The MechP model - Mechanistic modelling of *in-vitro* bidirectional permeability studies and *in vivo* absorption of metoprolol

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Background

Bidirectional transport assays can be used to obtain *in vitro* permeability estimates for use in Physiologically based pharmacokinetic (PBPK) models.

Assumptions made by the conventional analysis:

- Sink conditions are maintained → difficult to achieve experimentally, especially for highly permeable compounds → underestimated passive permeability
- No significant impact of the unstirred water layer (UWL) → the diffusion across the unstirred water layer can be the limiting factor for highly permeable compounds

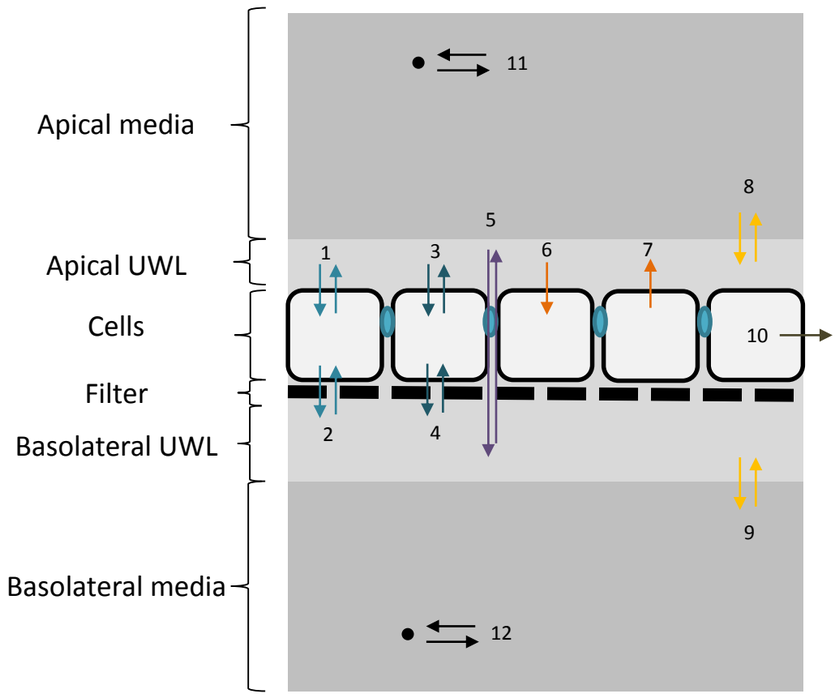
We developed a model that mechanistically describes the *in-vitro* permeability across Caco-2 cells for metoprolol, a highly permeable drug. The impacts of ionisation and UWL on the permeability were investigated.

Methods

In-vitro assay:

Data for the bidirectional transport of metoprolol across Caco-2 cell monolayers were previously generated [1]. Briefly, Caco-2 cells were seeded at a density of 1 x 10^5 cells/well onto 12-well Transwell® inserts and grown for 23±1 days prior to permeability experiments. Experiments were performed at 37°C, with apical and basolateral volumes of 0.5 and 1.5 mL, respectively, and stirred at 450 rpm (calibrated plate shaker BMG LabTechnologies GmbH, Offenburg, Germany). The basolateral compartment was buffered to a pH of 7.4; whereas a range of buffer pH values was investigate in the apical compartment (pH 5, 5.5, 6, 6.5, 7, 7.4, 7.7 and 8).

Figure 1: Structure of the Mechanistic permeability (MechP) model



1- Apical transcellular permeability of the non ionised form
2- Basolateral transcellular permeability of the non ionised form
3- Apical transcellular permeability of the ionised form
4- Basolateral transcellular permeability of the ionised form
5- Paracellular pathway
6- Uptake transporter (apical)
7- Efflux transporter (apical)
8- Apical UWL permeability
9- Basolateral UWL permeability
10- Metabolism
11- Apical protein binding
12- Basolateral protein binding

Data analysis (modelling):

A mechanistic model was developed in R software (version 3.3.1) and included 5 compartments, representing apical and basolateral bulk media and unstirred water layers in addition to the cell monolayer. The amount removed from the receiver well upon sampling was accounted for dynamically within the model.

