Modeling of Active Transport and Metabolism for *in vitro* Suspended and Sandwich Hepatocyte Assays Utilizing MembranePlus™

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Simulations Plus, Inc.
Overview

• MembranePlus™ – a software platform for simulation of drug transport in cell assays.

• Development of Hepatocyte Transport Model
  – Sandwich and plated hepatocytes.
  – Suspended hepatocytes.

• Model Case Studies
  – Sodium Taurocholate transport
  – Statins (uptake+metabolism)
  – IVIVE with combination of MembranePlus and GastroPlus
MembranePlus

**Data Analysis**
- Instant permeability output from molecular structure or experimental data.
- Unbound Intracellular concentrations (Membrane, cytosol, lysosome, etc.)
- $\textit{In vitro} \ K_m$ and $V_{\text{max}}$ for enzymes and transporters
- Parameter sensitivity analysis (on logP, shaking rate, pH etc.)
- Sandwich and suspended hepatocyte model (version 2 imminent)
- DDI predictions (Coming Soon in Version 3)

**Assay Prediction**
- Permeability
- Intracellular concentrations (Membrane, cytosol, lysosome)
- Virtual Trials (coming soon)
MembranePlus – Software Overview

ADMET Predictor™ Values

Applicable only to cell-based assays
MembranePlus – Membrane Entry/Exit Rate Structure Based Model

- Observed vs. Predicted on 44 training datasets

\[ \log(V_i) = \text{Intercept} + C1 \times \log P + C2 \times M \_ \text{RNG} + C3(\text{HBDH} - \text{HBD}) + C4 \times \text{HBAo} \]

Membrane entry and exit rates for anions and cations are determined based on logD vs. pH profile
MembranePlus – Lysosomal Trapping Model

Fig. 1. The basis of pH partitioning of lipophilic amines into lysosomes. The diagram illustrates the mechanism by which lipophilic amines (i.e., CADs) accumulate in lysosomes. From plasma (pH 7.4) and cytosol (~7.2), a lipophilic amine (logP > 1, pKa > 6.5) will readily diffuse across membranes in its unionized form (RNH₂) while maintaining Henderson-Hasselbalch equilibrium with its ionized form (RNH₃⁺, which cannot readily diffuse across membranes). After diffusion into the acidic environment of the lysosome (pH 4–5), the equilibrium between charged and uncharged species shifts in favor of the ionized form of the lipophilic amine, limiting diffusion of the drug back into the cytosol and, in effect, trapping the drug in lysosomes. For highly permeable lipophilic amines, the concentration of unionized drug (RNH₂) at equilibrium is assumed to be the same in all three compartments (lysosomes, cytosol, and plasma). The figure is not to scale; lysosomes make up about 1% of the hepatocyte volume.

Kazmi F., Drug Metab. Disp. 41(3):897 (2013)
Enzymes and Transporters - Equations

- Kinetics of carrier-mediated transport and metabolism is calculated using Michaelis-Menten kinetics:

\[
\nu_{\text{metab}} = \sum_i \left( \frac{V_{\text{max}}^i \times c_{\text{intra}u}}{K_m^i + c_{\text{intra}u}} \right) \\
\nu_{\text{efflux}} = \sum_i \left( \frac{V_{\text{max}}^i \times c_{\text{intra}u}}{K_m^i + c_{\text{intra}u}} \right) \\
\nu_{\text{influx}} = \sum_i \left( \frac{V_{\text{max}}^i \times c_{\text{buffer}}}{K_m^i + c_{\text{buffer}}} \right)
\]

- \( V_{\text{max}} \) units: \( \mu \text{M/s} \ (\mu \text{mol/L/s}) \)
- \( K_m \) units: \( \mu \text{M} \ (\mu \text{mol/L}) \)
- General units converter allows converting these into different types of units
- Transporter types: Influx and Efflux
- Transporter locations: Apical and Basolateral
Sandwich and Suspended Hepatocyte Model

• New to version 2.0 (expected May/June 2017)

• Model non-linear drug uptake and metabolism in:
  – Sandwich Hepatocytes
  – Plated Hepatocytes
  – Suspended Hepatocytes

• Inclusion of intracellular protein binding (free fraction)
Overview of Sandwich Hepatocyte Mechanisms

