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MembranePlus™: A Tool to Study *In Vitro*/*In Vivo* Transport and Drug-drug Interaction

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PURPOSE

To develop a mechanistic mathematical model for analysis of *in vitro* permeability assays that accounts for all mechanisms contributing to observed apparent permeability: passive paracellular and transcellular diffusion, carrier-mediated transport, as well as drug accumulation in membranes and some intracellular compartments (e.g., lysosomes). The model was validated by analyzing the effect of competitive inhibition of P-gp by vinblastine on the apparent permeability of indinavir in Caco-2 monolayers.

METHODS

MembranePlus™ (Simulations Plus, Inc.) was used to analyze the concentration-time profiles in donor and receiver compartments after apical and basolateral administration of 50 µg/mL (81.5 µM) indinavir alone and co-administration of 50 µg/mL (81.5 µM) indinavir with 70 µM vinblastine [1]. The physicochemical properties of indinavir and vinblastine were predicted by ADMET Predictor™ 6.0 (Simulations Plus, Inc.). The contribution of paracellular diffusion was estimated from drug properties and the experimental setup. Carrier-mediated transport was modeled with Michaelis-Menten kinetics. The indinavir P-gp Vmax/Km ratio, along with parameters accounting for passive transcellular diffusion and membrane accumulation were fitted to the indinavir-alone data. This basic model was then applied to simulate the inhibition of P-gp by vinblastine by fitting the Vmax/Km ratio for indinavir with competitive inhibition of P-gp by vinblastine. The model also includes various effects of major experiment-related parameters (e.g, shaking rate, solvent pH, filter support and sampling effects).

1. Compound Property

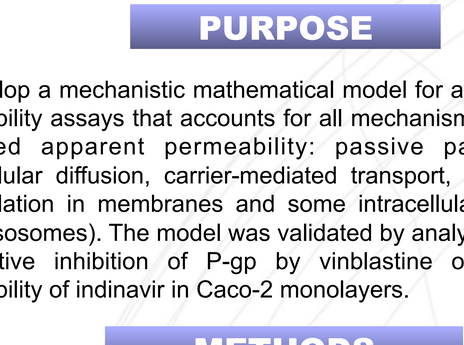


Figure 2. MembranePlus compound tab. The program allows two ways of accounting for passive diffusion through cell membranes: (1) S+ model as listed in Eqn. 1 and 2; and (2) direct transport parameter entries for all neutral and charged species.

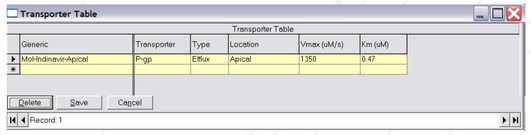
The membrane entrance and exit rates are given by Eqn. 1 and 2, as modified from [2] and [3]

$$k_i = \frac{\gamma P_{mem}}{\delta P_{mem} + 1}, k_o = \frac{\gamma}{\delta P_{mem} + 1} \quad \text{Eqn. 1}$$

$$P_{mem} = c(P_{oct})^m \quad \text{Eqn. 2}$$

2. Transporter Setup

A P-gp efflux transporter is set up in the program transporter table.



3. Experimental Setup

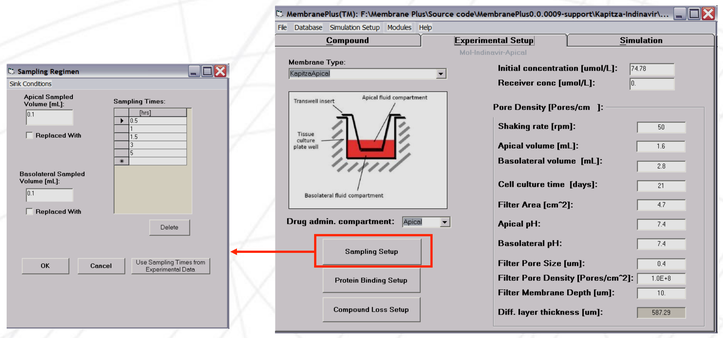


Figure 3. MembranePlus Experimental Setup tab. The program considers major experiment-related parameters, such as shaking rate, apical volume, basolateral volume, filter area, and filter support permeability and etc. As in [1], 0.1 mL samples were drawn from both and basolateral chambers at 0.5, 1, 1.5, 3 and 5 hours.

