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Metformin and cimetidine: Physiologically based pharmacokinetic modelling to investigate transporter mediated drug–drug interactions

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ABSTRACT

Metformin is used as a probe for OCT2 mediated transport when investigating possible DDIs with new chemical entities. The aim of the current study was to investigate the ability of physiologically-based pharmacokinetic (PBPK) models to simulate the effects of OCT and MATE inhibition by cimetidine on metformin kinetics. PBPK models were developed, incorporating mechanistic kidney and liver sub-models for metformin (OCT and MATE substrate) and a mechanistic kidney sub-model for cimetidine. The models were used to simulate inhibition of the MATE1, MATE2-K, OCT1 and OCT2 mediated transport of metformin by cimetidine. Assuming competitive inhibition and using cimetidine K_i values determined *in vitro*, the predicted metformin AUC ratio was 1.0 compared to an observed value of 1.46. The observed AUC ratio could only be recovered with this model when the cimetidine K_i for OCT2 was decreased 1000-fold or the K_i's for both OCT1 and OCT2 were decreased 500-fold. An alternative description of metformin renal transport by OCT1 and OCT2, incorporating electrochemical modulation of the rate of metformin uptake together with 8–18-fold decreases in cimetidine K_i's for OCTs and MATEs, allowed recovery of the extent of the observed effect of cimetidine on metformin AUC. While the final PBPK model has limitations, it demonstrates the benefit of allowing for the complexities of passive permeability combined with active cellular uptake modulated by an electrochemical gradient and active efflux.

1. Introduction

Metformin is widely prescribed as a first-line therapy for type II diabetes mellitus. As it is a hydrophilic base present in its cationic form at physiological pH, its pharmacokinetic behaviour is dictated largely by the influence of transporters (Graham et al., 2011). The physicochemical properties of metformin also determine that its elimination is predominantly by renal excretion (80%), with a minor contribution from metabolism (Sirtori et al., 1978; Tucker et al., 1981). While the magnitudes of documented drug–drug interactions (DDIs) affecting metformin as a victim are not of major clinical significance, the compound is recommended by the US FDA as a probe for OCT2 mediated transport when investigating possible DDIs with new chemical entities (U.S. Department of Health and Human Services et al., 2012). On this basis, a sound mechanistic understanding of DDIs with metformin is important. Such DDIs, involving raised drug exposure in the kidney, are not necessarily reflected in an equivalent change in systemic exposure (Sprowl and Sparreboom, 2014).

Within the proximal tubule cells of the kidney, metformin is actively transported across the basal membrane by OCT2 and effluxed into the tubular fluid at the apical membrane by MATE1 and MATE2-K (Koepsell et al., 2007; Pelis and Wright, 2011). In hepatocytes it has been shown to be a substrate for OCT1 expressed on the sinusoidal membrane (Koepsell, 2011). Although MATE1 is expressed on the canalicular membrane of hepatocytes, biliary clearance of metformin is

Abbreviations: AUC, area under the plasma concentration-time curve; B/P, blood-to-plasma ratio; CYP, cytochrome P450; CL_{int}, intrinsic clearance; C_{max}, maximum plasma concentration; CL_{PD}, passive permeability-surface area product; DDI, drug–drug interaction; fa, fraction absorbed; J_{max}, maximum rate of active transport; J_{OCT1}, rate of OCT1 transport; J_{OCT2}, rate of OCT2 transport; ka, absorption rate constant; K_i, inhibition constant; K_m, Michaelis constant; Kp, tissue to plasma partition coefficient; MATE, multidrug and toxin extrusion protein; Mech KiM, mechanistic kidney model; OAT, organic anion transporter; OCT, organic cation transporter; PE, parameter estimation; PerL, permeability-limited liver model; PMAT, plasma membrane monoamine transporter; PTC, proximal tubule cells; RAF, relative activity factor; t_{lag}, time-lag; t_{max}, time of maximum plasma concentration; V_{ss}, volume of distribution at steady-state; Φ_{df}, electrochemical driving force; Φ_m, membrane potential.

