Toxicology Letters 343 (2021) 34–43

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Incorporating renal excretion via the OCT2 transporter in physiologically based kinetic modelling to predict in vivo kinetics of mepiquat in rat

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HIGHLIGHTS

- This study incorporated active renal excretion via OCT2 of model compound mepiquat in a physiologically based kinetic model.
- The RPTEC cell line SA7K is proven to be a step forward in its use as a model to define kinetic parameters Vmax and Km for OCT2 uptake.
- Translation of in vitro data to in vivo data is accompanied with usage of adequate scaling factors.

ARTICLE INFO

Article history:
Received 11 December 2020
Received in revised form 19 February 2021
Accepted 22 February 2021
Available online 24 February 2021

Keywords:
Mepiquat
Active renal excretion
Physiologically based kinetic modeling
Renal proximal tubule epithelial cell line
Organic cation transporter 2
Scaling factor

ABSTRACT

The present study aimed at incorporating active renal excretion via the organic cation transporter 2 (OCT2) into a generic rat physiologically based kinetic (PBK) model using an in vitro human renal proximal tubular epithelial cell line (SA7K) and mepiquat chloride (MQ) as the model compound. The Vmax (10.5 pmol/min/mg protein) and Km (20.6 μM) of OCT2 transport of MQ were determined by concentration-dependent uptake in SA7K cells using doxepin as inhibitor. PBK model predictions incorporating these values in the PBK model were 6.7–8.4-fold different from the reported in vivo data on the blood concentration of MQ in rat. Applying an overall scaling factor that also corrects for potential differences in OCT2 activity in the SA7K cells and in vivo kidney cortex and species differences resulted in adequate predictions for in vivo kinetics of MQ in rat (2.3–3.2-fold). The results indicate that using SA7K cells to define PBK parameters for active renal OCT2 mediated excretion with adequate scaling enables incorporation of renal excretion via the OCT2 transporter in PBK modelling to predict in vivo kinetics of mepiquat in rat. This study demonstrates a proof-of-principle on how to include active renal excretion into generic PBK models.

1. Introduction

Physiologically based kinetic (PBK) modelling-facilitated reverse dosimetry is a useful tool for quantitative in vitro-in vivo extrapolation (QIVIVE) to predict in vivo toxicity (Louisse et al., 2017; Zhang et al., 2018). Jointly with the results of in vitro methods, QIVIVE provides an alternative strategy for risk assessment for human safety without the use of animal experiments, thus contributing to the 3Rs (replacement, reduction and refinement of animal experiments) in the field of toxicology (Bessems et al., 2015).

Until now, proofs-of-principle for QIVIVE based on PBK modelling facilitated reverse dosimetry have been provided for a number of adverse outcomes including liver toxicity (Ning et al., 2019), kidney toxicity (Abdullah et al., 2016), developmental toxicity (Louisse et al., 2015; Strikwold et al., 2017) and cardiotoxicity (Shi et al., 2020). These examples, however, did not relate to model compounds for which plasma and tissue concentrations depend on kinetics for excretion.

Abbreviations: PBK, physiologically based kinetic modelling; QIVIVE, quantitative in vitro-in vivo extrapolation; RPTEC, renal proximal tubule epithelial cells; OCT2, organic cation transporter 2; MQ, mepiquat; SA7K, generated human renal proximal tubule epithelial cell line; MATE, multidrug and toxin extrusion transporter; PBS, phosphate buffered saline; BSA, bovine serum albumin.

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http://dx.doi.org/10.1016/j.toxlet.2021.02.013

A few reasons for this lack of including excretion in the PBK models can be identified and relate to: 1) the assumption that for most compounds excretion is not a rate limiting step since molecular weights up to 2000 g/mol can easily pass the filter of the glomeruli (Fagerholm, 2007), 2) the fact that following metabolism to more water soluble and often less toxic metabolites, the role of excretion in determining the physiological concentrations of the relevant parent compound is often negligible (Fabian et al., 2019), and 3) the fact that there is a lack of adequate in vitro models for renal excretion due to its complexity (Bessems et al., 2015).

Renal excretion is the result of three main processes, 1) passive glomerular filtration; 2) active tubular secretion and 3) passive and active tubular reabsorption (Zeidel et al., 2014). This implies that developing in vitro models to predict renal excretion, should enable adequate description of all three processes which may be a challenge especially when active transport is involved. A study by Felmlee et al. (2013) has compared four mechanistic pharmacokinetic models for active secretion and reabsorption and concluded that especially the Vmax of active transporters in renal proximal tubules plays a pivotal role in the prediction of renal excretion.

The most abundant active drug transporter present in the renal proximal tubules of both human and rat is the organic cation transporter 2 (OCT2) located basolaterally (Motohashi and Inui, 2013). OCT2 secretes, together with the H^+/organic cation antiporter multidrug and toxin extrusion (MATE) transporter, cations to the renal lumen. Since OCT2 is Na^+-independent and electrogenic, its transport is driven by the inside-negative membrane potential that exists within the proximal tubule cells (Yin and Wang, 2016). Consequently, the route of renal excretion of cations via the OCT2/MATE complex should be considered when including renal excretion in a PBK model.

The aim of the present study was to develop a proof-of-principle to include active renal excretion in a rat PBK model using renal proximal tubule epithelial cells (RPTEC) to obtain kinetic parameters for OCT2 transport. To this end we used the SA7K cell line described by Li et al. (2017), which is an RPTEC cell line generated from human primary kidney proximal tubule epithelial cells by executing a zinc-finger nuclease-mediated knockout of a cell cycle protein making the cell line so-called pseudo-immortalized. The group of Li et al. (2017) was able to generate this cell line with preserved expression and activity of relevant transporters when cells are in culture, of which OCT2 was one. As a model compound we selected mepiquat chloride (MQ; molecular structure of mepiquat cation is shown in Table 1), a positively charged, non-metabolised compound that is excreted predominantly via the kidneys (Agency, 1997). Implementing the kinetic parameters of MQ transport via OCT2 into a rat PBK model together with the appropriate scaling factors should provide an improved prediction of the blood concentration–time curve compared to predictions made by a model that does not take active renal excretion into account.

2. Materials and methods

2.1. Chemicals

Doxepin hydrochloride was purchased from Carbosynth (Berkshire, UK). (±)Verapamil hydrochloride, bovine serum albumin (BSA) and formic acid were purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands). BASF SE kindly provided mepiquat chloride (MQ). Dimethyl sulfoxide (DMSO) used for dissolving doxepin and verapamil was purchased from Merck (Darmstadt, Germany). Acetonitrile was purchased from Biosolve (Valkenswaard, the Netherlands). Ultrapure water from a system of Arium Pro VF Sartonius (Rotterdam, the Netherlands) was used to dissolve MQ.

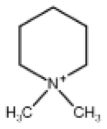
2.2. Cell culture

SA7K cells (Sigma-Aldrich, Zwijndrecht, the Netherlands), a human renal proximal tubule epithelial cell (RPTEC) line containing functional active transporters (Li et al., 2017) were cultured in MEMα (Sigma-Aldrich, Zwijndrecht, the Netherlands) supplemented with RPTEC Complete Supplement (Sigma-Aldrich, Zwijndrecht, the Netherlands), 2.5 mM L-glutamine (Gibco), 30 μg/ml gentamicin and 0.015 μg/ml amphotericin B at 37 °C with 5% (v/v) CO_2 and 95 % (v/v) humidity. For uptake studies cells were seeded in 6-well plates at a density of 1.5×10^6 cells/well and grown for 2 days prior to use with a medium change after one day. Cells used in this study were between 6–22 cell passages.

