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Transporter-based *in vitro-in vivo* extrapolation (IVIVE)

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Pharmaceuticals

Abstract:

The use of *in vitro* data to predict the pharmacokinetics (PK) of drugs whose disposition is mediated by transporters is complicated due to unknown transporter expression levels in individual tissues both *in vivo* as well as in various *in vitro* cell culture systems. The contribution of passive diffusion to drug transfer between extracellular and intracellular space in individual tissues is another important aspect to consider for drugs with low permeability and slow diffusion through cellular membranes. The permeability-surface area product (*PStc*) is commonly used to describe the rate of passive diffusion through membranes. Estimation of *PStc* values for different tissues incorporated in PBPK models is not well-established due to unknown physiological aspects of individual tissues, e.g., cell surface areas. We propose a new method for describing the passive diffusion in different tissues by scaling the *PStc* values to tissue cell volumes. The method was further extended to estimate the contribution of passive and carrier-mediated transport *in vivo* from *in vitro* measurements. Valsartan was used as a model compound with distribution and clearance dependent on transporter activity. GastroPlus™ 7.0 with its PBPKPlus™ Module (Simulations Plus, Inc., Lancaster, CA) was used to simulate plasma concentration-time (Cp-time) profiles utilizing physiologically based pharmacokinetic (PBPK) models based on both animal and human physiologies. Drug partitioning into the extracellular space was described by an extracellular-water:plasma partition coefficient [1], taking into account binding of drug to plasma and extracellular tissue proteins. Carrier-mediated transport kinetics in liver was estimated from previously reported *in vitro* parameters measured in cultured hepatocytes [2]. Passive diffusion between the extracellular and intracellular spaces in liver was described by *PStc*, which was also estimated from previously reported values measured *in vitro* [2]. Passive diffusion in other tissues was described by *PStc* values scaled from the liver *PStc* according to individual tissue volumes. Simulations using a combination of kinetic parameters from the relevant *in vitro* system (cultured hepatocytes) with physiologically based scaling of the passive diffusion rate across all tissues resulted in very good prediction of total valsartan exposure and provided the correct shape of the predicted Cp-time profile in rat and human based on *in silico* and *in vitro* parameters. This method is a promising tool for prediction of the pharmacokinetics of drugs whose disposition cannot be described by well-stirred tissue models, and it expands the predictive capabilities of PBPK modeling approaches for prediction of pharmacokinetics based on *in vitro* data to a wider range of compounds.

A previously reported method [2] for prediction of PK of compounds whose hepatic elimination is transporter-dependent utilized a PBPK model with permeability-limited liver tissue, but assumed perfusion-limited (well-stirred) models for all remaining tissues. The predicted Cp-time profiles were within the range of observed values, but the profile shape was not captured correctly (Figure 1) and required additional scaling factors.

Table 1. Previously reported *in vivo* kinetic parameters for liver uptake of valsartan extrapolated from *in vitro* measurements in cultured rat and human hepatocytes [2].

	rat	human
Km [ug/mL]	12.4	19.3
Vmax [mg/s]	0.0126	0.241
PStc [mL/s]*	0.0268	1.322

** PStc estimate is assumed to account for sinusoidal surface area*

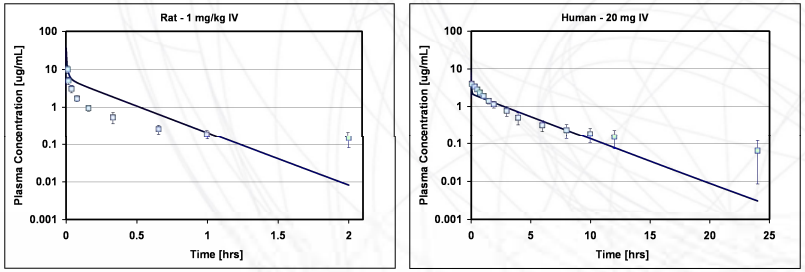


Figure 1. Plasma concentration-time profiles in rat (left) and human (right) predicted by a PBPK model with liver treated as permeability-limited tissue and all remaining tissues treated as perfusion-limited tissues (well-stirred tissue model). Observed profiles (points) are shown for comparison

Methods:

Our proposed method expands the previously reported method [2] by extrapolation of permeability-limited drug distribution into all tissues. It relies on the mechanistic analysis of *in vitro* measurements to obtain values of kinetic parameters for drug interactions with transporters as well as for passive diffusion through cell membranes.
In the new method, all tissues are treated as permeability-limited tissues (if the passive diffusion through the cell membrane is slow in liver tissue, it may be a limiting factor in all other tissues as well) and the *PStc* values for all tissues are estimated from liver *PStc* value as described below.

