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. Jmax/Km) 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.

\[
\text{REF} = \frac{\text{In Vitro Transporter Expression}}{\text{In Vivo Transporter Expression}} \quad \text{Eq. 1}
\]

These models have so far used relative measurements of intestinal transporter expression from immunoblottings 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; REFPGP) and Breast Cancer Resistance Protein (BCRP; REFBcrp) generated from human jejunal 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 jejunal enterocyte (n=3) and 21d Caco-­2 cell (n=3, P25-­35) membranes were quantified using the quantitative concatamer (QconCat) QTAP strategy. Human jejunal and Caco-­2 cell homogenate P-gp and BCRP expression data from immunoblottings were obtained from the literature³,⁴, REFPGP and REFBcrp 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 REFPGP 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-fold⁵), was investigated.

REFBCRP 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 jejunal was sensitive to alterations in small intestine transit time (SITT).

Results
There was a 5-­fold lower REFPGP (0.4) generated from absolute abundance data compared to that generated by immunoblotting (2), while REFBCRP 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>
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<tr>
<th>System</th>
<th>Absolute Abundance (QTAP)* (fmol/μg membrane protein)</th>
<th>Relative Abundance (Immunoblot) (relative to reference protein or normalised %)</th>
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<tr>
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<td>P-gp (± SD)</td>
<td>BCRP (± SD)</td>
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<tr>
<td>Jejunum</td>
<td>1.9 (± 1.1)</td>
<td>2.6 (± 0.8)</td>
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<tr>
<td>Caco-­2</td>
<td>4.7 (± 0.5)</td>
<td>1.2 (± 0.0)</td>
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*Mean of n=3 samples, Immunoblotting optical density values for jejunum (n=1) and Caco-­2 (n=3 lanes).

The QTAP-REFGP provided a 1.2 and 1.3-­fold higher area under the plasma concentration-­time curve (AUC) and maximal plasma concentration (Cmax) for a single oral dose (0.5 mg) of digoxin, respectively (Figure 1A), highlighting the sensitivity of model to laboratory-­specific REFPGP. Irrespective of the laboratory, the REFPGP was used to digoxin Cmax values within observed ranges⁶,⁷ (Figure 1B).

Results cont.
At present, the ability to investigate REFBcrp 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 2 - Observed (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-REFPGP of 2, (B) QTAP-REFPGP of 0.4 and (C) QTAP-REFPGP of 0.4 after optimising BCRP-Cmax 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 concentration interval (dashed lines) for 10 virtual trials of 8 individuals in each trial.

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<tr>
<td></td>
<td>Cmax (ng/mL)</td>
<td>AUC (ng*min/mL)</td>
<td>Cmax (ng/mL)</td>
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<tr>
<td></td>
<td>Mean (± SD)</td>
<td>Mean (± SD)</td>
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<td>601 (± 117)</td>
<td>544 (± 114)</td>
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<td>910 (± 144)</td>
<td>843 (± 140)</td>
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<td>217 (± 28)</td>
<td>240 (± 27)</td>
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</table>

At present, the ability to investigate REFBcrp 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-REFPGP of 1.2 (white bars) and QTAP-REFPGP 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) Cmax in 100 virtual healthy individuals (Mean ± SD).

The QTAP-REFBCRP (2.2) led to a maximum 1.5-fold lower fa than the immunoblot-REFBCRP in the distal jejunum at the fastest SITT. The difference in fa between REFs diminishes as SITT increases (Figure 3A). Using the QTAP-REFBCRP, a maximum 1.2-fold lower Cmax was observed at the slowest SITT, with a considerable overlap in fa and Cmax 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 REFPGP could be used (0.1-5) to attain observed digoxin Cmax.
- 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 Cmax and fa are more relevant than the differences in REFBcrp of the currently available scalars.

References