2.3. OCT2 uptake of MQ

To confirm OCT2 dependent uptake of MQ, SA7K cells were pre-incubated in pre-warmed (37 °C) uptake buffer (136 NaCl, 5.3 mM KCl, 1.1 mM KH_2PO_4, 0.8 mM $MgSO_4$ x 7 H_2O, 1.8 mM $CaCl_2$ x 2 H_2O, 11 mM D-glucose, 10 mM HEPES, pH 7.4) within the presence or absence of the OCT2 inhibitors doxepin and verapamil (Hacker et al., 2015; Li et al., 2017) (final concentration 10 μM and 100 μM, respectively, added from 200 times concentrated stock solutions in DMSO as starting concentrations based on uptake/inhibition studies using the SA7K cell line by Li et al. (2017)) for 10 min at 37 °C. After 10 min, substrate MQ (final concentration 1 μM and 10 μM added from 200 times concentrated stock solutions in ultrapure water) was added and the cells were incubated for 30 and 60 min. After incubation medium was removed and cells were washed twice with ice-cold PBS containing 0.2 % (w/v) BSA (to avoid unspecific binding) and once with ice-cold PBS alone. Cells were lysed with ultrapure water in a freeze-thaw cycle. Protein was measured using the Pierce BCA protein assay kit (Thermo Fischer Scientific Bleiswijk, the Netherlands). MQ present in the cell lysate was quantified using LC–MS.

Table 1
Molecular structure of mepiquat cation from mepiquat chloride (MQ), fragments and ESI-MS parameters used in this study.

Chemical	Molecular structure	MRM transition	Q1 pre Bias (V)	CE (V)	Q3 Pre bias (V)
MQ		114.1 → 98.10	−21.0	−28.0	−19.0
		114.1 → 70.10	−20.0	−36.0	−26.0
		114.1 → 58.10	−18.0	−26.0	−23.0
		114.1 → 42.10	−19.0	−47.0	−15.0

2.4. Time optimisation for OCT2 uptake

SA7K cells were pre-incubated in pre-warmed (37 °C) uptake buffer within the presence or absence of the OCT2 inhibitor doxepin (final concentration 100 μM added from a 200 times concentrated stock solution in DMSO) for 10 min at 37 °C. After 10 min, substrate MQ (final concentration 10 μM added from a 200 times concentrated stock solution in ultrapure water) was added and the cells were incubated for 2, 5, 10, 30 and 60 min. MQ present in the cell lysate was quantified using LC–MS.

2.5. Quantification of OCT2 mediated transport of MQ

Under time-optimised conditions, uptake experiments were continued following the same approach as described above in the absence and presence of the OCT2 inhibitor doxepin at 100 μM final concentration to quantify the OCT2 mediated transport of MQ. After 10 min incubation in the presence or absence of doxepin, substrate MQ (final concentrations 10, 25, 50, 100, 200, 300, 400 and 500 μM added from 200 times concentrated stock solutions in ultrapure water) was added to the cells. MQ present in the cell lysate was quantified using LC–MS. OCT2 mediated uptake of MQ was derived from the difference in transport in the absence and presence of the OCT2 inhibitor.

2.6. Liquid chromatography mass spectrometry (LC–MS/MS) analysis

MQ (Table 1) was analysed on an LC–MS/MS system (Shimadzu, Kyoto, Japan), which contained a Nexera XR LC-20AD SR UPLC system coupled to a triple quadrupole mass spectrometer LCMS-8045. A Luna Omega polar C18 column (Phenomenex) (100 × 2.1 mm, 1.6 μm particle size) was used for separation. The mobile phase consisted of water (A) and acetonitrile (B) both containing 0.1 % (v/v) formic acid (gradient: 0.00–6.00 min 25−100% B, 6.00–6.50 min 100 % B, 6.50–7.00 min 100−25% B, 7.00–10.50 min 25 % B) and was delivered at a flow rate of 0.3 mL/min. The injection volume was 5 μl and the column oven was set at 40 °C. Under these conditions the retention time of MQ was 0.83 min. The parameters used in multiple reaction monitoring (MRM) were as follows: positive-ion mode, electrospray ionisation source, nebulizer gas flowrate: 2.0 L/min, heating gas flow rate: 10.0 L/min, interface temperature: 300 °C, DL temperature: 250 °C, heating block temperature: 400 °C, drying gas flow rate: 10.0 L/min, dwell time: 10 ms, and fragments and ESI-S parameters are presented in Table 1.

2.7. Physiologically based kinetic (PBK) modelling

A physiologically based kinetic model for MQ in rat was developed. The model consists of separate compartments for GI-tract, liver, fat, blood and kidney (Fig. 1). All other organ tissues were placed under rapidly perfused tissue (brain, lungs, heart) or slowly perfused tissue (bone, skin, muscle). The physiological and anatomical parameters including tissue weight and tissue blood flow were obtained from the literature (Brown et al., 1997). Tissue: blood partition coefficients were calculated by a quantitative property-property relationship method described in literature (Rodgers and Rowland, 2006) and obtained via the QIVIVE toolbox developed by Punt et al. (2020). Toolbox input of MQ: LogP = -3.55 (safety data sheet BASF), molecular weight = 149.7 g/mol, pKa = not applicable, ticked box for the presence of quaternary N atom(s). Since MQ is hardly metabolised in the body, no equations and kinetic parameters for clearance were included in the model. For describing active renal excretion of MQ via OCT2, the kinetic parameters of OCT2 mediated membrane translocation of MQ need to be included in the model. Each compartment of the model

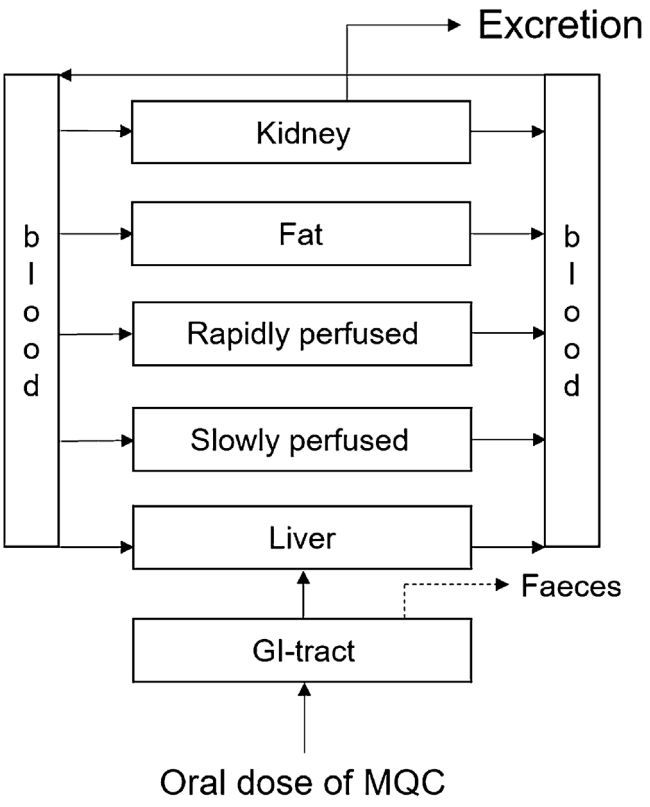


Fig. 1. Schematic overview of the conceptual PBK model for MQ including renal excretion.

contains its own set of mathematical equations. The equation to describe the amount of MQ excreted via the kidney included a term for glomerular filtration (Felmlee et al., 2013) and a term to describe active renal excretion, and was as follows:

$$AKe = (GFR \times (CVK \cdot Fub)) + \left(\frac{Vmax_{OCT2} \times CVK}{(Km_{OCT2} + CVK)}\right) \quad (1)$$

where AKe is the amount of MQ excreted via the kidney in time (μmol/hr), GFR is the glomerular filtration rate of rat (L/hr), CVK the venous concentration of MQ in the kidneys (μmol/L), $Vmax_{OCT2}$ and Km_{OCT2} the maximum rate (μmol/hr) and Michaelis-Menten constant (μmol/L) for the active transport of MQ by OCT2, and Fub the fraction unbound of MQ in blood, which was 1 (Neef and Meijer, 1984).

