MembranePlus™: A tool to Study *in vitro*/*in vivo* Transport and Lysosomal Trapping

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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, ionization effects, carrier-mediated transport, and metabolism, as well as drug accumulation in membranes and some intracellular compartments (e.g., lysosomes). The model was applied to analyze the effects of lysosomal trapping on the measured apparent permeability of propranolol.

METHODS

MembranePlus™ (Simulations Plus, Inc.) was used to analyze the concentration-time profiles in donor and receiver compartments after apical and basolateral administration of ibuprofen, testosterone and propranolol alone, as well as propranolol in the presence of bafilomycin [1]. The physicochemical properties of propranolol, ibuprofen, and testosterone were predicted by ADMET Predictor™ 6.0 (Simulations Plus, Inc.). The contribution of paracellular diffusion for each drug was estimated from drug properties and the experimental setup. Parameters accounting for passive transcellular diffusion and membrane/lysosomal accumulation were fitted to the propranolol, ibuprofen, and testosterone alone data. This basic model was then applied to explore the effect of bafilomycin on lysosomal pH and the subsequent change in propranolol accumulation in lysosomes. The model includes various effects of the experimental setup (i.e., shaking rate, solvent pH, filter support, sampling effects, etc.) on measured apparent permeability.

1. Compound Properties

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

The membrane entrance (Ki) and exit rates (Ko) are given by Eqn. 1 and 2, as modified from [2] and [3], where P_{oct}, P_{mem} are the octanol-water and membrane-water partition coefficients, and c, m, γ and δ are fitting coefficients.

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

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

2. Experimental Setup

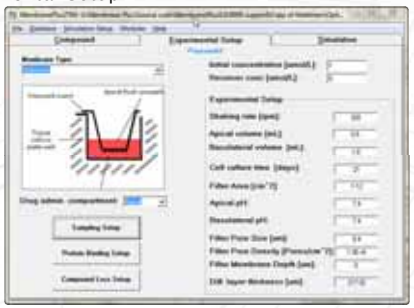


Figure 2. MembranePlus Experimental Setup tab. The program considers major experiment-related parameters, such as shaking rate, apical volume, basolateral volume, filter area, filter support permeability, etc.

3. Diffusion Model

The unstirred layer thicknesses are automatically calculated after the relevant experimental parameters are entered: shaking rate, apical and basolateral volumes, filter area. Therefore, in the current program, the apical and basolateral departments are separated into a well stirred layer and a diffusion layer. This diffusion layer is then divided into numbers of thinner sublayers to allow accurate calculation of diffusion across the layers according to Fick's Second Law, where c is the concentration and D is the diffusion coefficient.

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \qquad \text{(Fick's Second Law)}$$

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's mean projected radius and the hydrodynamically equivalent sphere radius. The filter permeability calculation was adopted from [5]. The total 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

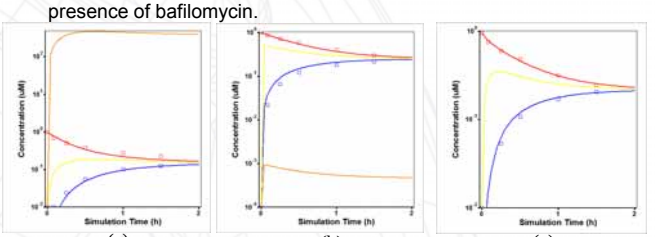
$$P_{Filter} = \frac{\varepsilon_F * D * F(r/R_F)}{h_F},$$

$$F(x) \rightarrow \text{Renkin Function}$$

where P_{para} and P_{Filter} are the paracellular and filter permeabilities, r is the molecular radius, R_F is the filter pore radius, ε_F is the filter porosity and h_F is the filter pore depth.

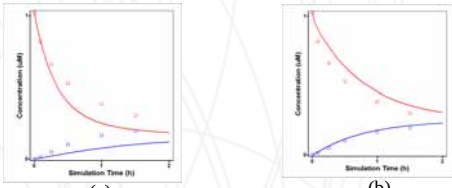
Results

The membrane transport model (c, m, γ and δ) was fitted to apical and basolateral administrations of ibuprofen, testosterone and propranolol simultaneously (6 profiles). The fitted concentration-time profiles showed a good match to the experimental data (Figure 3). The overall R^2 for all the concentrations was over 0.9. The same model was then applied to co-administration of propranolol and bafilomycin, suggesting that bafiloimycin caused an increase in lysosomal pH from 4 to 5.5 (Figure 4), which resulted in a good match of propranolol concentration-time profiles measured in the presence of bafilomycin.



(a) (b) (c)

Figure 3. Simulated (lines) and *in vitro* (squares) concentration-time profiles after apical administration of 1 μM (a) propranolol (b) ibuprofen and (c) testosterone. Simulated apical, basolateral, cytosol and lysosomal concentrations are shown in red, blue, yellow and orange respectively. Note that in (c), cytosol and lysosomal concentrations are overlapping. A similar match between simulated and observed profiles was obtained for basolateral administration (data not shown).



(a) (b)

Figure 4. Predicted (lines) and *in vitro* (squares) concentration-time profiles after apical administration of 1 μM propranolol in the presence of 100 nm bifilomycin. (a) lysosomal pH set to be 4. (b) lysosomal pH set to be 5.5. Simulated apical, basolateral concentrations are shown in red and blue, respectively. A similar match between simulated and observed profiles was obtained for basolateral administration (data not shown).

CONCLUSIONS

MembranePlus has enabled the ability to analyze *in vitro* experiments with respect to the variety of mechanisms affecting measured apparent permeability. The current study shows that it is a promising tool in drug research and development for *in vitro-in vivo* extrapolation, not only in prediction of absorption, but also other processes affecting drug distribution in different tissues (e.g., lysosomal trapping).

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References:

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[3] H. Kubinyi et al., Journal of Pharmaceutical Sciences 67 (2), 1977, 262-3
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