Challenges in the modelling of bidirectional transport experiments

Burt HJ[1], Harwood MD[1,2], Neuhoff S[1], Machavaram KK[1], Cain T[1], Rose R[1], Feng K[1], Wedagedera J[1], Patel N[1], Warhurst G[2], Barter ZE[1], Gardner I[1], Jamei M[1] and Rostami-Hodjegan A[1,3]

(1) Simcyp (A Certara Company), Sheffield, UK; (2) Gut Barrier Group, School of Translational Medicine, University of Manchester, Salford Royal Hospital NHS Trust, Salford, UK ; (3) Centre for Applied Pharmacokinetic Research, School of Pharmaceutical Sciences, University of Manchester, UK

Introduction

There is an increasing demand for modelling and simulation techniques to be applied to the analysis of *in vitro* pharmacokinetic experiments. For example, the International Transporter Consortium has recently advocated the use of models which account for multiple physical spaces within drug transport experiments[1]. In the current study, the impact of several considerations when modelling *in vitro* bidirectional transport data from Caco-2 cells was investigated using the model compound vinblastine, a P-glycoprotein (P-gp) substrate with negligible paracellular permeability.

Aims

- To simultaneously fit vinblastine concentration data generated at multiple concentrations and time points and in both apical-to-basolateral (A>B) and basolateral-to-apical (B>A) transport directions.
- To investigate the use of three-compartment models with different parameterisations to fit the data.
- To investigate the impact of accounting for dilution upon sampling and different parameter estimation methods on final parameter estimates.

Methods

Bidirectional Transport Studies:

Caco-2 cells between passages 25 to 35 were seeded at a density of 2.2 x 10^5 cells/cm^2 onto Transwell inserts (#3401) and grown for 21 days prior to transport experiments. Transport experiments were performed at 37°C using HBSS-MES (pH 6.5) and HBSS-HEPES (pH 7.4) buffer at volumes of 0.4 and 1.2 mL in apical and basolateral compartments, respectively.

Bidirectional transport studies (n ≥3 filters) were initiated by adding [^{3}H]-vinblastine to donor buffer. This was performed at 9 apical donor concentrations ranging from 0.03 to 1000 µM and 7 basolateral donor concentrations ranging from 0.03 to 250 µM. At 5 time points up to 1 hour, 200 µL of receiver buffer was sampled and replaced with an equal volume of blank buffer. [^{3}H]-vinblastine concentrations in the sampled buffer were determined by scintillation counting.

Bidirectional transport studies (n = 6 filters) were also performed in the presence of the P-glycoprotein inhibitor verapamil at 100 µM in both donor and receiver buffers. In this case [^{3}H]-vinblastine was added at a single donor concentration of 0.03 µM at time-zero, with the same sampling and analysis procedure as described earlier.

Data analysis:

Bidirectional transport studies were analysed by simultaneous fitting of vinblastine concentrations in apical and basolateral compartments using R (version 3.1.0, R Foundation for Statistical Computing). Fitting was attempted using the mean data from all replicates and with two models, both of which had a base structure of three-compartments. As the molecular radius of vinblastine (~10 Å) is greater than the Caco-2 pore radius (~4- 7 Å), paracellular permeability was assumed to be negligible and was not considered in either model.

In Model 1 (Figure 1) it was assumed that only unbound and unionised drug was able to passively permeate apical and basolateral membranes (P_{ap} and P_{bl}, respectively) and P-gp mediated apical efflux was assumed to act on unbound drug regardless of ionisation. The fraction of vinblastine (a diprotic base) unionised (f_{ui}) was calculated using standard Henderson-Hasselbalch equations applying pKa's of 5.4 and 7.57 and applying a pH of 6.5, 7.4 and 7.4 for apical, cell and basolateral compartments[2]. Cell volume (V_{cell}) was calculated from cell protein content and a scaling factor of 3.65 µL/mg protein[3].

In a 'reduced' Model 2, fu and f_{ui} in Model 1 were fixed to 1 in all compartments. In this case, any inter-compartment differences in binding and ionisation were subsumed into the estimates of P_{ap} , P_{bl} and $CL_{int,P\text{-}gp}$ in the model, meaning these were no longer designated as drug-specific parameters.