The glomerular filtration rate in the model was included as a formula depending on the body weight of rat and the glomerular filtration rate in rat, which was reported to be 5.2 ml/min/kg bw (Walton et al., 2004). The formula included was the following:

$$GFR = 0.0052 * BW * 60 \quad (2)$$

where GFR is the glomerular filtration rate (L/hr), BW the body weight (kg) and 60 to convert minutes to hours.

Besides renal excretion, a fraction of 10 % of an oral dose given is known to be excreted via the faeces, indicted in the model as Fef set at 0.1. Berkeley Madonna software (UC, Berkeley, CA, USA version 8.3.18) was used to solve the equations.

2.8. Plasma to whole blood

In the PBK model the blood compartment relates to whole blood. However six out of the eight rat in vivo kinetic data sets (provided by BASF) for model evaluation on time-dependent MQ concentrations were obtained in plasma (the other two data sets

were obtained in whole blood). Therefore, the MQ plasma concentrations were converted to whole blood concentrations assuming that the concentration in plasma is equal to the concentration in the erythrocytes using the following formula:

$$C_{blood} = C_{plasma} \times (1 - Hct) \quad (3)$$

where C_{blood} is the concentration of MQ in whole blood (μmol/L), C_{plasma} the concentration of MQ in plasma (μmol/L) and Hct the rat haematocrit, which was set at 40 %, the average of the range published (Probst et al., 2006). (See Fig. A.1 and A.2 in supplementary material for original plasma-concentration time data).

2.9. Scaling factor

To convert the in vitro obtained Vmax value for OCT2 uptake of MQ in SA7K cells expressed in pmol/min/mg protein into an in vivo Vmax expressed in μmol/hr/kidneys a scaling factor is needed. The scaling factor used in this study consisted of three parts. Part one of the scaling factor was applied to convert the activity expressed per mg protein present in the cells in vitro expressed in pmol/min/mg cellular protein to the activity expressed in a unit that represents the activity in the two whole kidneys expressed in pmol/min/g kidneys. Therefore, we need to know how the amount of protein in the cells in vitro relates to the amount of protein present in the kidneys. A study performed by Kumar et al. (2018) has experimentally quantified how much protein (mg) is present in 1 mg of kidney resulting in 0.3 mg protein/mg kidney, which equals to 300 mg protein/g kidney (units appropriate for our conversion). Using this conversion factor the activity expressed per mg protein in the cells in vitro was converted to the activity expressed per g kidney in vivo. Part two of the scaling factor was required because OCT2 might be only expressed in the cortex part of the kidneys and not in the whole kidneys. This assumption is based on the assumptions also made by Kumar et al. (2018) indicating that OCT2 is located in the RPTEC cells, which in their turn are located in the kidney cortex. This means that conversion of the in vitro OCT2 activity in the cells to an in vivo activity in the kidneys cannot be done based on the total kidney weight, but needs to take into account that only the cortex, that is 70 % of the total kidney weight will contain OCT2. Thus, to convert the activity in the two whole kidneys expressed in pmol/min/g kidneys, obtained by applying the first part of the scaling factor, to a Vmax expressed for the kidneys as a whole in pmol/min/whole kidneys requires multiplication by 0.7 times the weight of the kidney in grams. Finally, part three is required for the scaling that accounts for conversion of the expression and activity of OCT2 in SA7K cells compared to its expression and activity in the renal tubule cells in the kidney cortex. This third part includes i) the differences in the level of OCT2 expression, ii) potential differences in membrane potential between the relevant cells in vitro and in vivo and also, iii) given that the SA7K cell line is a human derived cell line and the PBK model relates to rat, interspecies differences between human and rat. The actual size of this third part of the overall scaling factor was quantified by fitting the PBK model based predictions to available in vivo data, altering the in vitro obtained Vmax in Berkeley Madonna to obtain the best fit. The overall scaling factor was obtained by multiplying the three factors (see results). The conversion of the Vmax for OCT2 mediated transport obtained in vitro to the Vmax for OCT2 mediated transport in vivo could thus be done using the following formula :

$$In\ vivo\ \text{Vmax} = \left(\frac{In\ vitro\ \text{Vmax}}{1,000,000}\right) \times 60 \times \text{SF} \times (\text{Volume of kidneys}) \times 1000 \quad (4)$$

where the factor 1,000,000 is used to convert pmol to μmol, 60 to convert minutes to hours, 1000 to convert kg kidney weight to g kidney weight and SF is the scaling factor encompassing the three parts mentioned above expressed in mg protein/g kidney.

The full model code is presented in Appendix B of the supplementary material.

2.10. Evaluation of the PBK model and sensitivity analysis

The developed PBK model for MQ was evaluated by comparing the predicted blood concentration time curve with available experimental data on the blood concentration time curve of MQ in rat (data from BASF). To visualise the effect of including the urinary excretion in the model the predicted blood concentration time curve was compared with the predicted blood concentration time curve obtained without including this active excretion. In addition, a sensitivity analysis was performed to assess model parameters that influence the model output maximum blood concentration (C_{max}) most. The sensitivity analysis was performed at dose levels used in the available rat studies including a low dose (1.2 mg/kg bw) and a high dose (12 mg/kg bw). Normalised sensitivity coefficients (SCs) were calculated for the model parameters based on the method reported in the literature (Evans and Andersen, 2000) as follows:

$$SC = (C' - C)/(P' - P) \times (P/C) \quad (5)$$

were C is the initial value of the model output, C' is the modified value of the model output resulting from an increase in the parameter value. P is the initial parameter value and P' is the modified parameter value after a 5% increase in its value.

2.11. Data analysis

The apparent Km and Vmax for OCT2 uptake of MQ were determined by non-linear regression analysis in GraphPad Prism version 5.04 using the formula:

$$V = \frac{Vmax \times [S]}{Km_{app} + [S]} \quad (6)$$

where V is the transport rate in pmol/min/mg protein, Vmax the maximum transport rate of MQ in pmol/min/mg protein, S the substrate concentration in μmol/L, Km_{app} the apparent Michaelis-constant in μmol/L.

Given that the kinetics were derived from curves obtained in the presence of the OCT2 inhibitor doxepin, the Km_{app} can be described as follows:

$$Km_{app} = Km\left(1 + \frac{[I]}{Ki}\right) \quad (7)$$

Rewritten:

$$Km = \frac{Km_{app}}{\left(1 + \frac{[I]}{Ki}\right)} \quad (8)$$

where Km is the Michaelis-Menten constant of MQ in μmol/L, I is the concentration of the inhibitor doxepin in μmol/L and Ki the inhibitor constant of doxepin for OCT2 in μmol/L.

As the inhibitory constant of doxepin for OCT2 was not available in the literature an alternative approach was used to obtain the Ki based on the half maximum inhibitory concentration (IC50) of doxepin for OCT2 (in the presence of substrate1-methyl-4-phenylpyridinium (MPP^+)). The following formulas apply (Burlingham and Widlanski, 2003):

$$IC50 = Ki\left(1 + \frac{[S]}{Km}\right) \quad (9)$$

Rewritten:

$$Ki = \frac{IC50}{\left(1 + \frac{[S]}{Km}\right)} \quad (10)$$

where IC50 is the half maximum inhibitory concentration of doxepin in μmol/L, Km the Michaelis-Menten constant of MPP^+ for OCT2 transport in μmol/L and [S] the concentration of MPP^+ in μmol/L.

Using data from Zolk et al. (2009) with: IC50 for doxepin = 13 μmol/L, Km of MPP^+ = 19.5 μmol/L and concentration MPP^+ S = 10 μmol/L, resulted in a Ki of doxepin for OCT2 of 8.6 μmol/L applying formula (10). The value for Ki was used to determine the Km of MQ for OCT2 applying formula (8).

