In Vitro-In Vivo Extrapolation of Intestinal Transporter Activity using an Absolute Transporter Abundance Approach within a PBPK Framework

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Abstract

This study describes an IVIVE strategy for scaling *in vitro* transporter kinetic data 'per pmol of transporter' from a cell monolayer *via* intestinal absolute abundance within the Simcyp Simulator (V17). The mathematical approach is provided, with derivation of the parameters that the user requires as model inputs. For the test compound digoxin J_{max} and K_m values for intestinal apical efflux transporter P-glycoprotein (P-gp) are required. A comparison of digoxin PBPK model performance in the Simcyp Simulator is provided against an independent set of clinical studies using the relative intestinal scaling approach[1] compared to the newly implemented absolute approach.

Background

The *In Vitro-In Vivo* Extrapolation (IVIVE) of intestinal transporter-mediated drug clearance within Physiologically-Based Pharmacokinetic (PBPK) models has historically relied upon transporter expression data from relative quantification approaches (*e.g.*, PCR or immunoblotting).

Transporter absolute abundances have recently become available[2] and can be utilised in IVIVE-PBPK strategies. This study defines a strategy for scaling *in vitro* intestinal transporter activity data to *in vivo* by describing the IVIVE algorithms and scaling factors together with the necessary parameter inputs. The utility of scaling *via* an absolute approach for intestinal P-gp within an IVIVE-PBPK strategy using the P-gp probe digoxin is shown.

Methods

The two approaches to scale gut transporters activity by IVIVE in the Advanced Dissolution and Absorption and Metabolism (ADAM) model of the Simcyp Simulator (V17, Sheffield, UK) are:

Option 1 – The relative approach *via* an Relative Expression Factor (**REF**)
Option 2 – The absolute approach *via* an Inter-System-Extrapolation Factor for Transporters (**ISEF,T**).

Option 1 – REF - Apical efflux transporter in ADAM model

Eq. 1

$$P_{app,trans,n,i}\left(\frac{10^{-6}\text{cm}}{\text{s}}\right) = \frac{J_{max}\left(\frac{pmol}{min}\right)}{A\ (cm^2)\cdot\left((K_m\ (\mu M)\cdot fu_{inc}) + Cu_{ent,i}(\mu M)\right)}\cdot REF$$

Eq. 2

$$P_{eff,trans,n}\left(10^{-4}\frac{cm}{s}\right) = 10^{A\cdot \boldsymbol{logP_{app,trans}}+B}$$

Eq. 3

$$\text{Segmental } CL_{int,T}(L/h) = P_{eff}(10^{-4}\ cm/s)\cdot S_i(M^2)\cdot F_{n,i}$$

$P_{app,trans,n,i}$ is the *in vitro* transporter-mediated apparent permeability in the i^{th} gut segment, J_{max} is the maximal flux of a transporter isoform, A is the Transwell filter surface area (SA), K_m is the Michaelis constant, fu_{inc} is the unbound fraction in the incubation and $Cu_{ent,i}$ the unbound enterocyte concentration in the i^{th} gut segment. The unit-less *REF* accounts for *in vitro*-to-*in vivo* transporter expression or activity differences, $P_{eff,trans,n,i}$ is the effective transporter-mediated permeability, where A and B are the slope and intercept values derived from a log-log plot between $P_{eff,man}$ and P_{app}[3] (at *in vitro* apical pH 7.4/basolateral 7.4 across Caco-2 cell monolayers). S_i is the gut segmental cylindrical SA (m^2) and $F_{n,i}$ is the relative segmental transporter expression.

Option 2 – ISEF,T - Apical efflux transporter in ADAM model

Eq. 4

$$CL_{int,T,in\ vitro,n}\left(\frac{uL/min}{pmol}\right) = \frac{J_{max}\left(\frac{pmol/min}{pmol}\right)}{\left((K_m\ (\mu M)\cdot fu_{inc}) + Cu_{ent,i}\ (\mu M)\right)}\cdot ISEF,T$$

Eq. 5 – Proximal Jejunum

$$CL_{int,T}(\text{L/h}) = CL_{int,T,in\ vitro}\left(\frac{\text{uL/min}}{\text{pmol}}\right)\cdot\left((F'_{n,i-J1}\cdot Phen_{,n})\cdot TMeP_{,J1}\right)$$

$F'_{n,i-J1}$ is the proximal jejunum absolute transporter abundance (pmol/mg total membrane protein), the $TMeP_i$ is the total membrane protein in given segment (mg) and $Phen_{,n}$ is the phenotype specific abundance. For absolute (*ISEF,T*) scaling, the J_{max} input units are **per pmol of transporter**. Eq. 5 represents scaling apical efflux clearance (CL) to the ADAM model proximal jejunum segment, this is the reference segment in ADAM and all other segmental transporter CL are scaled relative to this segment according to the segment-specific transporter expression ($F_{n,i}$) and the $TMeP_i$. The unit-less *ISEF,T* accounts for *in vitro*-to-*in vivo* transporter expression and activity differences per pmol of transporter[4].

Methods (continued)

Option 2 – ISEF,T - Total Membrane Protein Scalar Derivation

Eq. 6

$$TMeP_{,i,Ind} = TMePPI\ (\text{mg})\cdot\left(\frac{SA_{,i,S.I,Ind}}{SA_{,i,S.I,Avg}}\right)\cdot\left(\frac{SA_{,i,Ind}}{SA_{,i,Avg}}\right)\cdot\frac{SA_{,i,Ind}}{SA_{,i,S.I,Ind}}$$

$TMeP_{,i,Ind}$ is the total membrane protein (mg) for a given small intestine (SI) segment in an individual (*Ind*), the *TMePPI* (mg) is the population mean total membrane protein yield for the entire SI, the $SA_{,i,S.I}$ is the entire SI surface area (m^2) and the $SA_{,i}$ is the Surface Area (m^2) of each individual's gut segment *versus* the average individual (*Avg*). For the colon total membrane protein (*TMePPC*), the *TMePPI* is replaced with the yield for the colon.