The fraction ionised was calculated in each compartment and for each experiment based on the drug pKa and media pH values. An intracellular pH of 7 was assumed.

The total UWL thickness was predicted on the basis of stirring rate using data from Adson *et al.* [2].

The transcellular permeability of the ionised form ($P_{trans,i}$) was calculated using the permeability of the neutral form ($P_{trans,0}$) and an ionisation scalar describing the log decrease in permeability for cationic metoprolol compared to the neutral species. This scalar and $P_{trans,0}$ were estimated.

Simulations:

The permeability estimates obtained were implemented in the metoprolol compound file in the Simcyp Simulator v17. The Mechanistic passive regional permeability predictor (MechPeff) model was used to predict the effective permeability observed in human ($P_{eff,man}$). This $P_{eff,man}$ was used to predict the absorption using a first-order model and the Advanced Dissolution, Absorption and Metabolism (ADAM) model. Plasma concentration-time profiles of metoprolol after a single oral dose of 100 mg in CYP2D6 extensive metabolisers were simulated for 10 trials of 16 female subjects 18 – 40 years and compared to observed data from Sharma *et al.* [3] (Figure 5).

Results

The *in vitro* model was able to describe the decrease in metoprolol permeability with an increase in ionisation (figure 2 and 3).

Figure 2: Basolateral to apical experiments

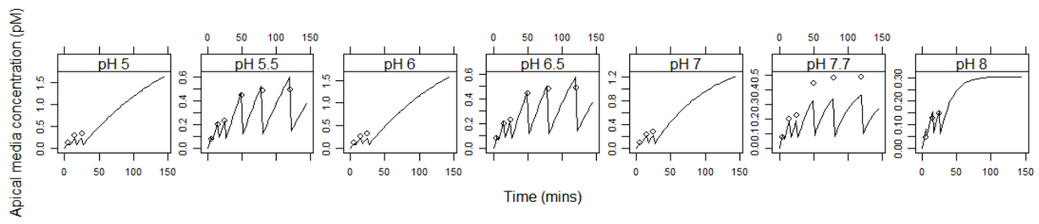


Figure 3: Apical to basolateral experiments

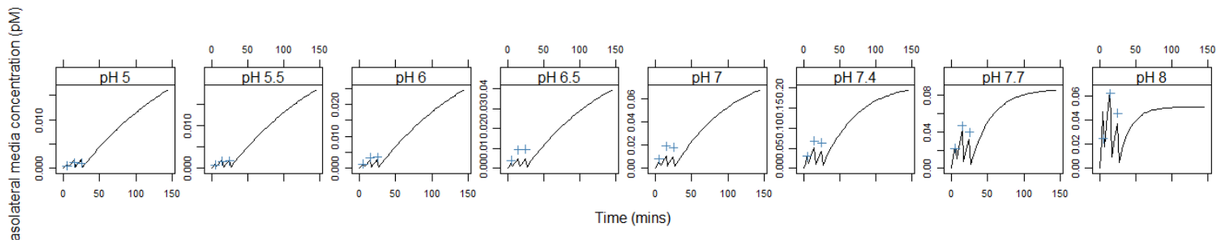
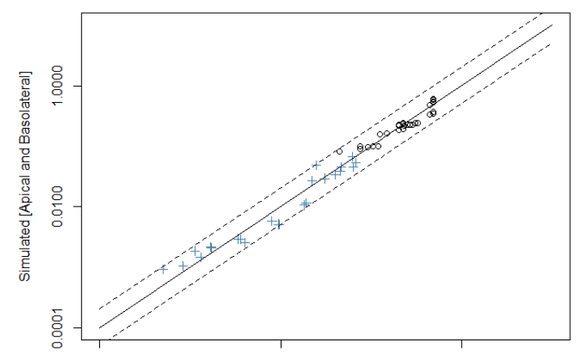