3. Results

3.1. OCT2 mediated uptake of MQ

To ensure that MQ is indeed an OCT2 substrate, the uptake of MQ was measured in the presence and absence of the OCT2 inhibitors doxepin and verapamil. Fig. 2 shows that the uptake of MQ at 1 μM (Fig. 2A) and 10 μM (Fig. 2B) by SA7K cells is inhibited in the presence of doxepin by 34–49% and verapamil by 59–73%. This confirms that MQ uses an organic cationic transport system, OCT2, to be taken up from the medium into the SA7K cells, which represents the first step in its active elimination via the kidneys.

3.2. Optimization of uptake time

To effectively determine kinetic parameter values for Vmax and Km, MQ uptake via OCT2 needed to occur under linear conditions with respect to time. Time-dependent uptake of MQ was studied in the absence and presence of the inhibitor doxepin at 100 μM. Although verapamil showed a slightly stronger inhibition on the OCT2 uptake of MQ, inhibition with doxepin was more consistent throughout the whole study. Given the Ki for doxepin mediated OCT2 inhibition of 8.6 μmol/L (see materials and methods) a concentration of 100 μM was used to effectively block OCT2 mediated transport. The total uptake and the uptake remaining in the presence of the inhibitor are displayed in Fig. 3A. To obtain the net OCT2 mediated active uptake, the uptake remaining in the presence of the inhibitor was subtracted from the total uptake, demonstrating linearity of the OCT2 mediated uptake up until 30 min (Fig. 3B). Therefore, we chose to work with 30 min as incubation time for the concentration-dependent uptake.

3.3. Kinetic constants for OCT2 mediated uptake of MQ

Under optimised conditions, concentration-dependent OCT2 uptake studies of MQ were performed. Fig. 4A shows the total uptake and the remaining uptake in the presence of doxepin of MQ. (A detailed overview of the uptake until 100 μM is found in Supplementary Fig. A.3). Fig. 4B presents the concentration dependent OCT2 mediated transport and reveals that the net OCT2-mediated uptake is following Michaelis-Menten kinetics. Parameter values were obtained following Eq. (6) with Vmax = 10.5 ± 3.5 pmol/min/mg protein and Km_{app} = 260 ± 193 μM. Using Eqs. (7) to (10), the calculated Km of MQ for OCT2 transport was 20.6 μM.

3.4. PBK model predictions of blood concentrations

With the input of the in vitro obtained kinetic parameter values, PBK model predictions were made. For the in vivo data set of 12 mg/kg bw there is no maximal blood concentration (C_{max}) for comparison, so it was decided to predict and compare the concentration detected at t = 1 h ($C_{t=1}$). Fig. 5A and B show that with the partial scaling factor (for protein and the cortex fraction of the kidney of 0.7) the PBK model is overpredicting with the predicted C_{max} being 6.7-fold higher than what was reported (Fig. 5A) (Table 2). When in absence of a C_{max} using $C_{t=1}$, the difference is somewhat bigger amounting to 8.4-fold (Fig. 5B). This indicates that scaling of the in vitro kinetic data from the SA7K cells with a protein factor and correction for the cortex fraction of the liver alone does not suffice to predict the excretion-dependent blood concentration in time of MQ, indicating that additional scaling is needed. To investigate what parameters would influence the predictions to the highest extent and could be used for this additional scaling first a sensitivity analysis was performed. This sensitivity analysis should also reveal whether further scaling of Vmax would likely influence the predictions.

3.5. Sensitivity analysis

Fig. 6 presents the results of the sensitivity analysis. Model parameters with an absolute value for the normalised sensitivity coefficient of ≥ 0.1 were considered. This reveals that the absorption rate constant ka and also the kinetic constants Vmax and Km for the OCT2 mediated transport are influential parameters. This indicates that further scaling of Vmax can be expected to have an effect on the accuracy of the predictions. Therefore it was investigated what extra scaling factor would be required to optimise the predictions, since that scaling factor

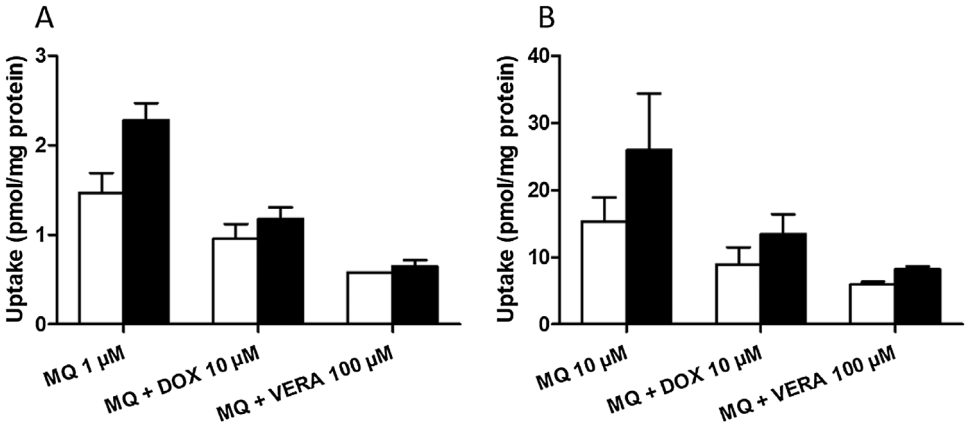


Fig. 2. Uptake and inhibition of MQ uptake in SA7K cells at 1 μM (A) and 10 μM (B) via OCT2 at 30 min (white bars) and 60 min (black bars). Inhibitors used were 10 μM doxepin and 100 μM verapamil. Each bar represents the mean ± SEM of two biological replicates, each with three technical replicates.

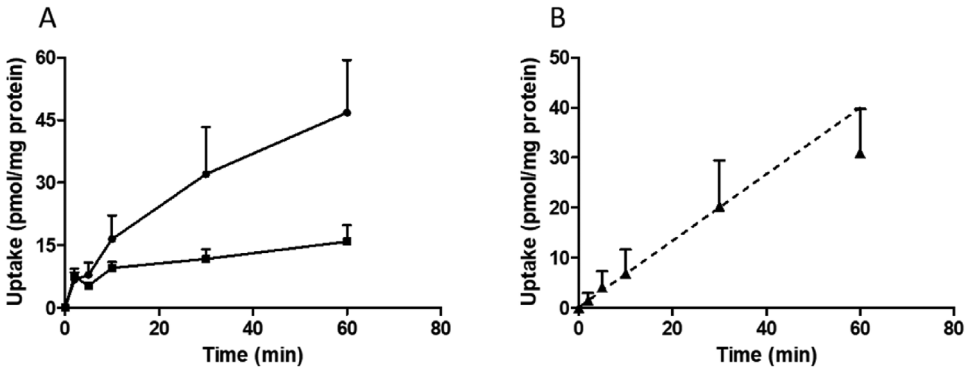


Fig. 3. Time-dependent OCT2 uptake (2, 5, 10, 30 and 60 min) of MQ (10 μM) into SA7K cells. (A) The uptake remaining in the presence of doxepin (100 μM) (squares) was subtracted from the total uptake (circles) to obtain (B) the net OCT2 mediated uptake of MQ. Each data point represents the mean ± SEM of three biological replicates, each with three technical replicates.

