Poster T4077

Modeling of Active Transport and Metabolism for Hepatocyte Assays with Application of In Vitro to In Vivo Extrapolation (IVIVE)

James Mullin[1], Viera Lukacova[1], Walter S. Woltosz[1], Michael B. Bolger[1]

[1]Simulations Plus, Inc.

2017 AAPS ANNUAL MEETING & EXPOSITION

CONTACT INFORMATION: jim@simulations-plus.com, Simulations Plus, Inc., 42505 10th Street West, Lancaster, CA 93534

DEVELOPING SCIENCE. IMPACTING HEALTH.

PURPOSE

Sandwich and suspended hepatocyte cultures are routinely used to assess either active transport and/or metabolism of drug molecules. In vitro assays that evaluate critical drug clearance and metabolism pathways are important in the prediction of hepatobiliary transport, drug-drug interactions, drug induced liver injury (DILI), and the relative importance between active transport and metabolism in hepatocytes. Physiologically based pharmacokinetic (PBPK) models provide quantitative in vivo simulation of drug disposition and drug-drug interactions if Km and Vmax values for the relevant transporters or enzymes can be extracted accurately from in vitro data. To that end, a fully mechanistic simulation of drug transport in both sandwich and suspended hepatocytes was developed in MembranePlus™ (Simulations Plus, Inc.) that allows simultaneous determination of Km and Vmax values for enzymes as well as both influx and efflux transporters. Both models account for additional processes such as drug diffusion through the unstirred boundary layer, sample volume loss, protein binding in both media and cytosol, lysosomal trapping, and drug partitioning into lipid bilayers. Two case studies are presented to demonstrate the applicability of these models. The active uptake and biliary secretion of sodium taurocholate is simulated in the sandwich hepatocyte model. The CYP2D6 metabolism of propafenone is evaluated in the suspended hepatocyte model and, in conjunction with a PBPK model, is used to predict the in vivo exposure.

OBJECTIVES

1. Determine intracellular Km/Vmax values for metabolism in suspended hepatocyte cell culture.
2. To facilitate the generation of metabolism inputs for GastroPlus™ (Simulations Plus, Inc.).
3. Calculate the biliary transport and disposition of sodium taurocholate.

METHODS

The mechanistic models for transport and metabolism in sandwich and suspended hepatocytes were implemented in MembranePlus version 2 and are shown in Figure 1.

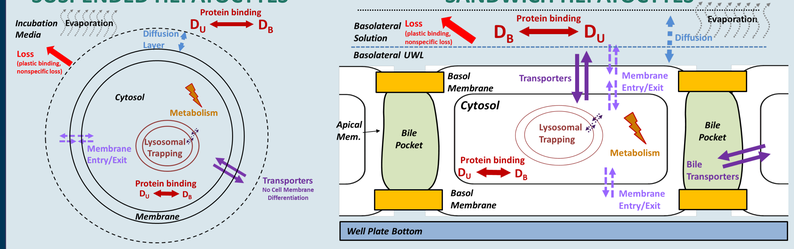

Figure 1. Physical model for drug transport in suspended hepatocytes (left) and sandwich hepatocytes (right).

The partial differential equations that describe drug transport are solved using the method of lines in rectangular or spherical 1D geometries. The sandwich hepatocyte model was utilized to determine the Vmax for active uptake and efflux of sodium taurocholate as shown in Figure 2. Because disposition data was only measured at one dose concentration, (1 μM) Km values for MRP4, OSTα/β, and BSEP mediated transport of sodium taurocholate could not be fitted and were used as obtained from literature[4,5].

METHODS (CONT.)

The Km values are shown in Table 3. ADMET Predictor™ 8.0 (Simulations Plus, Inc.) was used to determine physicochemical properties and passive membrane transport parameters for each case study as shown in Table 2. The suspended hepatocyte model was used to predict passive diffusion and CYP2D6 metabolism of propafenone across 4 dose levels from 0.05 to 5 μM. Experimental data for media, cell, bile concentrations, or plasma concentrations, as well as, *in vitro* experimental settings (Table 1) in these systems was obtained from literature data.[1,2,3]

Table 1. Cell assay parameters for MembranePlus simulations

Cell Assay Inputs	Na. Taur.	Propafenone	
Feed Solution Conc.	1	0.05, 0.2, 1, 5	μM
Protein in Basolateral Fluid (BSA)	4	NA	%
Well size	24	6	well
Volume	0.3	2	mL
Cell Volume	6.46	NA	pL
Cell Thickness/Diameter	18.6	16.79	micron
Cell Density	0.4	1	Mcell/well

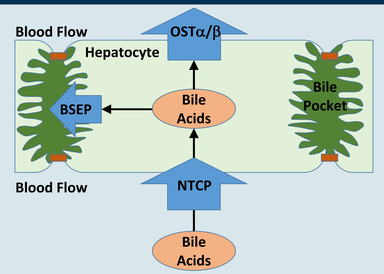

Figure 2. Schematic of sodium taurocholate uptake

Table 2. Properties of sodium taurocholate and propafenone for simulations in MembranePlus and GastroPlus