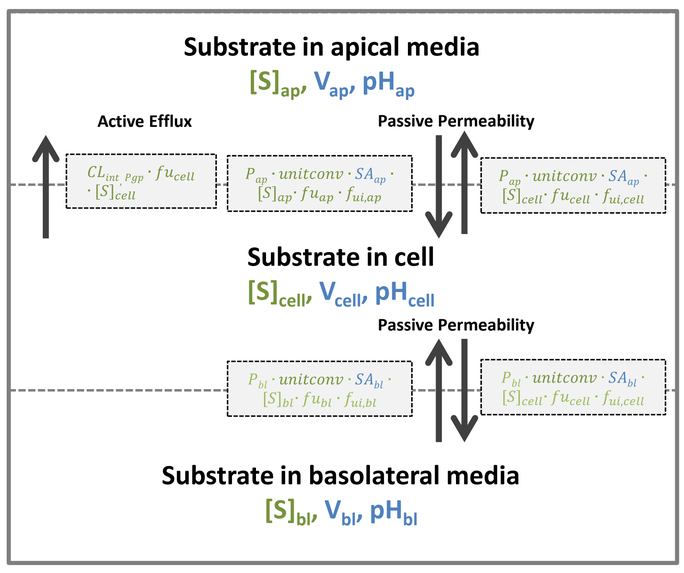

Figure 1. Model 1 used to fit vinblastine concentration data. System-specific parameters are coloured blue, drug-specific parameters are coloured green.
Where:
[S] = Substrate (vinblastine) concentration (µM)
P = Passive permeability (10^{-4} cm/sec)
$CL_{int,P\text{-}gp}$ = P-gp mediated efflux (µL/min)
fu = Fraction unbound
f_{ui} = Fraction unionised
SA = Surface area (cm^2)
V = Volume (µL)
unitconv = Unit conversion for P (60/10)
Subscripts ap, bl and cell, refer to apical, basolateral and cell values, respectively

Models were initially fit to vinblastine concentration data in the presence of verapamil (10 mean data points), in which case P-gp mediated apical efflux was assumed to be completely inhibited ($CL_{int,P\text{-}gp}$ fixed to zero) and P_{ap}, P_{bl} and fu (Model 1 only) were determined by regression. Following this, the estimated P_{ap} was fixed and the same model was to fit to all experiments in the absence of verapamil (80 mean data points) to obtain estimates of $CL_{int,P\text{-}gp}$, P_{bl} and fu (Model 1 only).

Initial fitting was performed using a basolateral surface area (SA_{bl}) that was 3-fold larger than the apical surface area[4]. In addition, a weighted least squares (WLS) estimation method was applied ($1/Y_{pred}^2$).

The impact of applying equal apical and basolateral surface areas and the use of ordinary least squares (OLS) and extended least-squares[5] (ELS, power variance model) parameter estimation methods were also investigated.

Results

- Using Model 1, it was not possible to obtain both a reasonable fit to the current set of vinblastine apical and basolateral concentration data and robust estimates for P_{ap}, P_{bl}, $CL_{int,P\text{-}gp}$ and fu (<30% CV).
- Reasonable fitting of vinblastine concentration data was possible in a limited case of Model 1 where $P_{ap} = P_{bl}$ and $fu_{cell} = 0.01$ (~fu_{plasma}). In this case, estimates of P, fu_{ap}, fu_{bl} and $CL_{int,P\text{-}gp}$ were obtained but parameter CV estimates were very large (Table 1).
- Using the 'reduced' Model 2 it was possible to obtain a good fit to vinblastine apical and basolateral concentration data (Figure 2) and reasonable parameter estimates for P_{bl} and $CL_{int,P\text{-}gp}$ (Table 1), although the CV for the P_{ap} estimate was relatively high (66%).

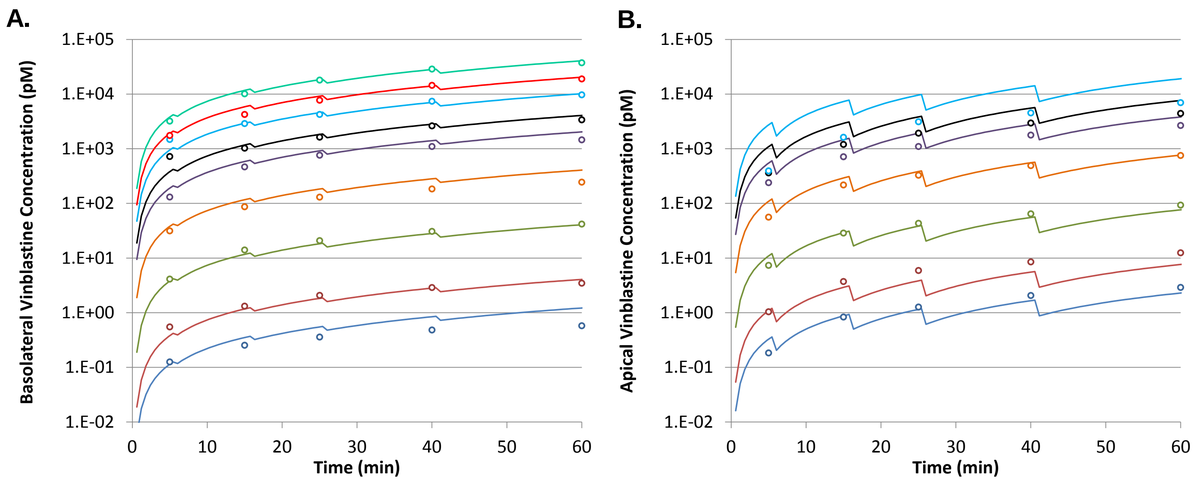

Figure 2. Mean observed and model predicted vinblastine concentrations from (A) A>B experiments and (B) B>A experiments following simultaneous fitting of all data using Model 2.