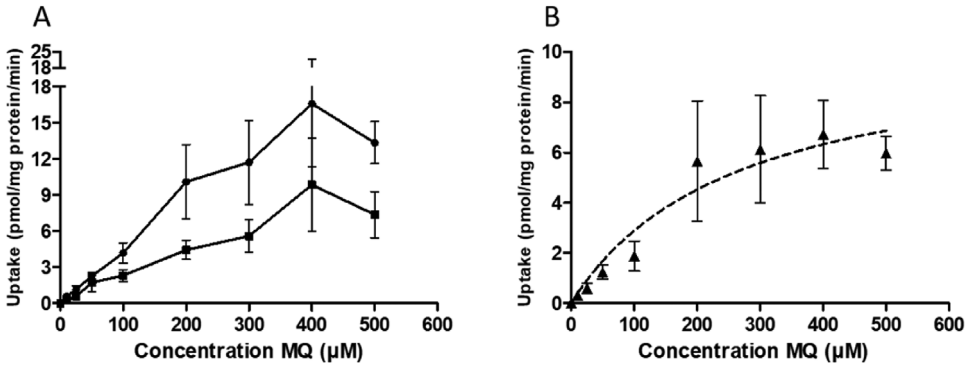


Fig. 4. Concentration-dependent OCT2 uptake of MQ into SA7K cells. (A) The uptake remaining in the presence of doxepin (100 μM) (squares) was subtracted from the total uptake (circles) to obtain (B) the net uptake of MQ (triangles), which follows Michaelis-Menten kinetics with a Vmax of 10.5 pmol/min/mg protein and a Km_{app} in the presence of inhibitor amounting to 260 μM resulting in a Km of MQ for OCT2 transport of 20.6 μM. Each data point represents the mean ± SEM of four biological replicates, each with three technical replicates.

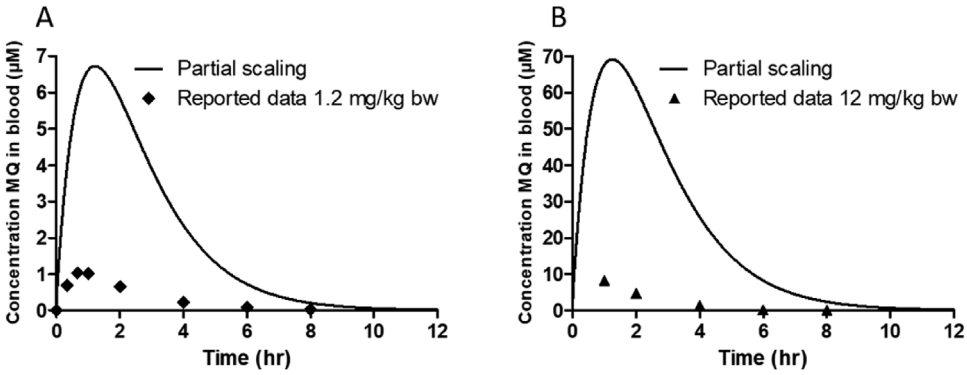


Fig. 5. Predicted and observed blood concentrations (corrected from reported plasma concentrations) of MQ in rats upon oral administration using scaling of the in vitro Vmax obtained in SA7K cells for only a protein factor and correction for the cortex fraction of the kidney. The symbols represent the in vivo data obtained at a dose of 1.2 mg/kg bw (diamonds) and 12 mg/kg bw (triangles). The lines represent the predictions based on partial scaling of the Vmax (SF; 300*0.7 mg protein/g kidney). Each data point represents the mean ± SEM of four replicates of rat studies, each study containing five animals.

would be of use in future studies using the SA7K cells as an in vitro model to define PBK model parameters for taking active OCT2 mediated excretion into account.

3.6. PBK model predictions of the blood concentrations with further scaling of Vmax and ka

Given the deviation between observed and predicted plasma levels it was considered that additional scaling of the Vmax obtained in the SA7K cells when converting it to the Vmax in vivo was required to account for i) the level of expression of OCT2 in the SA7K cells as compared to renal tubule cells in vivo, ii) the effect of differences in the negative membrane potential driving the activity of OCT2 (Kumar et al., 2018) in the SA7K cells and the in vivo situation and iii) species dependent differences in these factors given that the SA7K are human derived cells and in vivo data relate to rat. Given the absence of actual data on these factors the additional scaling factor was determined by defining the Vmax

Table 2
Predicted maximum blood concentration (C_{max} and $C_{t=1}$[a]), and time of maximum blood concentration (T_{max}) with the fold difference (predicted/observed) of PBK model predictions of orally administered MQ in rats.

		Partial scaling		Full scaling		Fitted ka		Passive elimination	
1.2 mg/kg bw	Observed	Predicted	Fold difference	Predicted	Fold difference	Predicted	Fold difference	Predicted	Fold difference
C_{max} (μmol/hr)	1.0	6.7	**6.7**	2.3	**2.3**	1.0	**1.0**	7.0	**7.0**
T_{max} (hr)	0.67	1.10	**0.75**	0.46	**0.69**	0.67	**1.00**	1.27	**1.90**
12 mg/kg bw	Observed	Predicted	Fold difference	Predicted	Fold difference	Predicted	Fold difference	Predicted	Fold difference
$C_{t=1}$[a] (μmol/hr)	8.2	69.1	**8.4**	26.6	**3.2**	11.2	**1.4**	70.2	**8.5**

[a] $C_{t=1}$ is the concentration MQ at t = 1 h. In the data set at dose 12 mg/kg bw no actual C_{max} is presented.

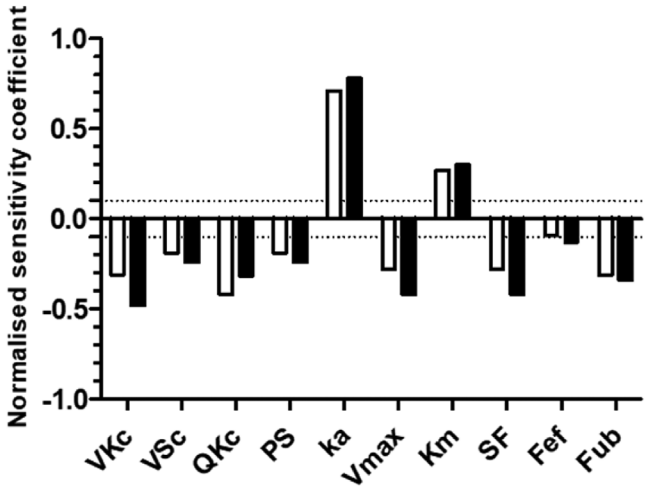


Fig. 6. Normalized sensitivity coefficients of PBK model parameters for the predicted C_{max} of MQ in blood after an oral administration of 1.2 mg/kg bw (white bars) and 12 mg/kg bw (black bars). Only model parameters with normalised sensitivity coefficients with an absolute value higher than 0.1 are shown. VKc = volume of the kidneys, VSc = volume of the slowly perfused tissues, QKc = fraction of blood flow to the kidneys, PS = partition coefficient of slowly perfused tissue, ka = absorption rate constant, Vmax = maximum rate of MQ transport via OCT2, Km = Michaelis-Menten constant of MQ transport via OCT2, SF = scaling factor, Fef = Fraction excreted to faeces, Fub = fraction unbound.

required to obtain the optimal fit for the PBK model predictions, in terms of C_{max}, the time at which C_{max} is obtained (T_{max}) and the profile for the time-dependent decrease in plasma concentrations after the C_{max} has been reached. This was achieved by applying an extra scaling factor of 100 resulting in a Vmax amounting to 1050 pmol/min/mg protein. Multiplying this additional scaling factor with the factor for protein content (300 mg protein/g kidney) and the factor 0.7 for cortex content of the kidney results in an overall scaling factor of 21,000 mg protein/g kidney. In this scaling factor de virtual amount of protein exceeds the kidney weight because the substantially lower expression level of OCT2 in the SA7K cells as compared to kidney tissue requires a virtually large amount of SA7K protein to equal the amount OCT2/g kidney.