For a single cell, a general relationship between cell volume and surface area can be written as:

$$SA_{Cell} = k \times Vol_{Cell}$$

Assuming that all cells in a tissue have similar shape, the relationship can be extended to total surface area and volume of all tissue cells:

$$SA_{Tissue} = k \times Vol_{TissueCells}$$

The calculation for permeability-surface area product then becomes:

$$PStc_{Tissue} = Perm \times k \times Vol_{TissueCells}$$

$$PStc_{Tissue} = SpecPStc \times Vol_{TissueCells}$$

SA_{Cell}, SA_{Tissue} – surface area of cell and tissue membranes
Vol_{Cell}, $Vol_{TissueCells}$ – volume of one cell and all tissue cells
k – *SA* to *Vol* scaling factor
$Perm$ – permeability

Where *SpecPStc* - "*Specific PStc*" or "*PStc per mL of cell volume*" – is constant across all tissues under assumptions that:

- Average cell sizes in all tissues can be treated as the same, so the same scaling factor, k, between the cell volume and the surface area can be used for all tissues
- Composition of membranes is similar enough across all tissues to allow using similar passive permeability through membranes in all tissues

Liver $PStc_{Tissue}$ was obtained from *in vitro* data as reported previously [2] in rat and human hepatocytes and was used to calculate *SpecPStc*.

Hepatocyte surface area consists of three distinct interfaces, sinusoidal, lateral, and bile canalicular. Two functional interfaces, sinusoidal and bile canalicular, are important in the calculation of valsartan uptake into hepatocytes and secretion into the bile. In the calculation of *SpecPStc*, we assumed that the liver $PStc_{Tissue}$ estimated from *in vitro* data accounts only for sinusoidal membrane surface area.

Contributions of sinusoidal and bile canalicular interfaces to the total surface area of hepatocytes were previously reported as 72% and 13% [3-4], or 47% and 23% [5], respectively. Both sets of reported estimates were used in calculation of *SpecPStc* (Table 2) and subsequent calculation of $PStc_{Tissue}$ values for all remaining tissues.

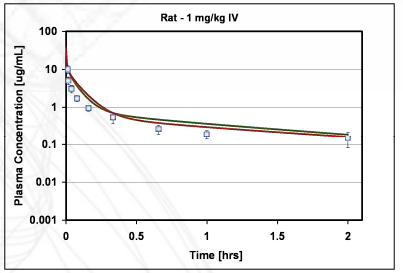
Table 2. *SpecPStc* values for rat and human calculated from their respective liver *PStc* values (Table 1), assuming various contributions of sinusoidal interface to total hepatocyte surface area

	***SpecPStc* [mL/s/mL cell volume]**	
Sinusoidal interface	**rat**	**human**
72%	4.30×10^{-3}	1.55×10^{-3}
47%	6.59×10^{-3}	2.37×10^{-3}

References:

1. Poulin P., J Pharm Sci 2002, 91: 129-156
2. Poirier A., J Pharmacokinet Pharmacodyn 2009, 36: 585-611
3. Weibel ER., J Cell Biol 1969, 42: 68-91
4. Blouin A., J Cell Biol 1977, 72: 441-455
5. Hubbard AL., J Cell Biol 1983, 96: 217-229

Results:



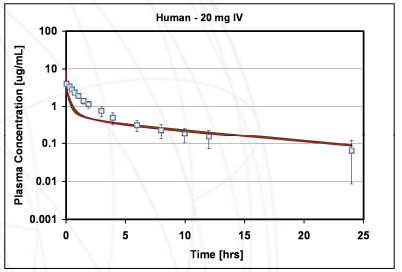


Figure 2. Plasma concentration-time profiles in rat (top) and human (bottom) predicted by a PBPK model with all tissues treated as permeability-limited where *PStc* values for individual tissues were estimated by scaling liver *PStc* as described under the Methods section. Red and green lines are showing predictions obtained assuming that sinusoidal surface area represents 47% and 72% of total hepatocyte surface area, respectively. Observed profiles (points) are shown for comparison

Conclusions:

- New proposed method resulted in considerably improved prediction of valsartan pharmacokinetics
- The predicted profiles reasonably matched total exposure as well as shape of the Cp-time profiles without additional scaling factors that would need to be obtained by fitting to *in vivo* data
- Further validation of the method will be done using data for other compounds whose hepatic clearance is dependent on transporter uptake

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