- Collagen is assumed to not affect transport processes
- Model is also applicable for plated hepatocytes when bile volume is not considered
Overview of Suspended Hepatocyte Mechanisms

- Incubation Media
- Evaporation
- Membrane Entry/Exit
- Transporters
- No Cell Membrane Differentiation
- Loss (plastic binding, nonspecific loss)
- Diffusion Layer

Components:
- Cytosol
- Metabolism
- Lysosomal Trapping
- Protein binding
- Membrane

Symbol definitions:
- $D_U$
- $D_B$
Physiological Settings

- Plated hepatocytes can be modeled by setting bile volume and area percentages to 0.
- Bile pockets contract and open/release drug to the media thus a first order bile efflux rate is available.
Case Study 1: Sodium Taurocholate Uptake Into Bile
Sodium Taurocholate

- Experimental data from Qualyst Guo, ISSX 2014
- All properties except \(K_m\) values from ADMET Predictor

AP 7.2 = ADMET Predictor v. 7.2
S+ stands for properties predicted with Simulations Plus models
S+Sw = solubility in pure water

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S+IS (mg/mL) @ pH 2.78</td>
<td>0.0184</td>
</tr>
<tr>
<td>S+pKa (Acid)</td>
<td>1.1</td>
</tr>
<tr>
<td>S+SF</td>
<td>906</td>
</tr>
<tr>
<td>S+pH</td>
<td>2.78</td>
</tr>
<tr>
<td>S+logP</td>
<td>0.82</td>
</tr>
<tr>
<td>S+Peff (x10^-4 cm/s)</td>
<td>0.36</td>
</tr>
<tr>
<td>DiffCoef (x10^-5 cm/s)</td>
<td>0.53</td>
</tr>
<tr>
<td>S+fumic</td>
<td>0.911</td>
</tr>
</tbody>
</table>

Fig. 3. Observed (circles) and simulated (lines) \(d_8\)-TCA accumulation in lysate [from cells+bile and cells] (black) and efflux buffer [Ca\(^{2+}\)-containing and Ca\(^{2+}\)-free] (red). Representative graph from one of \(n=3\) experiments. Recovered estimates for CL (mean and SD):

<table>
<thead>
<tr>
<th>((\mu)L/min/mg protein)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL(_{\text{Uptake}})</td>
<td>6.97</td>
<td>1.39</td>
</tr>
<tr>
<td>CL(_{BL})</td>
<td>0.379</td>
<td>0.12</td>
</tr>
<tr>
<td>CL(_{\text{Bile}})</td>
<td>0.827</td>
<td>0.30</td>
</tr>
</tbody>
</table>

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS
Model of Sodium Taurocholate Bile Canalicular Uptake in Sandwich Hepatocytes

OST alpha/beta

- Guo, 2014 ISSX Poster
**Na\(^{+}\) Taurocholate Model Assumptions**

- **Assumptions**
  - No protein binding for Na Taurocholate
  - No stirring
  - Complete monolayer of cells (100% viability)
  - ADMET Predictor values for properties and transport model parameters calculated using calibration

- Literature values for $K_m$ were used as a starting point for building the model.

- $K_m$ values are similar across species
  - Swift-Mol-Pharm-2010-7(2)-491–500

### Cell Assay Inputs

<table>
<thead>
<tr>
<th>Feed Solution Conc.</th>
<th>1,2.5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>4 %</td>
</tr>
<tr>
<td>Well size</td>
<td>24 well</td>
</tr>
<tr>
<td>Volume</td>
<td>0.3 mL</td>
</tr>
<tr>
<td>Cell Vol.</td>
<td>6.46 pL</td>
</tr>
<tr>
<td>Cell Layer Thickness</td>
<td>18.6 micron</td>
</tr>
<tr>
<td>Cell Den</td>
<td>0.4 Mcell/well</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transporter</th>
<th>$K_m$ (µM)</th>
<th>Cells</th>
<th>Literature Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>OST alpha/beta</td>
<td>25.8</td>
<td>Human</td>
<td>Swift-Mol-Pharm-2010-7(2)-491–500</td>
</tr>
<tr>
<td>Overall Uptake</td>
<td>19</td>
<td>Rat</td>
<td>Schwarz-Eur-J-Biochem-1975-55-617-623</td>
</tr>
<tr>
<td>NTCP</td>
<td>6</td>
<td>Human</td>
<td>J-Exp-Biol-2001-204-1673-1686</td>
</tr>
<tr>
<td>BSEP</td>
<td>5</td>
<td>Rat</td>
<td>J-Exp-Biol-2001-204-1673-1686</td>
</tr>
</tbody>
</table>
TCA Results 1 μM Donor
Experimental $K_m$ Values

