Laboratory differences in relative expression factors generated for intestinal P-glycoprotein and Breast Cancer Resistance Protein: Relevance to *in vitro-in vivo extrapolation*

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Introduction

In Vitro-In Vivo Extrapolation (IVIVE) data from cell–based transport assays can be included within Physiologically-Based Pharmacokinetic (PBPK) models that aim to predict time-dependent profiles of drug disposition.

Drug-dependent kinetic transporter data (*i.e.* J_{max}/K_m) are thereby combined with system-dependent data (*e.g.* tissue transporter expression in a population).

Relative Expression Factors (REFs, Eq. 1), are also required to estimate the mass of drug transferred across a membrane *in vivo* when scaling from *in vitro* data.

$$REF = \frac{In\ Vivo\ \text{Transporter Expression}}{In\ Vitro\ \text{Transporter Expression}} \qquad \text{Eq. 1}$$

These models have so far used relative measurements of intestinal transporter expression from immunoblotting[1] rather than absolute abundances from quantitative targeted absolute proteomics (QTAP) techniques to generate intestinal REFs.

Aims

To assess the impact of scalars for P-glycoprotein (P-gp; $REF_{iP\text{-}gp}$) and Breast Cancer Resistance Protein (BCRP; REF_{iBCRP}) generated from human jejunum and Caco-2 cells, using different samples and different methods from 2 independent laboratories, and to quantify transporter expression on PBPK outcomes.

Methods

P-gp and BCRP absolute abundances from human distal jejunum enterocyte (n=3)[2] and 21d Caco-2 cell (n=3, P25-35) membranes were quantified using the quantitative concatamer (QconCAT) QTAP strategy. Human jejunum and Caco-2 cell homogenate P-gp and BCRP expression data from immunoblotting were obtained from the literature[3,4]. $REF_{iP\text{-}gp}$ and REF_{iBCRP} from QTAP and immunoblot data were calculated according to Equation 1.

IVIVE-PBPK simulations using the Simcyp population-based simulator (Version 14 Release 1) were performed to assess the impact of QTAP or immunoblotting-based $REF_{iP\text{-}gp}$ on the plasma concentrations of the P-gp probe digoxin. To verify the relative contribution of intestinal P-gp to the overall intestinal transport of digoxin, the DDI with rifampicin, a P-gp inducer (3.5-fold[5]), was investigated.

REF_{iBCRP} was used to assess the regional-specific absorption and plasma concentrations of a theoretical compound (TC-1); a highly permeating, basic compound with BCRP intrinsic clearance, in which jejunal absorption was highest and the fraction of dose absorbed (fa) in the jejunum was sensitive to alterations in small intestine transit time (SITT).

Results

There was a 5-fold lower $REF_{iP\text{-}gp}$ (0.4) generated from absolute abundance data compared to that generated by immunoblotting (2), while REF_{iBCRP} generated from QTAP analysis (2.2) was 1.9-fold higher compared to that obtained from immunoblotting (1.2) (Table 1).

It is noteworthy that for QTAP analysis, membrane fractions were isolated for abundance quantification, whereas for immunoblotting homogenates were used.

Table 1. The mean absolute abundance and relative expression for P-gp and BCRP in human jejunum and Caco-2 cells with their associated REFs.

	Absolute Abundance (QTAP)* (fmol/µg membrane protein)		Relative Abundance (Immunoblot) (relative to reference protein or normalised %)	
System	P-gp (± SD)	BCRP (± SD)	P-gp†	BCRP‡
Jejunum	1.9 (± 1.1)	2.6 (± 0.8)	2064[3]	100%[4]
Caco-2	4.7 (± 0.5)	1.2 (± 0.0)	1014[3]	84%[4]
REF	**0.4**	**2.2**	**2.0**	**1.2**

*Mean of n=3 samples, †immunoblotting optical densitometry values for jejunum (n=1) and Caco-2 (n=3 lanes), ‡immunoblotting densitometry after normalisation against the jejunum (n=2 lanes). Caco-2 (n=1 P65 & n=1 P75)

The QTAP-$REF_{iP\text{-}gp}$ provided a 1.2 and 1.3-fold higher area under the plasma concentration-time curve (AUC) and maximal plasma concentration (C_{max}) for a single oral dose (0.5 mg) of digoxin, respectively (Figure 1A), highlighting the sensitivity of model to laboratory-specific $REF_{iP\text{-}gp}$. Irrespective of the laboratory, the $REF_{iP\text{-}gp}$ led to digoxin C_{max} values within observed ranges[6, 7] (Figure 1B).