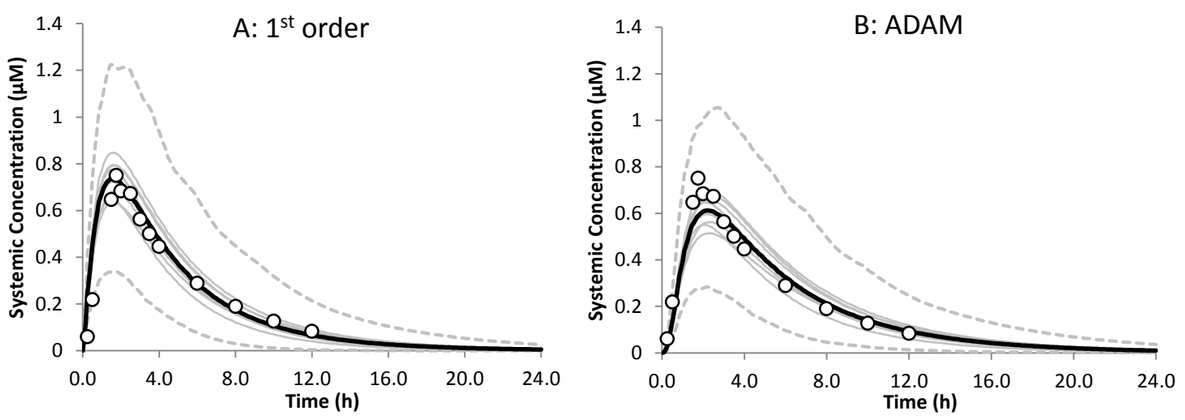
Figure 4: Simulated vs observed concentrations



The difference between the observed and predicted *in-vitro* concentrations was less 2-fold (figure 4). The geometric mean fold error (GMFE) was 1.27 and the geometric fold bias (GMFB) was 1.02.

The $P_{trans,0}$ estimate of 40000 x 10^{-6} cm/s and ionisation scalar of 3.4 predicted an $P_{eff,man}$ in the jejunum I of 2.95 x 10^{-4} cm/s when applied in the metoprolol PBPK model (figure 5).

Figure 5: In-vivo PK profile



Model	1st order	ADAM								
		Overall	Duodenum	Jejunum I	Jejunum II	Ileum I	Ileum II	Ileum III	Ileum IV	Colon
Predicted fa	0.95	0.92	0.08	0.39	0.18	0.06	0.05	0.04	0.03	0.1
Predicted ka	1.31 h^{-1}									

Simulated (black line) and observed (data points) mean plasma concentration-time profile of metoprolol after a single oral dose of 100 mg in CYP2D6 extensive metabolizer using a first-order model and ADAM model (**A** and **B**, respectively). The grey lines represent the predictions from 10 trials of 16 female subjects 18 – 35 years (Sharma *et al.* 2005).

Discussion

When *in vitro* permeability estimates were applied in the metoprolol PBPK model, the predicted *in-vivo* absorption was in accordance with clinical data, indicating that this approach could be used to generate robust inputs for PBPK models from the in-vitro Caco-2 cell model. This "MechP" model will be implemented in Simcyp® In Vitro Data Analysis (SIVA) toolkit.

References

1. Neuhoff S, Ungell A-L, Zamora I, Artursson P. Pharm Res. 2003 Aug;20(8):1141–8.
2. Adson A, Burton PS, Raub TJ, Barsuhn CL, Audus KL, Ho NF. J Pharm Sci. 1995 Oct;84(10):1197–204.
3. Sharma A, Pibarot P, Pilote S, Dumesnil JG, Arsenault M, Bélanger PM, Meibohm B, Hamelin BA.. J Pharmacol Exp Ther. 2005 Jun 1;313(3):1172–81.

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