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negligible (Tucker et al., 1981). In the gut wall, paracellular transfer appears to be significant (Proctor et al., 2008), while metformin has also been shown to be a substrate for OCT1, OCT3 and PMAT at the apical membrane of enterocytes (Muller et al., 2005; Zhou et al., 2007). Metabolism of metformin by CYP3A4 has been reported based on studies with recombinant enzyme (Choi et al., 2010).

Co-administration of cimetidine, pyrimethamine, trimethoprim, lansoprazole, dolutegravir and vandetanib with metformin has been shown to increase its AUC by 1.2 to 1.7-fold (Ding et al., 2014; Grun et al., 2013; Johansson et al., 2014; Kusuhara et al., 2011; Somogyi et al., 1987; Wang et al., 2008; Zong et al., 2014). All of these compounds are inhibitors of OCT2, while cimetidine, pyrimethamine and trimethoprim are also reported to be inhibitors of MATE transporters. Cimetidine K_i values for MATE1 and MATE2-K are, respectively, 62- and 40-fold lower than those for OCT2 inhibition, suggesting that the latter is unlikely to be significant after therapeutic doses of cimetidine (Ito et al., 2012; Tsuda et al., 2009). Similarly, pyrimethamine K_i values for MATE1 and MATE2-K are 118- and 193-fold lower than those for OCT2 inhibition, respectively (Ito et al., 2010; Kusuhara et al., 2011).

The incorporation of permeability-limited uptake models within conventional PBPK models has been shown to improve insight into transporter mediated DDIs in the liver (Li et al., 2014). However, examples where this approach has been used to simulate transporter mediated DDIs in the kidney are rare. Posada et al. (2015) used a mechanistic kidney model to simulate the increase in pemetrexed exposure due to inhibition of renal OAT3 uptake by ibuprofen. Hsu et al. (2014) investigated DDIs between probenecid and three renally cleared drugs using a similar approach. However, *in vitro* data for the inhibition of specific transporters by probenecid were not applied, obscuring understanding of the impact of the relative inhibition of uptake and efflux transporters. In addition, permeability-limited PBPK models typically assume that active transport will follow first-order or Michaelis–Menten kinetics, with little acknowledgement of the mechanism(s) of transport. The driving force for transport varies greatly amongst the known drug transporters. For example, despite OCT and MATE both being solute carriers, transport by the former is driven by the electrochemical gradient across the cell membrane while the latter is an antiporter of protons (Pelis and Wright, 2011).

We describe an attempt to simulate reported effects of cimetidine on the kinetics of metformin using a PBPK model incorporating active uptake and efflux in the kidney and permeability-limited uptake in the liver based on available *in vitro* and *in vivo* data. A novel feature of the final model for metformin was the incorporation of an electrochemical driving force for uptake by OCT1 and OCT2.

2. Material and methods

Full PBPK models were developed for metformin and cimetidine in the Simcyp Simulator® Version 14 (Simcyp Ltd., A Certara Company, Sheffield, UK) (Fig. 1). For metformin, renal and hepatic disposition were described by a mechanistic kidney model (Mech KiM) (Neuhoff et al., 2013a) and a permeability-limited liver model (PerL) (Jamei et al., 2014; Neuhoff et al., 2013b), respectively. For cimetidine, renal disposition was described by Mech KiM. For both compounds, distribution to all other organs was assumed to be perfusion-limited, with tissue-to-plasma partition coefficients (Kp's) predicted using the method of Rodgers et al. (Jamei et al., 2014; Rodgers and Rowland, 2007). A schematic representation of the processes governing the renal secretion of metformin and cimetidine in the proximal tubule of the kidney is shown in Fig. 2.

2.1. Model development

2.1.1. Metformin

Data used in the development of the metformin model are summarised in Table 1. Where these data were obtained from more than one study, weighted mean values were calculated based on the number of observations in each study. Absorption following an oral dose of metformin was described as a first-order process after a lag time (t_{lag}) with a mean fraction absorbed (fa) of 0.7 (Pentikainen et al., 1979; Tucker et al., 1981). Estimates of ka were obtained by fitting a one-compartment PK model to mean plasma metformin concentrations reported after administration of 500 to 1500 mg immediate release tablets using Phoenix® WinNonlin® software (Version 6.3, Pharsight, A Certara Company). A weighted mean value of ka was determined based on the number of individuals in each study.

