Transporter inhibition: modelling *in-vitro* Transwell assays

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Background

Transporter inhibition can have an impact on the **disposition of a drug as well as on its safety and efficacy.** Being able to have reliable estimates of inhibition parameters for use in Physiologically based pharmacokinetic (PBPK) models is key to evaluate the drug-drug interaction (DDI) potential.

Assumptions made by the conventional analysis are:

- Sink conditions are maintained → difficult to achieve experimentally, especially for highly permeable compounds → underestimated passive permeability
- Driving concentration for the transporter inhibition is the nominal concentration → for efflux transporters the intracellular concentration or the membrane concentration is relevant

It has been shown for the substrates that using **modelling** to estimate the intracellular concentration **decreases the inter-laboratory variability** and tends to give **lower and more consistent Km estimates** [1]. The similar conclusions were recently made for inhibition parameters [2] and could explain the overestimation of Ki values frequently observed.

We developed a model that mechanistically describes the efflux transport across Caco-2 cells for digoxin and quinidine, two P-gp substrates. The Ki_{P-gp} value for quinidine was also estimate using the *in-vitro* drug-drug interaction (DDI) with digoxin.

Methods

In-vitro assays

Data for the bidirectional transport of quinidine and digoxin across Caco-2 monolayers were previously generated [3]. 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 was stirred at 450 rpm (calibrated plate shaker (BMG LabTechnologies GmbH, Offenburg, Germany). The basolateral and apical compartment were buffered to a pH of 7.4.

Table 1: protocol

Assay	Concentrations	Sampling time
Digoxin alone	**0.059, 1, 10, 100 µM**.	5,15,25,50,80, and 120 min
Quinidine alone	**0.001, 0.05, 1, 10, 100 µM**	
Digoxin + Quinidine	**0.059/100, 0.059/10, 0.02/50 µM**	5, 15, 25, and 50 min

Sampling of (Apical to basolateral) A-B experiments was conducted by moving the Transwell insert to a new well containing blank buffer and retaining the previous well, thereby representing complete removal of drug from basolateral buffer. Sampling of (basolateral to apical) B-A experiments was conducted by removal of 400 µl of apical buffer and replacement with an equal volume of blank buffer.

Data analysis:

A mechanistic model was developed in R software (version 3.5.1) and included **3 compartments**, representing apical and basolateral media in addition to the cell monolayer **for the substrate and the inhibitor**. No assumption about sink conditions was done and the passive diffusion (CL_{PD}) was estimated.

The **driving concentration for P-gp as well as the perpetrating concentration for P-gp inhibition was assumed to be the intracellular concentration.**

The **impact of sampling** on the concentrations measured was accounted for in the model.