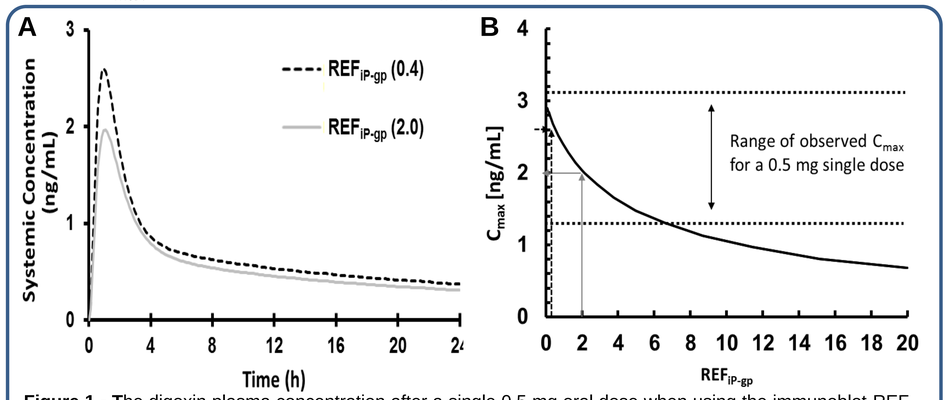

Figure 1 - The digoxin plasma concentration after a single 0.5 mg oral dose when using the immunoblot-$REF_{iP\text{-}gp}$ of 2 (solid line) and QTAP-$REF_{iP\text{-}gp}$ of 0.4 (dashed line) **(A)**. Sensitivity analysis of digoxin C_{max} to the $REF_{iP\text{-}gp}$ **(B)**. The range of observed C_{max} values are given between the dotted lines. The simulated C_{max} using an immunoblot-$REF_{iP\text{-}gp}$ (grey arrows) and QTAP-$REF_{iP\text{-}gp}$ (dashed arrows) are indicated.

The P-gp kinetic data for digoxin were obtained from the same laboratory as the immunoblotting-$REF_{iP\text{-}gp}$[3]. When increasing the QTAP-$REF_{iP\text{-}gp}$ from 0.4 to 1.4, to reflect rifampicin induced P-gp expression, this failed to capture the observed DDI[5], yet the DDI was recovered by inducing the immunoblot REF of 2 by 3.5-fold to 7 (Figures 2A & B). As expected, to recover the rifampicin DDI when using the induced QTAP-$REF_{iP\text{-}gp}$ (1.4), a 4.2-fold higher J_{max} (1874 pmol/min/cm^2) value is required (Figure 2C), which was determined utilising the parameter estimation module within the Simcyp simulator.

References

1. Neuhoff *et al*., 2013, J Pharm Sci, 102: 3145; **2**. Harwood *et al*., 2015, J Pharm Biomed Anal, 110: 27; **3**. Troutman & Thakker 2003, Pharm Res, 20: 1210; **4**. von Richter *et al*., 2009, Naunyn Schiedebergs Arch Pharmacol, 379: 11; **5**. Greiner *et al*., 1999, J Clin Invest, 104:, 147; **6**. Reitman *et al*., 2011, Clin Pharmacol Ther, 89: 234; **7**. Versufyft *et al*., 2003, CPT, 73: 51

Results cont.