- Experimental $K_m$ values utilized from literature and $V_{max}$ values fit to data.

- We have obtained superior model results with new datasets but they can not be shown until published.

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Type</th>
<th>$K_m$ (Exp) (μM)</th>
<th>$V_{max}$ (μmol/s/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP</td>
<td>Efflux</td>
<td>5</td>
<td>6.83E-03</td>
</tr>
<tr>
<td>OST alpha/beta</td>
<td>Efflux</td>
<td>25.8</td>
<td>9.63E-02</td>
</tr>
<tr>
<td>NTCP</td>
<td>Influx</td>
<td>6</td>
<td>3.88E-02</td>
</tr>
</tbody>
</table>
Case Study 2: Quantification of Influx Transport vs. Metabolism Statin Compounds

Suspended Hepatocytes
Quantify the Relative Importance of Influx Transport (OATP1B1) vs. Metabolism

- Media and whole cell concentration data for atorvastatin, cerivastatin, and indomethacin.
- Used a simple compartmental model to extract clearance values

Atorvastatin

- **Solubility**
  - $S^+$ Sol = 0.18 mg/mL @ pH 4.5
  - Sol (water exp) = 0.11 mg/mL
  - Sol (pH 1.2) = 0.01 mg/mL
  - Sol (pH 7.4) = 0.70 mg/mL
- $S^+$SF = 285
  - SF (Exp) = 70
- $S^+$ LogP 4.28
  - LogP exp = 4.18 (biabyte)
- $S^+$ pKa (Acid) = 4.71, 11.05
  - pKa Exp = 4.51 (Schonherr_Eur-J-Pharm-Biopharm_2015-155-170)
- OATP1B1 Substrate ($K_m = 0.77 \mu M$)
  [Vildhede_Drug Metab Disp_2014_42.7_1210-1218]
- Caco-2 Papp (MembranePlus) = 4.80E-05 cm/s
- $S^+$ Metabolism (2C9)
  - Experimental (3A4) ($K_m = 44.9 \mu M$)
  [Park_Xenobiotica_2008_38.9_1240-1251]

Cerivastatin

- **Solubility**
  - $S^+$ Sol = 0.2 mg/mL @ pH 4.92
- $S^+$SF = 280.7
- $S^+$ LogP 3.04
  - LogD exp = 3.26 @ pH 5 (biabyte)
- $S^+$ pKa (Acid/Base) = 4.39, 5.39
- OATP1B1/1A1? Substrate ($K_m = 5.86 \mu M$)
- Caco-2 Papp (MembranePlus) = 7.5E-08 cm/s
- $S^+$ Metabolism (3A4) ($K_m = 11.8 \mu M$)

AP 7.2 = ADMET Predictor v. 7.2
$S^+$ stands for properties predicted with Simulations Plus models
$S^+$Sw = solubility in pure water
Model Results

Atorvastatin

Lipid bilayer permeability model was fit due to poor prediction from structure.
**Model Results, Cont.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atorvastatin</th>
<th>Cerivastatin</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{max}$ 3A4 (umol/s/L)</td>
<td>0.119</td>
<td>3.74E-02</td>
<td></td>
</tr>
<tr>
<td>$V_{max}$ OATP1B1 (umol/s/L)</td>
<td>31.9</td>
<td>295.28</td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}_{\text{met}}$ (uL/min/Mcell)</td>
<td>63 (68 Lit.)</td>
<td>20 (17 Lit.)</td>
<td></td>
</tr>
<tr>
<td>$\text{Cl}_{\text{upt}}$ (uL/min/Mcell)</td>
<td>471 (375 Lit.)</td>
<td>704 (413 Lit.)</td>
<td></td>
</tr>
</tbody>
</table>

*At a cell concentration of $1 \times 10^6$ cells/mL.