Using this overall scaling factor of 21,000 mg protein/g kidney in the PBK model, new predictions were made. Fig.7A and B show that the model predictions improve compared to the reported in vivo kinetic data on the blood concentration of MQ resulting in a 2.3 and a 3.2-fold difference for the C_{max} and $C_{t=1}$, respectively (Table 2). This confirms the influence and importance of Vmax on the model outcome as already shown in the previous section. Furthermore, a full match between the PBK model predictions and the reported data could be obtained by also fitting the other influential parameter ka, the absorption rate constant for uptake of MQ from the gastrointestinal tract into the liver. Fitting the ka (default ka = 1 h^{-1}, reflecting efficient uptake (Punt et al., 2008)), thereby reducing the rate of intestinal uptake, appears essential in approaching the shape of the reported in vivo kinetic concentration response-curve best. Using the optimised scaling factor and a ka of 0.36 h^{-1} the predicted C_{max} was predicted 1 on 1 with the in vivo C_{max} and $C_{t=1}$ was 1.4-fold different from the in vivo $C_{t=1}$, respectively, while in this case the T_{max} of MQ in blood fits one to one (Table 2).

Finally, to further evaluate the importance of active transport in systemic clearance of MQ, Fig. 8 compares the contribution of glomerular filtration and active excretion via OCT2 to the total amount of MQ excreted after 24 h as predicted by the newly defined PBK model at different dose levels. It reveals that when a partial scaling factor for Vmax is applied the contribution of active transport via OCT2 is minimal (1–10 %). This explains directly why the C_{max} and $C_{t=1}$ were overpredicted by the PBK model and

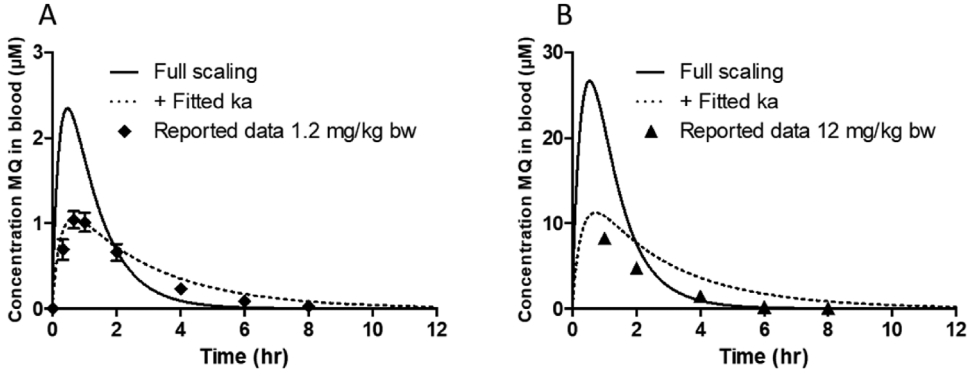


Fig. 7. Predicted and observed blood concentrations (corrected from reported plasma concentrations) of MQ in rats upon oral administration. The symbols represent the in vivo data obtained at a dose of 1.2 mg/kg bw (diamonds) and 12 mg/kg bw (triangles). The lines represent the different predictions based on full scaling of the Vmax (SF; 21,000 mg protein/g kidney) (black lines) and the full scaling plus an added fit of the absorption constant (ka) (0.36 h^{-1}) (dotted lines). Each data point represents the mean ± SEM of four replicates of rat studies, each study containing five animals.

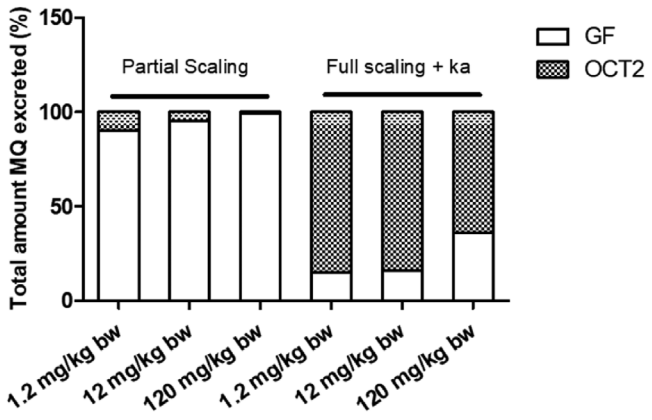


Fig. 8. Contribution (%) of glomerular filtration (GF) and OCT2 mediated excretion to the total amount of MQ excreted after 24 h as predicted by the PBK model when using different scaling factors. Included are partial scaling of the Vmax (SF; 300* 0.7 mg protein/g kidney) and full scaling of the Vmax (SF; 21,000 mg protein/g kidney) combined with a fitted ka. The total amount excreted was predicted for the following doses: 1.2 mg/kg bw, 12 mg/kg bw and 120 mg/kg bw.

illustrates the need for further scaling of Vmax. Furthermore, the results presented in Fig. 8 also reveal that in the final PBK model, including the full scaling factor for Vmax in combination with the fitted ka, the contribution of OCT2 to the total clearance of MQ is 85 % at a dose of 1.2- and 12 mg/kg bw corroborating that MQ clearance is predominantly dependent on OCT2 transport rather than glomerular filtration. Additionally, the model shows that at higher dose, 120 mg/kg bw, the active transport system may become saturated, resulting in a decrease in the relative contribution of OCT2 transport to the total clearance to 64 %.

4. Discussion

Our study aimed at incorporating renal active excretion into PBK modelling using the RPTEC cell line SA7K to determine the values of kinetic parameters Vmax and Km for OCT2 transport of the model compound MQ. The SA7K cell line is a renal tubular kidney cell line generated with preserved expression and activity of OCT2 (Li et al., 2017). This is the uptake transporter of interest in our study since the model compound chosen, MQ, is a cation that is not cleared from the systemic circulation by metabolism but predominantly via active excretion via the kidneys (Agency, 1997). MQ was also chosen as the model compound for the present study because for this compound in vivo kinetic data for PBK model evaluation were available. The SA7K cell line is a kidney cell line and its use eliminates the need for scaling for differences in tissue type (Chan et al., 2019; Chapy et al., 2015). Nevertheless, there is still a need to consider interspecies differences since the SA7K cells represent a human RTPEC cell line, while the PBK model developed in the present study refers to rat. However, given that no rat RPTEC cell line with preserved expression and activity of transporters upon cultivation is available the SA7K cell line was considered the best available in vitro model.

In this SA7K model system MQ was shown to be excreted via active transport involving the OCT2 transporter since its transport was inhibited by the OCT2 inhibitors doxepin and verapamil. The Michaelis-Menten kinetic parameter values for Vmax and Km of OCT2 transport of MQ as determined in our present study amounted to 10.5 pmol/min/mg protein and 20.6 μM respectively. Implementing these values into the PBK model together with the partial scaling factor (300 mg protein/g kidney) and correcting for the fact that only 70 % of the kidney volume consists of cortex where the RPTEC are located (Kumar et al., 2018) resulted in a predicted C_{max} that was 6.7-fold higher than the reported C_{max} value and 8.4-fold higher than the $C_{t=1}$ from the study where a C_{max} was not observed. This resulted in the understanding that scaling for protein and cortex volume alone would not allow use of the SA7K cell model data for in vivo predictions. Further optimisation of the scaling factor by fitting the Vmax to the experimental data resulted in an overall optimised scaling factor of 21,000 mg protein/g kidney, and model predictions for C_{max} and $C_{t=1}$ that deviated from reported values only 2.3 and 3.2-fold, respectively. The importance of Vmax as an influential parameter in the PBK model was in line with a previous study reporting that in the compared reabsorption and secretion models Vmax is critically important in determining renal excretion (Felmlee et al., 2013). Furthermore, this previous study also reported that when using higher dose levels the contribution of glomerular filtration to the renal clearance was increased due to saturation of the active transport system as shown by our model as well. Moreover, the study indicated that to incorporate in vitro obtained Vmax data for active transport into PBK models additional scaling is required. Comparison of available in vivo data to the predictions by our initial PBK model also indicated that additional scaling of Vmax was essential.

