp abundances are available from the same laboratory (Oswald et al., 2013) as for this study, however these were quantified from Caco-2 cells grown on plastic and not filter systems, therefore are not relevant for transporter assays and IVIVE	(Drozdzik et al., 2014)
P-gp (MDR1) & BCRP	Protein	QTAP	Y	**Inclusion:** Data available using the same techniques from the same two laboratories for both human jejunum and Caco-2 cell monolayers	(Harwood et al., submitted) (currently unavailable in PubMed database)

In Vitro-In Vivo Extrapolation Scaling Factors for Intestinal P-glycoprotein and Breast Cancer Resistance Protein: Part II. The Impact of Cross-Laboratory Variations of Intestinal Transporter Relative Expression Factors on Predicting Drug Disposition.
Matthew D Harwood, Brahim Achour, Sibylle Neuhoff, Matthew R Russell, Gordon Carlson, Geoffrey Warhurst, Amin Rostami-Hodjegan. Drug Metabolism & Disposition.

TECHNICAL NOTE: RATIONALE FOR BUILDING TC

Compounds that interact with BCRP are generally low permeability compounds that generally require apical intestinal uptake transporters to gain access to the enterocyte, consequently there are no selective BCRP compounds on the market. In addition, due to the limited availability of intrinsic kinetic data ($CL_{int,T}$, or J_{max} & K_m) for compounds that interact with BCRP in Caco-2 monolayers, a theoretical compound (TC) was built to assess the impact of laboratory-specific REFs for BCRP generated by different laboratories and different methods on the same samples. TC was designed to possess high 'passive' lipoidal bilayer permeability (apparent permeability; 115 x 10^{-6} cm/s), so access to the binding site of BCRP via the enterocytes cytoplasm was not limited by its' permeability through the membrane. As the BCRP REFs generated by LC-MS/MS quantification were based on human jejunum samples, it was important to ensure that an TC was absorbed primarily in the jejunum, therefore after sensitivity analysis assessing the relationship between fa/C_{max}, passive permeability and intestinal BCRP $CL_{int,T}$, an intestinal $CL_{int,T}$ for BCRP was of 17 μL/min/cm^2 was assigned. Finally, to reflect the negligible metabolism observed for acidic BCRP substrates such as rosuvastatin, there was no gut metabolism assigned for TC.

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FIGURE

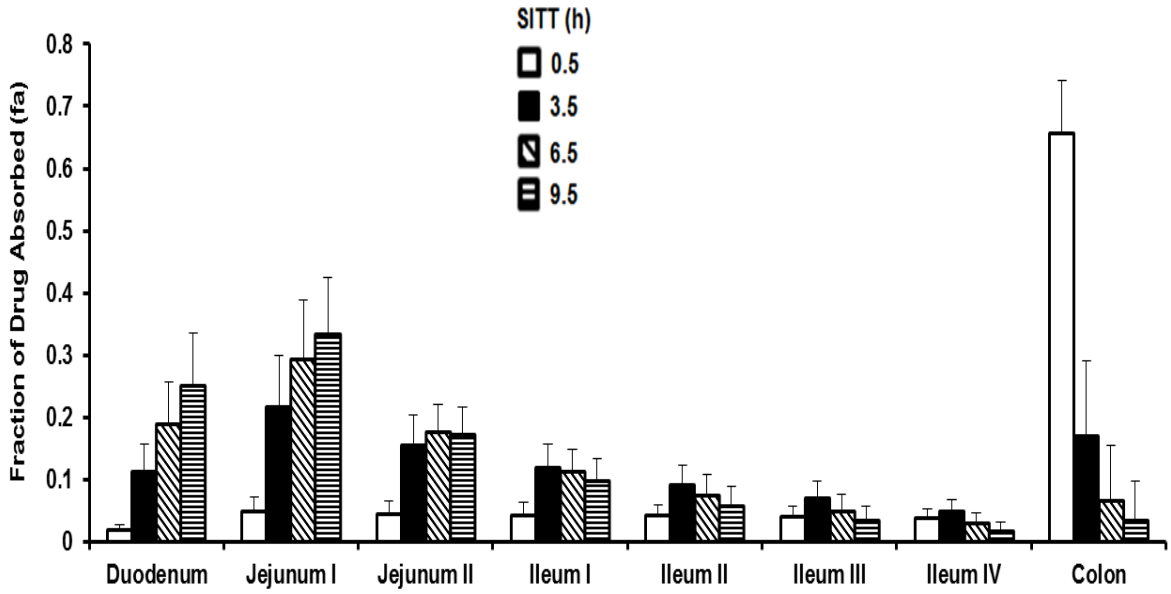

Figure S1. Assessing the impact of small intestinal transit time (SITT) (0.5, 3.5, 6.5 and 9.5 hours) on the regional fa in all intestinal compartments. The passive P_{app} (Caco-2, 115.2 x 10^{-6} cm/s), an intestinal $CL_{int,BCRP}$ of 17 µL/min/cm^2 and REF_{iBCRP} of 1.11 were used. The values are given as a mean of 100 individuals ± standard deviation.

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