Model Comparison

- The mechanistic model in MembranePlus achieved similar result as the simpler compartmental model with fewer fitted parameters

<table>
<thead>
<tr>
<th></th>
<th>Compartmenal model*</th>
<th>Membrane Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular volume</td>
<td>Fitted</td>
<td>System parameter</td>
</tr>
<tr>
<td>Cell membrane volume</td>
<td>Fitted</td>
<td>System parameter</td>
</tr>
<tr>
<td>Membrane/water partitioning (kmem)</td>
<td>Fitted</td>
<td>Predicted from compound properties</td>
</tr>
<tr>
<td>Active uptake</td>
<td>Fitted</td>
<td>Fitted</td>
</tr>
<tr>
<td>Passive diffusion</td>
<td>Fitted</td>
<td>Predicted from compound properties (atorvastatin only)</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Fitted</td>
<td>Fitted</td>
</tr>
</tbody>
</table>

Case Study 3: *In Vitro* to *In Vivo* Extrapolation Using MembranePlus and GastroPlus
Propafenone Metabolism
Suspended Hepatocytes

- Reports indicate it is a CYP2D6 substrate and has saturable dose dependent kinetics.
- Have IV data to compare predicted $K_m$ and $V_{max}$ to.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S+Sw (mg/mL) @ pH 10.28</td>
<td>0.49</td>
</tr>
<tr>
<td>S+pKa (Base)</td>
<td>9.47</td>
</tr>
<tr>
<td>S+SF</td>
<td>114</td>
</tr>
<tr>
<td>S+logP</td>
<td>3.03</td>
</tr>
<tr>
<td>S+Peff (x10-4 cm/s)</td>
<td>1.76</td>
</tr>
<tr>
<td>DiffCoef (x10-5 cm/s)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

**Table of Properties**

- **S+Sw** = solubility in pure water
- S+ stands for properties predicted with Simulations Plus models

**Diagram:**

- Solubility (mg/mL) vs pH
- pH range: 0.0 to 14.0
- Solubility range: 0.0 to 50.0
Propafenone Human Hepatocyte Data

- 100 rpm
- 2 mL incubation
- 1 Mcell/mL

Fig. 2. A, disappearance of propafenone in cryopreserved human hepatocytes at various initial concentrations. Propafenone at concentrations of 0.05, 0.2, 1, and 5 μM was incubated with $1 \times 10^6$ cells/ml human hepatocytes individually prepared from three donors. Each point represents the mean ± S.D. of the remaining percentage of substrate to the initial concentration in hepatocytes from three donors. B, relationship between initial substrate concentration and depletion rate constant of propafenone in cryopreserved human hepatocytes. Each point represents the mean ± S.D. of depletion rate constants of three donors. The line represents the curve predicted from eq. 1.

Fit to *In Vitro* Data

- $K_m = 0.0146 \mu M$
- $V_{\text{max}} = 9.27E-02 \mu\text{mol/s/L cytosol}$

- $V_{\text{max}}$ Converts to:
  - $2.17E-02 \text{ nmol/min/Mcell}$

$K_m$ calculated was lower than the paper which is indicative of the true intracellular $K_m$ based on unbound intracellular concentration.
In vivo $K_m / V_{max}$ Conversion

GastroPlus

Km and Vmax values exported to table!
Prediction of 70 mg IV Bolus Dose

- PBPK model with default parameters (Lukacova method) in GastroPlus with the *in vitro* extrapolated CYP2D6 clearance

Data: Hollmann, Cardiac Arrhythmias, Springer 125-132
Conclusion

• MembranePlus has multiple capabilities to assess
  – Passive and active drug transport in various assays
  – Interplay between metabolism and passive/active drug transport:
    • Sandwich Hepatocytes
    • Plated Hepatocytes
    • Suspended Hepatocytes
  – Drug disposition in Bile
    • Sandwich Hepatocytes
  – Drug concentration within cellular structures
    • Lysosomes, lipid bilayers
  – Extract relevant parameters for IVIVE in GastroPlus
  – Additional validation with datasets and software release imminent