Results

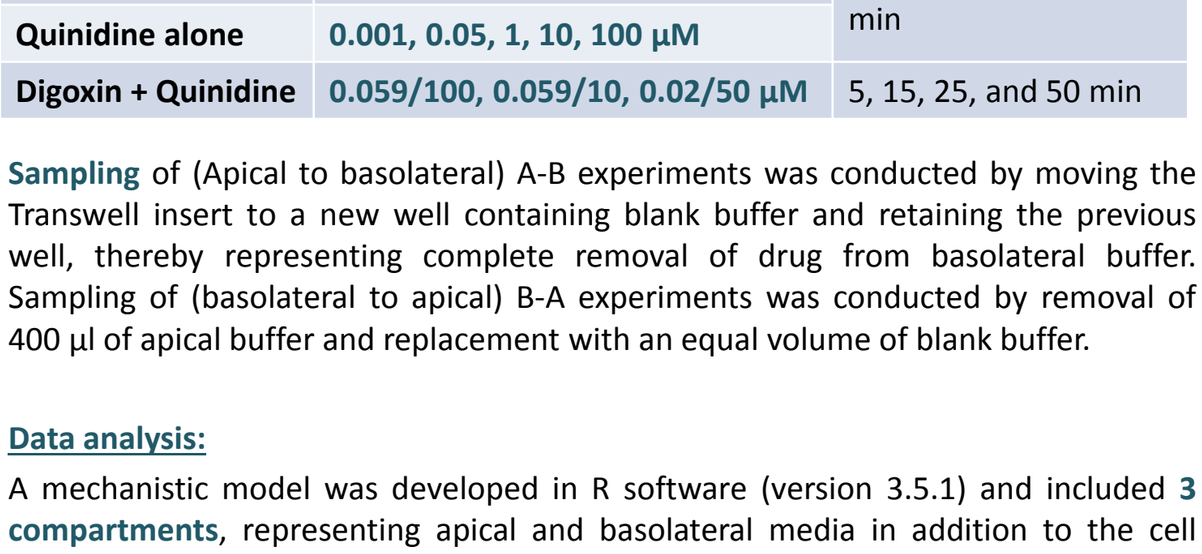

Figure 1: Bidirectional transport of digoxin

Results

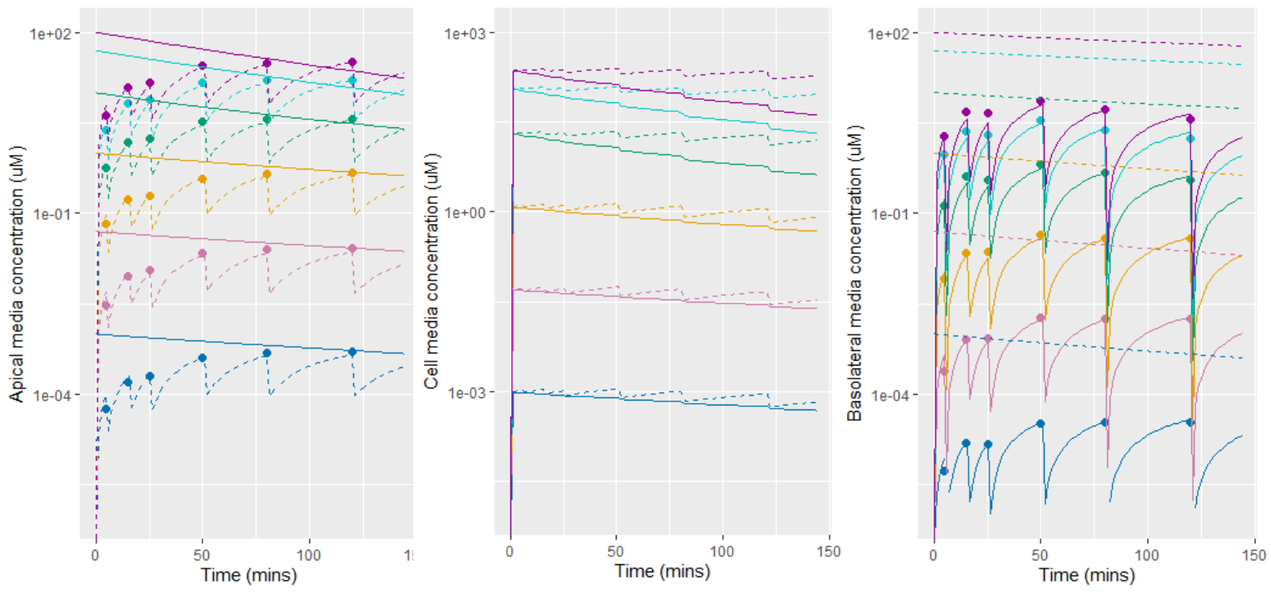

Figure 2: Bidirectional transport of quinidine

The model was able to describe the disposition of digoxin and quinidine alone (Fig 1 and 2). The geometric mean fold error (GMFE) between observed and model predicted digoxin concentrations was 1.29 and the geometric fold bias (GMFB) was 1.15. For quinidine, the GMFE was 1.16 and GMFB was 1.002.

Table 2: Results

Drug	Km (µM) (RSE%)	J_{max} (pmol/min) (RSE%)	CL_{PD} (10^{-6} cm/sec) (RSE%)
Digoxin	**18** (41%)	**253** (34%)	**41** (37%)
Quinidine	**0.278** (44%)	**11.3** (37%)	**201** (6%)

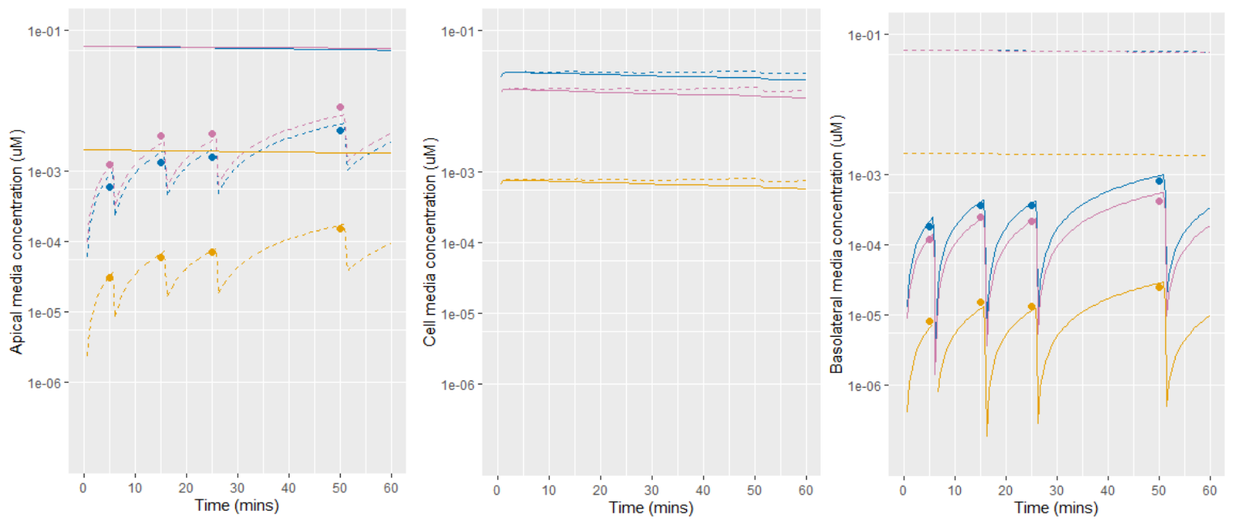

Figure 3: Bidirectional transport of digoxin in presence of quinidine

Once the disposition of the substrate and inhibitor alone were known digoxin disposition in presence of quinidine was fitted (Fig 3). The model was able to describe accurately the observed data with a GMFE of 1.18 and GMFB of 1.06. The **Ki** was estimated to **3.45 µM** (RSE%: 21%).

Conclusions

- The model was able to estimate J_{max}, Km, and CL_{PD} for digoxin and quinidine with reasonable accuracy.
- With only 3 experiment the present data set would have not allowed to estimate a Ki_{P-gp} value using the conventional approach, however we were able to estimate with good precisiona Ki_{P-gp} value for quinidine.
- This model will be available in SIVA 4

SIVA Toolkit

References

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3. Neuhoff S, Ungell A-L, Zamora I, Artursson P. pH-dependent bidirectional transport of weakly basic drugs across Caco-2 monolayers: implications for drug-drug interactions. Pharm Res. **2003** Aug;20(8):1141–8.

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