Property	Na. Taur.	Propafenone
S+Sw (mg/mL) @ pH	0.0184 @ pH 2.78	0.49 @ pH 10.28
S+pKa (Base)	1.1	9.47
S+SF	906	114
S+logP	0.82	3.03
S+Peff (x10-4 cm/s)	0.36	1.76
DiffCoef (x10-5 cm/s)	0.53	0.65
Rbp	0.59	0.81
Fu plasma	16.8	19.03

RESULTS

In sandwich hepatocyte cells, Vmax values of 0.0404, 0.0863, and 0.0737 μmol/s/L cytosol were obtained for NTCP (influx), BSEP (bile efflux), and OSTa/b (efflux) transport of sodium taurocholate. The fitted concentration profiles and Vmax values are shown in Figure 3 and Table 3, respectively. The resulting prediction of media and cell concentration including bile had a mean absolute error of 13.6%. The accuracy figure encompasses both the uptake and efflux (or wash) phase where the cells are exposed to drug-free buffer. In suspended hepatocytes, Km and Vmax values of 0.0146 μM and 0.0927 μmol/s/L cytosol were obtained for the CYP2D6 metabolism of propafenone and the fitted predictions are shown in Figure 4. The resulting parameters were used in the GastroPlus PBPK model to predict propafenone PK as shown in Figure 5. The IVIVE was reasonably accurate with a predicted and observed AUC of 1343 and 1032 ng-hr/mL (23% error). The result indicates that the metabolic clearance predictions obtained from MembranePlus analysis of in vitro data and the Lukacova method for tissue partition coefficients are an acceptable combination in this case and show the utility of both software tools for IVIVE.

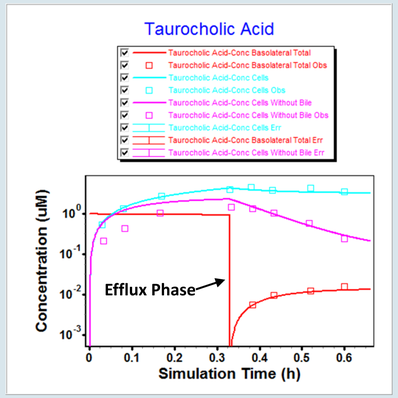

Figure 3. Model results for extraction of Vmax values. For biliary excretion of sodium taurocholate

Table 3. Fitted Vmax values for sodium taurocholate disposition in sandwich hepatocytes

Transporter	K_m Exp (μM)	V_{max} Fit (μmol/s/L)	K_m Literature Source
BSEP	25.8	6.83E-03	Swift-Mol-Pharm-2010-7(2)-491–500
OSTa/b	6	9.63E-02	J-Exp-Biol-2001-204-1673-1686
NTCP	5	3.88E-02	J-Exp-Biol-2001-204-1673-1686

RESULTS (CONT.)

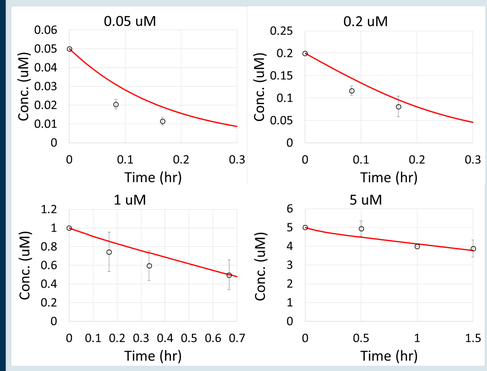

Figure 4. Km/Vmax fitting results for propafenone in suspended hepatocyte culture (left)

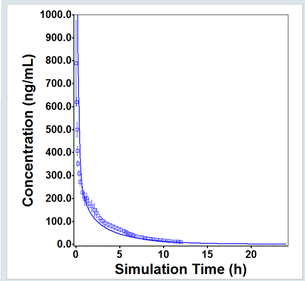

Figure 5. GastroPlus IVIVE prediction of 70 mg IV bolus dose in human (right)

CONCLUSION

The sandwich and suspended hepatocyte models within MembranePlus are new utilities for scientists to analyze their in vitro experiments and extract relevant Km and Vmax parameters for active transport and metabolism. We have shown that the models and parameters extracted from MembranePlus can then be utilized in a PBPK model to predict in vivo exposure. In the future, we will expand the capabilities of the new hepatocyte models to determine relevant in vitro drug-drug interaction parameters for predictions of in vivo DDI potential.

REFERENCE

1. Guo, Cen, et al. ISSX (2014).
2. Komura, Hiroshi, et al., Drug metabolism and disposition 33.6 (2005): 726-732.
3. Schlepper and Olsson, Cardiac Arrhythmias, Springer-Verlag (1983): 125-132.
4. Swift, et al., Molecular Pharmaceutics 7.2 (2009): 491-500.
5. St-Pierre, et al., Journal of Experimental Biology 204.10 (2001): 1673-1686.

SimulationsPlus
SCIENCE + SOFTWARE = SUCCESS