MembranePlus is an advanced, yet easy-to-use, modeling and simulation software program that unlocks important information from your in vitro permeability assay studies. With MembranePlus, all relevant experimental and cellular processes, such as protein binding, lysosomal trapping, pH difference, shaking rate, paracellular permeability, and carrier-mediated transport & metabolism, are integrated to simulate drug concentrations from in vitro cell-based/non-cell-based assays and calculate the corresponding permeability & additional in vivo rate parameters. The combination of MembranePlus and GastroPlus™ brings you closer to accurately predicting in vitro – in vivo extrapolation (IVIVE) of absorption processes [both passive and carrier-mediated].

Permeability is a critical parameter for absorption, since molecules need to permeate through cellular membranes to reach target sites in the body. For over 20 years, significant research has been devoted to in vitro permeability studies, with several guidelines being published and all pharmacopeia describing appropriate methods for testing.

MembranePlus has two primary purposes:

1.) Simulation of in vitro permeability
   a. Predict various permeability processes [e.g., paracellular, transcellular]
   b. Estimate different intracellular concentrations:
      i. Membrane
      ii. Cytosol
      iii. Lysosome
   c. Assess impact of experimental variability on the predicted outcomes

2.) Analysis of measured in vitro permeability data
   a. Identify paracellular and transcellular permeability
   b. Calculate in vitro Km and Vmax for enzymes and transporters
   c. Fit parameters to build a robust model and learn from the data

MembranePlus is going to be very helpful for:

• Users of GastroPlus looking to define inputs for more accurate in vivo absorption predictions in the Advanced Compartmental Absorption and Transit (ACAT™) model.
• Discovery scientists looking to categorize compounds into high and low permeability classes
• DMPK researchers that focus on in vitro transporter/metabolism kinetic studies
• CROs which provide in vitro Caco-2, PAMPA, or MDCK permeability services, to assist with the optimization of the experimental design for client studies
Which experiments can we simulate?

MembranePlus has default models describing several common in vitro systems:
1.) PAMPA (built-in 12, 24, and 96 wells)
2.) Caco-2 (built-in 12, 24, and 96 wells)
3.) MDCK (built-in 12, 24, and 96 wells)

Of course, use MembranePlus to customize any cell-monolayer based in vitro system to mimic your in-house lab!

For the experiment, MembranePlus allows you to control the following during your simulations:
1.) pH in donor and receiver compartments
2.) Addition of albumin to donor or receiver compartments
3.) Shaking/stirring rates
4.) Volume of media in donor and receiver compartments
5.) Sampling protocols that may impact volume or drug concentrations, including:
   a. Withdrawal of sample volume
   b. Replacement of sample volume with blank buffer or original donor solution
   c. Moving the insert to a different plate with blank buffer in receiving compartment
6.) Cell culture time
7.) Filter surface area
8.) Filter pore size and density
What drug information is needed for the simulation?

Basic physicochemical properties (e.g., logP/logD, pKa[s], and molecular weight) can be defined through *in vitro* measurements or predicted from chemical structure (through ADMET Predictor™). If including enzymes or transporters in your assay, information about Vmax and Km needs to be entered (or can be "fitted" with measured concentration-time data from your permeability studies).

Lysosomal Trapping - An important consideration

In MembranePlus, lysosomal trapping, a process which can impact a compound’s distribution in tissues, can be studied in the simulations.
With MembranePlus, you can run the program in one of several modes:

- **Single simulation**: based on your drug properties (whether measured or predicted through ADMET Predictor™) and experimental setup, easily run a simulation to predict the time course changes in concentrations (e.g., apical, basolateral, cellular, or lysosomal). Also calculate the different permeabilities (transcellular and paracellular).

- **Parameter Sensitivity Analysis**: during early drug development, researchers have a large number of compounds to evaluate and limited resources. With Parameter Sensitivity Analysis, quickly assess the impact of changes to certain properties (e.g., physicochemical or experimental) on critical endpoints. This can help guide your resource allocation plans and identify which experiments should be done next.

- **Batch Simulations**: quickly screen a library of compounds based on predicted permeability or run the same compound through a series of different experiments.

- **Optimization**: an important feature of MembranePlus which turns the program into a fitting routine. Using your measured concentration vs. time data, fit parameters to build more robust models and “learn” from your chemical series.

Regardless of the mode in which you run MembranePlus, report-quality results can be easily generated and shared with others.
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 physiochemical properties of indinavir and vinblastine were predicted by ADMET Predictor™ 8.0 (Simulations Plus, Inc.). The contribution of paracellular diffusion was estimated from drug properties and the experimental setup. Carrier-mediated transport was modeled with Fickian-Nernstian kinetics. The indinavir P-gp Vmmax/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 Vmmax/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 defects).

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 Eqs. 1 and 2; and (2) direct transport parameter entries for all neutral and charged species.

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

\[ k_e = \frac{\gamma}{\text{Vm} + 1} \]
\[ k_i = \frac{\gamma}{\text{Vm} + 1} \]

Eqs. 1

\[ P_m = \frac{\gamma}{\text{Vm} + 1} \]

Eqs. 2

2. Transporter Setup

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

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 apical and basolateral chambers at 0.5, 1, 2, 3, and 4 hours.

4. Paracellular and filter permeability

Both paracellular and filter permeabilities are accounted for in the program. The default paracellular model is the Zinman 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:

\[ \frac{1}{P_{m_{en}}} = \frac{1}{P_{m_{ap}}} + \frac{1}{P_{m_{filter}}} \]

where

\[ P_{m_{en}} = \frac{1}{P_{m_{ap}}} \left( 1 + \frac{R_{m}}{R_{m_{0}}} \right) \]

\[ F(x) \to \text{Rankin Function} \]

References


Results

For indinavir-alone data was 96.2% (A→B) and 86% (B→A) and 97% for vinblastine inhibition data was 91.1% (A→B) and 89.1% (B→A). The ratio between the two transport fluxes has been calculated to be ~50, indicating a strong P-gp inhibition effect by vinblastine.

Conclusions

MembranePlus accurately simulated the results of in vitro experiments with respect to a variety of mechanism 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.

simulationsplus.inc
MembranePlus™: A tool to Study in vitro/in vivo Transport and Lysosomal Trapping

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2. Experimental Setup

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

3. Diffusion Model

The unidirectional 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 compartments are separated into a well-stirred layer and a diffusion layer. This diffusion layer is then divided into a number of thinner sublayers to allow accurate calculation of diffusion across the layers according to Fick’s Second Law, where \( \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \) (Fick’s Second Law).

4. Paracellular and filter permeability

Both paracellular and filter permeabilities are accounted for in the model. The default paracellular model is the Zimm 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

\[
\text{Permeability} = \frac{1}{\mu} \int_0^1 \left( \frac{F(r)}{\mu F(r)} \right) \, \text{d}r
\]

where \( P_{r_{in}} \) and \( P_{r_{out}} \) are the paracellular and filter permeabilities, \( r \) is the molecular radius, \( R_{f} \) is the filter pore radius, \( \mu \) is the filter porosity, and \( h \) is the filter pore depth.

RESULTS

The membrane transport model (0.7, 0.5, and 0.6) 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 R² for all the concentrations was over 0.9. The same model was then applied to co-administration of propranolol and sulfadiazine, suggesting that sulfadiazine 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 sulfadiazine.

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-vivo extrapolation, not only in prediction of absorption, but also in processes affecting drug distribution in different tissues (e.g., lysosomal trapping).