The reasons potentially underlying the required further optimisation of the scaling factor are many-fold. First of all the level of expression of OCT2 in the SA7K cells as compared to renal tubule cells in vivo may be lower (Chan et al., 2019; Jamei et al., 2014). In addition, also the difference in activity of the OCT2 transporter is important since gene expression is not equal to activity (Izumi et al., 2018; Poirier et al., 2009; Vogel and Marcotte, 2012). Another factor potentially affecting the difference in OCT2 activity in the SA7K cells and in vivo renal tubule cells may be related to potential differences in the negative membrane potential driving the activity of OCT2. Kumar et al. (2018) for example showed that a twofold factor was applied to correct the plasma membrane potential of their in vitro systems OCT2-expressing HEK293 and MDCKII cells (35 mV) to human RPTEC cells (70 mV). The third factor is related to species differences since the SA7K cells are human derived cells and the in vivo data and the PBK model predictions relate to rat.

A species difference in OCT2 activity may arise from: i) the amino acid identity of organic cation transporters and their expression. The amino acid identity of human OCT2 compared with rat OCT2 is 90 % (Hayer-Zillgen et al., 2002; Koepsell et al., 2003). However, where humans have only OCT2 abundantly expressed in the RPTEC, rats also have OCT1 and to a lesser extent OCT3 expressed in the RPTEC (Chu et al., 2013; Slitt et al., 2002). Since there is a broad overlap of substrates for OCT1–3 there is a probability that MQ in rats has affinity for and will be transported also by OCT1 and OCT3 (Nies et al., 2011; Volk, 2013); ii) the difference in the amount of expression of OCT2 in human RPTEC compared with rat RPTEC in the kidneys. A study by Basit et al. (2019) determined the kidney cortex transporters in different species using quantitative proteomics. According to their results the abundance of OCT2 in human kidney cortex is 164.2 pmol/gram kidney and in rat kidney cortex 253.5 pmol/gram kidney, pointing at a 1.5 fold higher expression level in rat than human. Thus, it can be foreseen that the interspecies differences may account for part of the extra 100-fold scaling factor.

Furthermore, depending on the compound of interest additional transporters may be involved in the active elimination. The membranes of the RPTEC (and many other cell types) contain non-selective cation channels, which transport cations such as Na^+, K^+ or Ca^{2+} but also small compounds such as glycine ethyl ester and choline, hence non-selective (Flockerzi, 2008). MQ is also a small cationic organic compound and could potentially be transported by these non-selective cation channels (Koepsell et al., 2003), and this factor may also contribute to the extra scaling factor.

A further increase of the scaling factor did reduce the fold difference in predicting the C_{max} but at the same time resulted in deviation from the overall fit of the curve with T_{max} being underpredicted and the clearance at prolonged time points being somewhat overpredicted. Fitting of the other influential parameter ka, could result in accurate predictions of the whole dose response curve with C_{max} and $C_{t=1}$ predicted with a 1.0 and 1.4-fold difference, respectively. With respect to further optimisation of this ka value the report from the US EPA only qualitatively describes absorption to be rapid with a bioavailability of 85 % at the highest dose tested (12 mg/kg bw) (Agency, 1997). Our data reveal that with an absorption rate of 0.36 h^{-1} the in vivo reported data are fitted best. Whether further support for such a value can be provided has to await further studies.

This study is one of the few studies attempting on reporting on usage of an in vitro system with expression of active transporters to incorporate active renal excretion into generic PBK modelling for a compound of which the plasma and tissue concentration are known to depend on the kinetics of excretion. In the past, several attempts were made to include active renal clearance into a PBK model. One of the first models was proposed by Russel et al. (1987) including overall active tubular secretion of phenolsulfonphthalein (also known as Phenol red) in a dog PBK model. A more recent study by Huang and Isoherranen (2018) reported on a mechanistic kidney model to predict renal clearance with inclusion of active excretion based on literature-available in vitro transporter data from transfected cell lines and in vitro permeability data from Caco-2 cells. Although the authors were able to predict the renal clearance within two-fold, their model contained 35 compartments, making it complex rather than generic.

To summarize, we have used an in vitro approach with a fairly novel RPTEC cell line SA7K to obtain values for kinetic parameters Vmax and Km for renal OCT2 mediated active excretion of a model compound, MQ, to define the parameters needed to incorporate active excretion into a rat PBK model that can predict how in vivo plasma concentrations rely on active renal excretion. The use of this cell line is a step forward in studying renal transport since most kidney cell lines lose their expression of transporters when in culture (NRK-52E, Caki-1, IHKE-1 and even primary kidney cells) (Lechner, 2014). Although the designers of the SA7K cell line state that their cell line is suitable for transport studies, we note that even though the cell line is kidney derived, the results of the present study reveal that there are still a set of challenges to overcome when translating data obtained in this in vitro model to the in vivo situation. While we were able to determine parameter values of Vmax and Km suitable for PBK modelling, several additional factors had to be considered when translating the in vitro OCT2-mediated MQ uptake measured in the SA7K cell line to kinetic data for in vivo OCT2-mediated uptake. To find out if the overall scaling factor now obtained of 21,000 mg protein/g kidney will allow use of the SA7K model also for prediction of in vivo renal excretion of other OCT2 substrates requires more QIVIVE studies on OCT2 substrates using this SA7K cell line. All in all this study demonstrates a proof-of-principle on how to include active renal excretion into generic PBK models.

Declaration of Competing Interest

The authors report no declaration of interest.

Acknowledgement

This work was supported by BASF SE.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2021.02.013.