The corresponding equations are available for apical uptake transporters using relevant concentrations for Options 1 and 2.

The REF and ISEF,T scaling approach described herein were compared using the P-gp probe substrate digoxin[1] within the Simcyp Simulator V17. The key intestinal transporter inputs for model comparisons are given in **Table 1**.

Table 1. Key intestinal parameters used in the two digoxin PBPK models

Parameter	Value	Reference
J_{max} (**pmol/min/cm²**) – REF approach	434	5
J_{max} (**pmol/min/pmol P-gp**) – ISEF,T approach	517	5, 6
K_m (µM) – REF or ISEF,T approach	177	5
IVIVE scalar (unit less)	2 (REF) & 1 (ISEF,T)	7 & scaled value
P-gp-F'$_{n,i-J1}$ (pmol/mg total membrane protein)	0.4	8

The J_{max}-REF approach[5] was converted to *'per pmol of P-gp'* based on the scaled P-gp abundance in 21-day filter-grown Caco-2 cells (0.84 pmol P-gp/monolayer)[6], assuming the same activity *in vitro* and *in vivo,* and applied to J_{max}-ISEF, T (**Table 1**), as this data was unavailable in the original study[8].

Results and Conclusions

The simulated digoxin C_{max} (2.69 ± 0.53 ng/mL) using the ISEF,T approach compared favourably with observed C_{max} (range 1.39-3.0 ng/mL, *n*=10 studies, 0.5 mg single dose) and the simulated REF approach (C_{max} 1.82 ± 0.59) (**Fig. 1**).

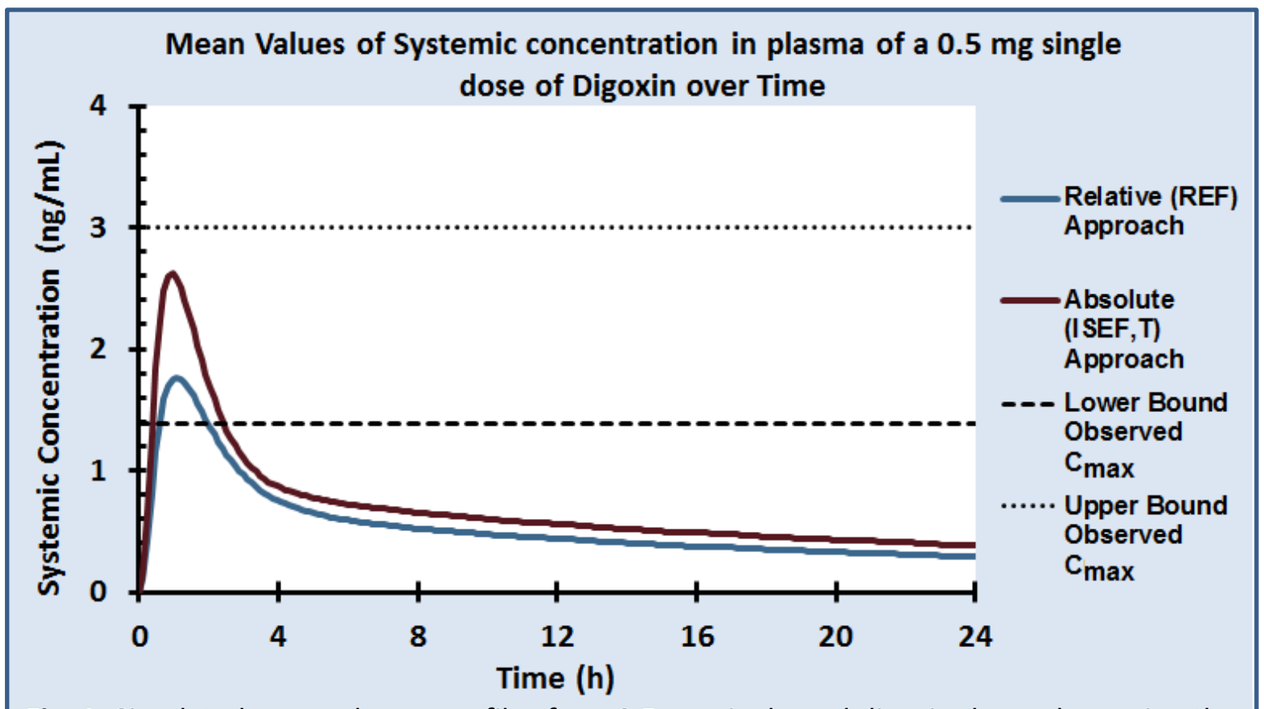

Fig. 1. Simulated mean plasma profiles for a 0.5 mg single oral digoxin dose when using the REF (—) or ISEF,T (—) approach for gut P-gp scaling in 100 virtual healthy volunteers. The range of observed C_{max} from clinical studies at this dose is given between the dotted and the dashed lines.

This is the first reported study to describe the approach and utility of IVIVE scaling of intestinal transport proteins *via* absolute transporter abundances within a PBPK model. This approach offers a mechanistic means to reveal the significance of intestinal transporter activity on drug absorption.

References

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