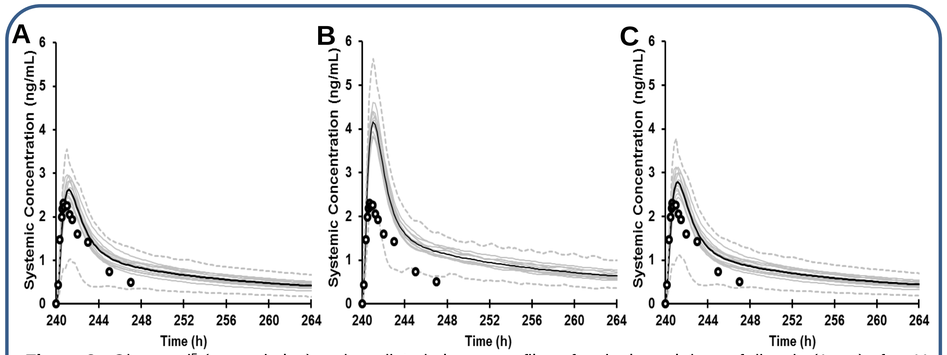

Figure 2 - Observed[5] (open circles) and predicted plasma profiles of a single oral dose of digoxin (1 mg) after 11 doses of rifampicin (600 mg once daily), **(A)** using an immunoblot-$REF_{iP\text{-}gp}$ of 2, **(B)** QTAP-$REF_{iP\text{-}gp}$ of 0.4 and **(C)** QTAP-$REF_{iP\text{-}gp}$ of 0.4 after optimising BCRP J_{max} using Simcyp's in-built parameter estimation module. The overall means (thick lines), the individual trials (thin lines) and the 95th and 5th percentiles of the confidence interval (dashed lines) for 10 virtual trials of 8 individuals in each trial.

At present, the ability to investigate REF_{iBCRP} on IVIVE-PBPK pharmacokinetic outcomes is problematic, due to the limited availability of robust kinetic data for BCRP in filter-grown cell monolayers. Therefore, the theoretical BCRP compound, TC-1, was built as a high permeating, weakly basic compound with significant jejunal efflux.

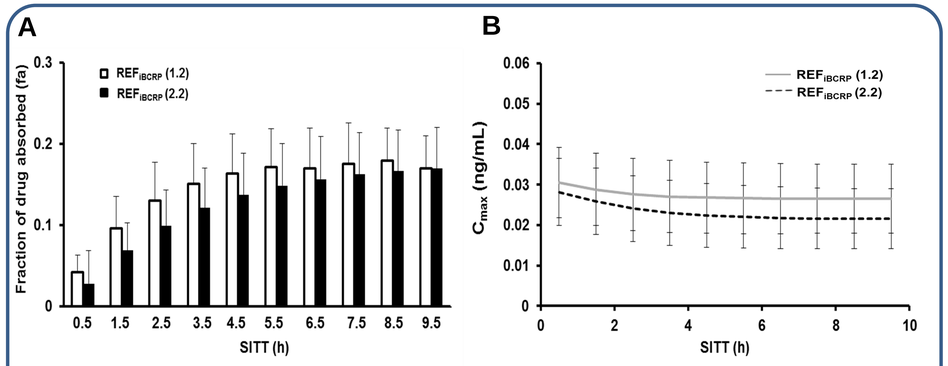

Figure 3 - The impact the immunoblot-REF_{iBCRP} of 1.2 (white bars) and QTAP-REF_{iBCRP} of 2.2 (black bars) on the fraction of drug absorbed **(A)** with varying small intestinal transit times (SITT) in the distal jejunum for TC-1 and **(B)** C_{max} in 100 virtual healthy individuals (Mean ± SD).

The QTAP-REF_{iBCRP} (2.2) lead to a maximum 1.5-fold lower fa than the immunoblot-REF_{iBCRP} in the distal jejunum at the fastest SITT. The difference in fa between REFs diminishes as SITT increases (Figure 3A). Using the QTAP-REF_{iBCRP}, a maximum 1.2-fold lower C_{max} was observed at the slowest SITT, with a considerable overlap in fa and C_{max} demonstrated across a population of 100 virtual individuals (Figure 3A & B).

Discussion & Conclusion:

- Laboratory-specific differences in REFs may lead to different IVIVE-PBPK outcomes.
- A wide-range of $REF_{iP\text{-}gp}$ could be used (0.1-to-5) to attain observed digoxin C_{max}.
- Only a specific REF in combination with the corresponding *in vitro* kinetic data will allow a realistic recovery of the active contribution to the overall membrane transport.
- Inter-individual variability in physiological parameters governing C_{max} and fa are more relevant than the differences in REF_{iBCRP} of the currently available scalars.