References

Abdullah, R., Alhusainy, W., Woutersen, J., Rietjens, I.M., Punt, A., 2016. Predicting points of departure for risk assessment based on in vitro cytotoxicity data and physiologically based kinetic (PBK) modeling: the case of kidney toxicity induced by aristolochic acid I. Food Chem. Toxicol. 92, 104–116.
Agency, U.E.P., 1997. Reregistration eligibility decision(RED) mepiquat chloride. Washington, D.C. 20460, 1–136.
Basit, A., Radi, Z., Vaidya, V.S., Karasu, M., Prasad, B., 2019. Kidney cortical transporter expression across species using quantitative proteomics. Drug Metab. Dispos. 47, 802–808.
Bessems, J., Coecke, S., Gouliarmou, V., Whelan, M., Worth, A., 2015. EURL ECVAM strategy for achieving 3Rs impact in the assessment of toxicokinetics and systemic toxicity. Publ. Off. Europe. Union..
Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol. Ind. Health 13, 407–484.
Burlingham, B.T., Widlanski, T.S., 2003. An intuitive look at the relationship of Ki and IC50: a more general use for the dixon plot. J. Chem. Educ. 80, 5.
Chan, J.C.Y., Tan, S.P.F., Upton, Z., Chan, E.C.Y., 2019. Bottom-up physiologically-based biokinetic modelling as an alternative to animal testing. ALTEX 36, 597–612.
Chapy, H., Klieber, S., Brun, P., Gerbal-Chaloin, S., Boulenc, X., Nicolas, O., 2015. PBPK modeling of irbesartan: incorporation of hepatic uptake. Biopharm. Drug Dispos. 36, 491–506.
Chu, X., Bleasby, K., Evers, R., 2013. Species differences in drug transporters and implications for translating preclinical findings to humans. Expert Opin. Drug Metab. Toxicol. 9, 237–252.
Evans, M.V., Andersen, M.E., 2000. Sensitivity analysis of a physiological model for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): assessing the impact of specific model parameters on sequestration in liver and fat in the rat. Toxicol. Sci. 54, 71–80.
Fabian, E., Gomes, C., Birk, B., Williford, T., Hernandez, T.R., Haase, C., Zbranek, R., van Ravenzwaay, B., Landsiedel, R., 2019. In vitro-to-in vivo extrapolation (IVIVE) by PBTK modeling for animal-free risk assessment approaches of potential endocrine-disrupting compounds. Arch. Toxicol. 93, 401–416.
Fagerholm, U., 2007. Prediction of human pharmacokinetics - renal metabolic and excretion clearance. J. Pharm. Pharmacol. 59, 1463–1471.
Felmlee, M.A., Dave, R.A., Morris, M.E., 2013. Mechanistic models describing active renal reabsorption and secretion: a simulation-based study. AAPS J. 15, 278–287.
Flockerzi, V., 2008. Non-selective cation channels. In: Offermanns, S., Rosenthal, W. (Eds.), Encyclopedia of Molecular Pharmacology.. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp. 870–871.
Hacker, K., Maas, R., Kornhuber, J., Fromm, M.F., Zolk, O., 2015. Substrate-dependent inhibition of the human organic cation transporter OCT2: a comparison of metformin with experimental substrates. PLoS One 10, e0136451.
Hayer-Zillgen, M., Bruss, M., Bonisch, H., 2002. Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. Br. J. Pharmacol. 136, 829–836.
Huang, W., Isoherranen, N., 2018. Development of a dynamic physiologically based mechanistic kidney model to predict renal clearance. CPT Pharmacometrics Syst. Pharmacol. 7, 593–602.
Izumi, S., Nozaki, Y., Kusuhara, H., Hotta, K., Mochizuki, T., Komori, T., Maeda, K., Sugiyama, Y., 2018. Relative activity factor (RAF)-Based scaling of uptake clearance mediated by organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 in human hepatocytes. Mol. Pharm. 15, 2277–2288.
Jamei, M., Bajot, F., Neuhoff, S., Barter, Z., Yang, J., Rostami-Hodjegan, A., Rowland-Yeo, K., 2014. A mechanistic framework for in vitro-in vivo extrapolation of liver membrane transporters: prediction of drug-drug interaction between rosuvastatin and cyclosporine. Clin. Pharmacokinet. 53, 73–87.
Koepsell, H., Schmitt, B.M., Gorboulev, V., 2003. Organic cation transporters. Rev. Physiol. Biochem. Pharmacol. 150, 36–90.
Kumar, V., Yin, J., Billington, S., Prasad, B., Brown, C.D.A., Wang, J., Unadkat, J.D., 2018. The importance of incorporating OCT2 plasma membrane expression and membrane potential in IVIVE of metformin renal secretory clearance. Drug Metab. Dispos. 46, 1441–1445.
Lechner, C.A., 2014. Nierenzellen Als In-vitro-Modell Zur Evaluierung Der Renalen Sekretion Von Arzneistoffkandidaten the Faculty of Bio Sciences Vol. PhD.. Universität Heidelberg.
Li, S., Zhao, J., Huang, R., Steiner, T., Bourner, M., Mitchell, M., Thompson, D.C., Zhao, B., Xia, M., 2017. Development and application of human renal proximal tubule epithelial cells for assessment of compound toxicity. Curr. Chem. Genom. Transl. Med. 11, 19–30.
Louisse, J., Bosgra, S., Blaauboer, B.J., Rietjens, I.M., Verwei, M., 2015. Prediction of in vivo developmental toxicity of all-trans-retinoic acid based on in vitro toxicity data and in silico physiologically based kinetic modeling. Arch. Toxicol. 89, 1135–1148.
Louisse, J., Beekmann, K., Rietjens, I.M., 2017. Use of physiologically based kinetic modeling-based reverse dosimetry to predict in vivo toxicity from in vitro data. Chem. Res. Toxicol. 30, 114–125.
Motohashi, H., Inui, K., 2013. Organic cation transporter OCTs (SLC22) and MATEs (SLC47) in the human kidney. AAPS J. 15, 581–588.
Neef, C., Meijer, D.K., 1984. Structure-pharmacokinetics relationship of quaternary ammonium compounds. Correlation of physicochemical and pharmacokinetic parameters. Naunyn Schmiedebergs Arch. Pharmacol. 328, 111–118.

Nies, A.T., Koepsell, H., Damme, K., Schwab, M., 2011. Organic cation transporters (OCTs, MATEs), in vitro and in vivo evidence for the importance in drug therapy. Handb. Exp. Pharmacol. 105–167.

Ning, J., Chen, L., Strikwold, M., Louisse, J., Wesseling, S., Rietjens, I., 2019. Use of an in vitro-in silico testing strategy to predict inter-species and inter-ethnic human differences in liver toxicity of the pyrrolizidine alkaloids lasiocarpine and riddelliine. Arch. Toxicol. 93, 801–818.

Poirier, A., Cascais, A.C., Funk, C., Lave, T., 2009. Prediction of pharmacokinetic profile of valsartan in human based on in vitro uptake transport data. J. Pharmacokinet. Pharmacodyn. 36, 585–611.

Probst, R.J., Lim, J.M., Bird, D.N., Pole, G.L., Sato, A.K., Claybaugh, J.R., 2006. Gender differences in the blood volume of conscious Sprague-Dawley rats. J. Am. Assoc. Lab. Anim. Sci. 45, 49–52.

Punt, A., Freidig, A.P., Delatour, T., Scholz, G., Boersma, M.G., Schilter, B., van Bladeren, P.J., Rietjens, I.M., 2008. A physiologically based biokinetic (PBBK) model for estragole bioactivation and detoxification in rat. Toxicol. Appl. Pharmacol. 231, 248–259.

Punt, A., Pinckaers, N., Peijnenburg, A., Louisse, J., 2020. Development of a web-based toolbox to support quantitative In-Vitro-to-In-Vivo extrapolations (QIVIVE) within nonanimal testing strategies. Chem. Res. Toxicol..

Rodgers, T., Rowland, M., 2006. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. J. Pharm. Sci. 95, 1238–1257.

Russel, F.G., Wouterse, A.C., van Ginneken, C.A., 1987. Physiologically based pharmacokinetic model for the renal clearance of salicyluric acid and the interaction with phenolsulfonphthalein in the dog. Drug Metab. Dispos. 15, 695–701.

Shi, M., Bouwmeester, H., Rietjens, I., Strikwold, M., 2020. Integrating in vitro data and physiologically based kinetic modeling-facilitated reverse dosimetry to predict human cardiotoxicity of methadone. Arch. Toxicol. 94, 2809–2827.

Slitt, A.L., Cherrington, N.J., Hartley, D.P., Leazer, T.M., Klaassen, C.D., 2002. Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. Drug Metab. Dispos. 30, 212–219.

Strikwold, M., Spenkelink, B., de Haan, L.H.J., Woutersen, R.A., Punt, A., Rietjens, I., 2017. Integrating in vitro data and physiologically based kinetic (PBK) modelling to assess the in vivo potential developmental toxicity of a series of phenols. Arch. Toxicol. 91, 2119–2133.

Vogel, C., Marcotte, E.M., 2012. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat. Rev. Genet. 13, 227–232.

Volk, C., 2013. OCTs, OATs, and OCTNs: structure and function of the polyspecific organic ion transporters of the SLC22 family. WIREs Membr Transp Signal 3, 14.

Walton, K., Dorne, J.L., Renwick, A.G., 2004. Species-specific uncertainty factors for compounds eliminated principally by renal excretion in humans. Food Chem. Toxicol. 42, 261–274.

Yin, J., Wang, J., 2016. Renal drug transporters and their significance in drug-drug interactions. Acta Pharm. Sin. B 6, 363–373.

Zeidel, M.L., Hoenig, M.P., Palevsky, P.M., 2014. A new CJASN series: renal physiology for the clinician. Clin. J. Am. Soc. Nephrol. 9, 1271.

Zhang, M., van Ravenzwaay, B., Fabian, E., Rietjens, I., Louisse, J., 2018. Towards a generic physiologically based kinetic model to predict in vivo uterotrophic responses in rats by reverse dosimetry of in vitro estrogenicity data. Arch. Toxicol. 92, 1075–1088.

Zolk, O., Solbach, T.F., Konig, J., Fromm, M.F., 2009. Structural determinants of inhibitor interaction with the human organic cation transporter OCT2 (SLC22A2). Naunyn Schmiedebergs Arch. Pharmacol. 379, 337–348.