Mechanistic modelling of electrochemical gradient driven transport of metformin by OCT2

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Introduction

Metformin is cleared predominantly by the kidneys and is a substrate for organic cation transporter 2 (OCT2) mediated active uptake into proximal tubule cells and multidrug and toxin extrusion transporters, MATE1 and MATE2-K, mediated efflux into urine. A previous study has suggested that the influence of membrane potential on the kinetics of OCT2 uptake should be considered when attempting to describe the drug-drug interaction between metformin and cimetidine (an inhibitor of MATE1/2-K and to a lesser extent OCT2) using a mechanistic physiologically-based pharmacokinetic model [1]. In order to confirm this observation *in vitro*, the kinetics of OCT2 mediated transport of metformin were investigated in OCT2 transfected HEK293 cells.

HEK293 cells expressing hOCT2 and plasmid vector alone (mock cells) [2] were plated in a 24-well tissue culture plates at a density of 1 x 10^5 cells/cm^2 and cultured for 2 days prior to uptake experiments.

Uptake experiments (n = 2) were conducted with [^{14}C]-metformin at concentrations of 0.01, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 and 10 mM in transport buffer adjusted to pH 7.4. At incubation times of 0.5, 1, 1.5, 2, 5, 10, 20 or 30 minutes, cells were washed and lysed. The radioactivity and protein content of the lysate was measured. The amount of metformin in cell lysates were calculated from scintillation count data and converted to a cell concentration on the basis of protein concentration at the end of the incubation and an estimate of HEK293 cell volume (6.4 µL/mg protein [3]).

The kinetics of metformin uptake were first determined using a conventional approach, using the initial linear rate of uptake. J_{max} (pmol/min/million cells) and K_m (µM) were estimated by fitting the Michaelis-Menten equation to the rate at each media concentration.

In addition, the cell concentration data were modelled using a series of two-compartment (media and cell) models in which the kinetics of OCT2 transport kinetics were defined in different ways. The passive permeability of metformin was determined by fitting data from mock cells, with the resulting CL_{PD} value fixed when fitting data from OCT2-HEK293 cells. Model fitting was performed using R (version 3.4.0).

$$V_{media} \cdot \frac{d[S]_{media}}{dt} = -rate\ of\ OCT2\ uptake + CL_{PD} \cdot no.cells \cdot ([S]_{cell} - [S]_{media})$$

$$V_{cell} \cdot no.cells \cdot \frac{d[S]_{cell}}{dt} = rate\ of\ OCT2\ uptake + CL_{PD} \cdot no.cells \cdot ([S]_{media} - [S]_{cell})$$

Model 1 – Michaelis-Menten transport kinetics:

In Model 1 the rate of OCT2 mediated uptake was described by Michaelis-Menten kinetics, based on media concentrations.

$$rate\ of\ OCT2\ mediated\ uptake = \frac{J_{max} \cdot no.cells \cdot [S]_{media}}{K_m + [S]_{media}}$$

Where J_{max} is the maximum rate of uptake (pmol/min/million cells), K_m is the Michaelis constant (µM), $[S]_{media}$ is the media concentration of substrate (metformin, µM) and no.cells is the number of cells (million).

Model 2 – Electrochemical gradient (ECG) driven transport:

In model 2, the rate of OCT2 mediated transport was defined based on the electrochemical gradient of metformin across the cell membrane. This model was previously used to fit metformin uptake data at a single concentration[1].

$$rate\ of\ OCT2\ mediated\ uptake = -\Phi_{df} \cdot J_{OCT2} \cdot no.cells$$

$$\Phi_{df} = \Phi_m - \frac{R \cdot T}{z \cdot F} \cdot ln\left(\frac{[S]_{media}}{[S]_{cell}}\right)$$

Where J_{OCT2} is the rate (pmol/min/volt/million cells) of OCT2 mediated transport, Φ_{df} is the electrochemical driving force (Volts), Φ_m is the membrane potential (Volts), R is the universal gas constant (8.314 Joules.Kelvin^{-1}. mol^{-1}), T is temperature (310.15 Kelvin in the experiment at 37 ℃), z is the valence of the ionic species (+1 for metformin), F is Faraday's constant (96490 coulombs.mol^{-1})

Results

Conventional analysis of the initial slope of metformin uptake resulted in apparent J_{max} and K_m value estimates (± SE) of 7.09 ± 0.78 nmol/min/10^6 cells and 1.1 ± 0.42 mM, respectively, which are in agreement with other published studies of metformin in this system.

Model 1 showed a poor fit to metformin cell concentration data at time points at 5 minutes or later. The resulting estimates of OCT2 J_{max} and K_m (± SE) were 13.6 ± 1.57 nmol/min/million cells and 1.72 ± 0.30 mM, respectively.