4. Paracellular and filter permeability

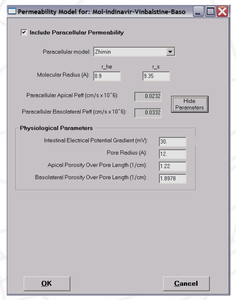
Both paracellular and filter permeabilities are accounted for in the program. The default paracellular model is the Zhimin model [4], which accounts for the molecule mean projected radius and the hydrodynamically equivalent sphere radius. The filter permeability calculation was adopted from [5]. Therefore, the effective paracellular permeability is given by

$$\frac{1}{P_{para}^{eff}} = \frac{1}{P_{para}} + \frac{1}{P_{Filter}} \Rightarrow P_{para}^{eff} = \frac{P_{para} P_{Filter}}{P_{para} + P_{Filter}}$$

where

$$P_{Filter} = \frac{\varepsilon_F * D * F(r/R_F)}{h_F},$$

$F(x) \rightarrow$ Renkin Function



RESULTS

R^2 for indinavir-alone data was 99.2% (A->B) and 88% (B->A) and R^2 for vinblastine inhibition data was 91.1% (A->B) and 85.1% (B->A). The ratio between the two transport fluxes has been calculated to be ~50, indicating a strong P-gp inhibition effect by vinblastine.

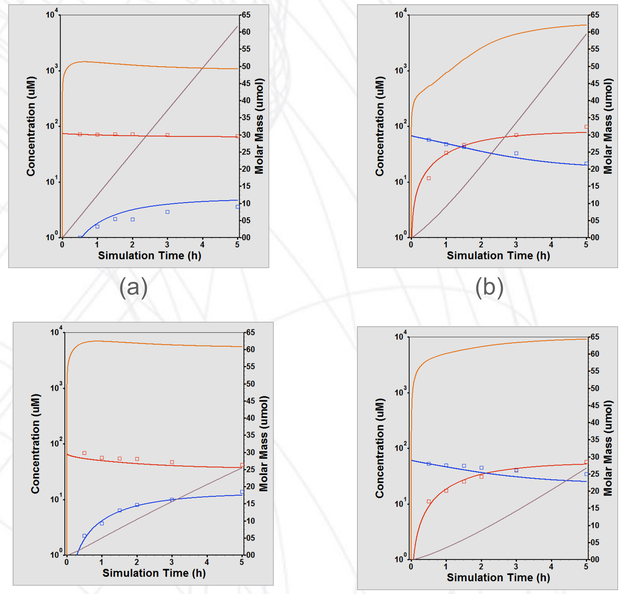


Figure 3. 5-hour simulation of indinavir profiles after (a) apical administration of indinavir, (b) basolateral administration of indinavir, (c) apical administration of indinavir and vinblastine and (d) basolateral administration of indinavir and vinblastine. Red: apical concentration; blue: basolateral concentration; orange: lysosomal concentration; purple: amount transported by P-gp transporter.

CONCLUSIONS

MembranePlus accurately simulated the results of *in vitro* experiments with respect to a variety of mechanisms affecting measured apparent permeability. It is a promising tool in drug research and development. By separating the system-specific from drug-specific parameters in description of drug permeation through the cell membranes it allows obtaining "clean" drug-specific properties (i.e. intracellular Km for efflux transporters) that will allow more direct *in vitro-in vivo* extrapolation and predictions of absorption and drug-drug interactions.

References:

[1] S. Kapitza et al, European Journal of Pharmaceutics and Biopharmaceutics 66, 2007, 146-158
[2] S. Balaz et al., General Physiology and Biophysics 6, 1987, 65-77
[3] H. Kubinyi et al., Journal of Pharmaceutical Sciences 67 (2), 1977, 262-3
[4] H. Zhimin et al., Transactions of Tianjin University 1 (1), 1995, 42-47
[5] A. Adson et al., Journal of Pharmaceutical Sciences 84 (10), 1995, 1197-1204

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