Model	Parameter	Units	Estimate	CV (%)
1	P_{ap}, P_{bl}	10^{-4} cm/sec	0.0835	>500%
	fu_{ap}	-	0.129	>500%
	fu_{bl}	-	0.00180	>500%
	$CL_{int,P\text{-}gp}$	µL/min	2.64	>500%
2	P_{ap}	10^{-4} cm/sec	0.0952	66.4
	P_{bl}	10^{-4} cm/sec	$1.66x10^{-4}$	7.6
	$CL_{int,P\text{-}gp}$	µL/min	1.45	12.8

Table 1. Estimated parameters following simultaneous fitting of A>B and B>A vinblastine experiments.

- Repeating the fitting procedure with Model 2 but applying equal apical and basolateral surface areas rather than a 3-fold higher in the case of basolateral, resulted in poorer fit of B>A experiments (Figure 3).

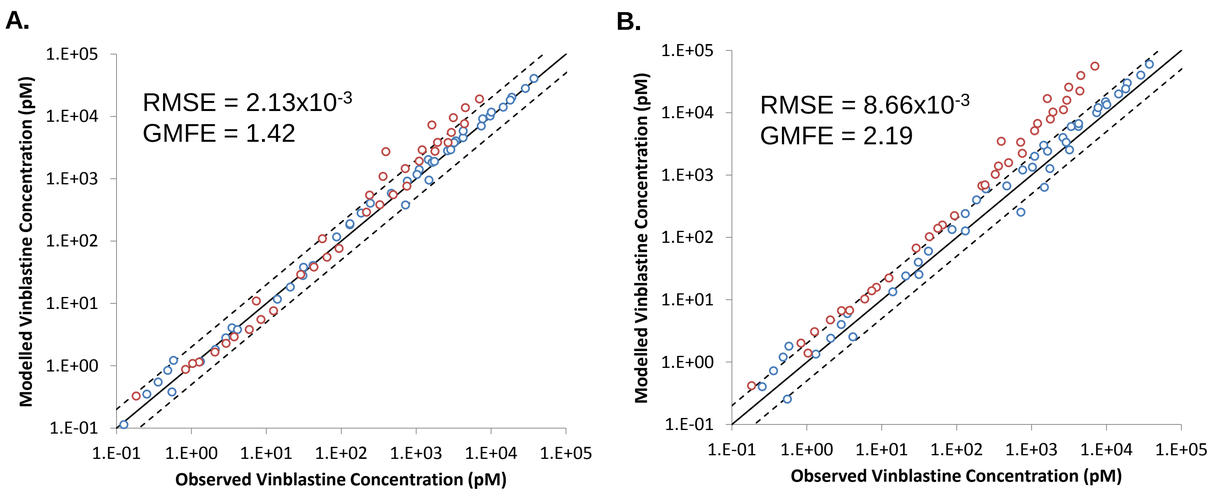

Figure 3. Mean observed and model predicted vinblastine concentrations following simultaneous fitting of A>B (blue points) and B>A (red points) experiments using Model 2 and assuming (A) a 3:1 basolateral/apical surface area ratio or (B) equal basolateral and apical surface areas. The solid line represents unity and dashed lines represent 2-fold either side of unity.

- Repeating the fitting procedure with Model 2 but applying different parameter estimation methods had a significant impact on the final estimate for active efflux, root mean squared error (RMSE) and geometric mean fold error (GMFE). This is likely due to the large range in observed data (more than 7 orders of magnitude).

Estimation Method	$CL_{int,P\text{-}gp}$ (µL/min)	CV (%)	RMSE	GMFE
WLS	1.45	12.8	$2.12x10^{-3}$	1.42
OLS	0.183	20.9	$5.13x10^{-4}$	1.56
ELS	0.377	18.9	$5.86x10^{-4}$	1.46

Table 2. Estimated $CL_{int,P\text{-}gp}$ and residual error following simultaneous fitting of A>B and B>A vinblastine experiments using the reduced Model 2 and different parameter estimation methods.

Conclusion

- The current study highlights the difficulty in obtaining drug and system-specific parameters (*e.g.* Model 1) when fitting raw data from bidirectional transport assays.
- Incorporation of asymmetrical apical and basolateral surface areas from an external source improved the fitting of vinblastine transport data.
- Given the wide range of observed data, the choice of parameter estimation method was found to significantly affect parameter estimates.

References

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