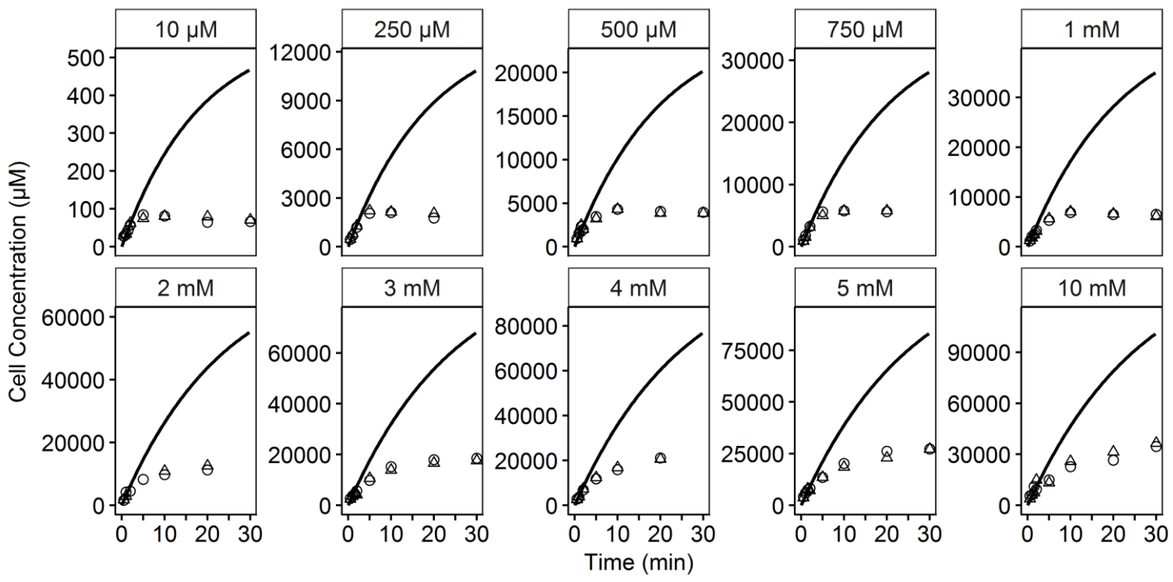

Figure 1. Observed (points) and Model 1 fitted (lines) metformin concentrations in OCT2-HEK293 cells. Circles and triangles represent replicates 1 and 2, respectively.

In contrast, Model 2 was able to reasonably fit metformin cell concentration data for the full duration of the assay. The resulting estimates of J_{OCT2} and Φ_m (± SE) were 150 ± 7.4 nmol/min/mV/million cells and 54.7 ± 0.99 mV, respectively.

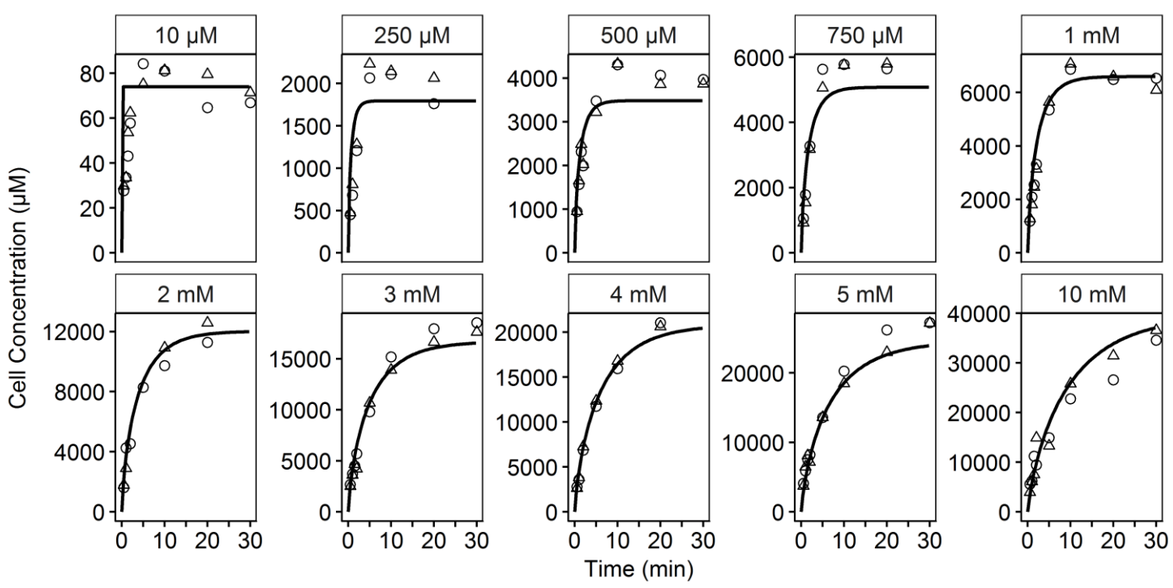

Figure 2. Observed (points) and Model 2 fitted (lines) metformin concentrations in OCT2-HEK293 cells. Circles and triangles represent replicates 1 and 2, respectively.

- Model 1 was unable to fit metformin cell accumulation data at time points later than 5 minutes.
- Model 2 was able to account for the impact of intracellular metformin accumulation on the driving force for OCT2 transport and provided a much better fit to data.
- These results support the application of Model 2 in the previous PBPK model of the DDI between metformin and cimetidine.

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2. Arakawa H, Omote S, Tamai I, (2017) Inhibitory Effect of Crizotinib on Creatinine Uptake by Renal Secretory Transporter OCT2. *J Pharm Sci,* **[Early Online]**.
3. Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M and Tsuji A (1997) Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett* **419:**107-111.