Total metabolic clearance in the liver was incorporated in the model based on back calculation of intrinsic metabolic clearance from the total blood clearance after an intravenous dose (32.0 $L \cdot h^{-1}$) after subtraction of renal clearance with respect to blood (26.1 $L \cdot h^{-1}$), using the method described by Jamei et al. (2014) (Jamei et al., 2014; Pentikainen et al., 1979; Sirtori et al., 1978; Tucker et al., 1981). A fixed average blood-to-plasma ratio of metformin was determined from paired AUC values in blood and plasma after intravenous and oral dosing (Robert et al., 2003; Sambol et al., 1995; Tucker et al., 1981).

The steady state volume of distribution of metformin (V_{ss}) was predicted using the method of Rodgers and Rowland (2007).

Data describing the OCT2, MATE1 and MATE2-K mediated transport of metformin were obtained from a study using transfected HEK293 cells (Ito et al., 2012). Rates of transport were extracted from Eadie–Hoftsee plots and converted from units of $\mu L \cdot min^{-1} \cdot mg\ protein^{-1}$ to $\mu L \cdot min^{-1} \cdot 10^6\ cells^{-1}$ on the basis that 1 million HEK293 cells contain 0.93 mg of total protein (Lazorova, 2010). Estimates of the maximum rate of active transport (J_{max}), Michaelis constant (K_m) and the passive permeability-surface area product (CL_{PD}) were obtained by nonlinear least squares regression using Equation 1. As saturation of transport was not observed at therapeutic concentrations ($K_m > 300\ \mu M$ in all cases) intrinsic clearances ($\mu L \cdot min^{-1} \cdot 10^6\ PTC^{-1}$) for OCT2 uptake and the sum of MATE1 and MATE2-K (MATE1/2-K) efflux were applied in Mech KiM.

$$\text{uptake rate} = \frac{J_{max} \cdot [S]}{K_m + [S]} + CL_{PD} \cdot [S] \quad (1)$$

where the rate of uptake is in units of $pmol \cdot min^{-1} \cdot 10^6\ cells^{-1}$, [S] is the substrate concentration (μM), J_{max} is the maximum rate of active transport ($pmol \cdot min^{-1} \cdot 10^6\ cells^{-1}$), Km is the Michaelis constant (μM) and CL_{PD} is the passive permeability-surface area product ($\mu L \cdot min^{-1} \cdot 10^6\ cells^{-1}$).

The passive permeability of metformin in the kidney ($CL_{PD,kidney}$) was obtained by multiplying permeability in a PAMPA system ($5 \times 10^{-7}\ cm \cdot s^{-1}$ (Balimane and Chong, 2008)) by an estimated total nephron surface area. In Mech KiM, the lengths and diameters of the proximal tubule, loop of Henle, distal tubule and collecting duct are defined for a typical nephron (Neuhoff et al., 2013a). From these, the surface area of a single nephron was calculated and scaled to a total nephron surface area of 291 cm^2 based on the number of nephrons (1.6×10^6) in a representative healthy Caucasian (Neuhoff et al., 2013a).

Hepatic uptake by OCT1 and passive permeability in the liver were applied in the PerL model using estimates of intrinsic clearance and $CL_{PD,liver}$, respectively, from experiments with cryopreserved human hepatocytes (Sogame et al., 2009).

To account for discrepancies in both transporter expression and activity between *in vitro* transfection systems and *in vivo* proximal tubule cells/hepatocytes, relative activity factors (RAFs) were estimated as scalars for each active transport process (Harwood et al., 2013). Currently, experimental RAF values for the relevant transporters are not available. Therefore, operational values were determined by fitting a training set of mean plasma drug concentration-time and urinary excretion data collated from one study in which a 250 mg intravenous bolus dose of metformin was administered (Tucker et al., 1981) and three studies in which 500 mg oral doses (Caille et al., 1993; Karttunen et al., 1983;