Electronic Supplementary Material: Supplementary material on compound background information, model assumptions, sensitivity analysis, and additional simulations

Title:

Evaluation of BCRP-Related DDIs Between Methotrexate and Cyclosporin A Using Physiologically Based Pharmacokinetic Modelling

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1 Compound ADME (and models)

1.1 Methotrexate

Methotrexate (MTX) is a folate antagonist used to treat a wide range of neoplastic conditions [1], as well as autoimmune conditions such as rheumatoid arthritis (RA) and psoriasis [2]. For autoimmune diseases such as RA, MTX is administered orally (PO) or subcutaneously as a prodrug at a 7.5–25 mg weekly dose and becomes active upon (reversible) polyglutamation [2].

MTX is metabolised in the liver by aldehyde oxidase (AOX), forming 7-OH-MTX (a non-active metabolite causing MTX-related adverse events), and by folylpolyglutamate synthetase,

forming active polyglutamated metabolites, which are converted back to MTX by hydrolases. MTX is discussed to be a substrate of organic anion transporting polypeptides (OATP) 1Bs and OATs [3,4], but conclusions on pharmacogenomic impact of polimorphisms remain controversial [5]. In addition, clinical DDIs of CsA on MTX through OATP1B have not been studied specifically or not mentioned as a relevant interaction pathway [6]. It is mainly excreted through the kidney (90%), predominantly by glomerular filtration rate (GFR), but renal clearance exceeds creatinine clearance by 28%, suggesting an active elimination process(es). Ten per cent of MTX is excreted through the liver by biliary clearance (1.17–3.49 L/h) [7].

1.2 Cyclosporin A

Cyclosporin A (CsA) is a calcineurin inhibitor (it inhibits T-cell activation intracellularly as cyclosporin–cyclophilin complex) [8], and is isolated from the fungus *Beauveria nivea* [9]. It is known for its immunomodulatory properties that prevent organ transplant rejection and treat various inflammatory and autoimmune conditions [10]. Cyclosporin A is administered PO and intravenously at a 5–15 mg/kg daily dose for predominant use in organ transplantation (small therapeutic range: TDLO (toxic dose low) = 12 mg/kg). Cyclosporin A was initially approved for use by the Food and Drug Administration (FDA) in 1983 and is now available in various products.

Cyclosporin A is a relatively large molecule (>1000 g/mol) but is highly permeable with low solubility, which might be absorption-rate limiting (Biopharmaceutics Classification System II). As solubility is bile-acid-dependent, it exhibits a food effect. A second peak is occasionally observed, following intake in fed state, which may be due to bile acid-enhanced solubility and absorption (not observed after intravenous dosing due to low biliary excretion). The absorption of Sandimmune Soft Gelatin Capsules and Oral Solution have been reported to be erratic, according to the Novartis prescribing information (product label). Cyclosporin A exhibits low to no excretion in urine nor in bile [11].

Cyclosporin A is mainly metabolised in the liver with a predominant fraction of CYP3A4-mediated metabolism (metabolites M1 + M17 [+ M21] and a minor CYP3A5 involvement [M17; negligible]). While its metabolites are cleared via biliary excretion, there is no relevant biliary excretion of the parent (<1.5%). Although CsA is deemed a P-glycoprotein substrate, no or only minor renal clearance is reported (UW DIDB; (University of Washington-Drug Interaction Database), and its pharmacokinetics are unchanged in renally impaired patients [11].

The fraction unbound in plasma (f_u) of CsA is highly variable [12] within a range of 3.5–17% [12–14]. Cyclosporin A is >80% lipoprotein-bound, and variations in measured f_u values correlate with blood lipoprotein (e.g. cholesterol) levels. Cyclosporin A rapidly binds (~10 min) to red (RBCs) and white blood cells [11], and this binding consists of a combination of cellular uptake and intracellular bindin [15]. At therapeutic concentrations, 50–90% of CsA is bound to (or internalised by) erythrocytes [11]. Cyclosporin A binds intracellularly to the CsA binding partner cyclophilin (CyP) in a 1:1 fashion (at concentrations of 1 mg/L) [11] with a K_d = 36.8 nM [16]. A total binding capacity of 2.56 mg/L (~2 µmol/L) in packed erythrocytes is reported (although it is unclear if this is fully attributable to binding to CyP A, as CyP A levels in RBCs are only 0.4 µmol/L [15]).

Cyclosporin A exhibits a clear saturation of CsA partitioning to blood cells above concentrations of 1000 µg/L [11], with blood/plasma ratios switching from ~1.5 at concentrations below 100 ng/ml to <0.5 at concentrations above 1000 ng/ml [11]. This has been observed for high intravenous (IV) doses (e.g. 4 mg/kg). However, the causes for the nonlinearity of partitioning are still not fully elucidated in the literature and might be due to saturation of binding partner cyclophilin and/or phospholipid binding (partitioning) and/or facilitated saturating uptake transport to RBCs.

2 Study Data

The following tables provide an overview of published studies, which report the pharmacokinetics of MTX (Table S1) and CsA (Table S2) in humans.

Table S1 Overview of literature reports contributing data to the development of the clinical physiologically based pharmacokinetic model for MTX in humans

#	Reference	Population	Route	Dosage	Analytes	#Subjects /arm	Matrix	Use in model development
1	Baluom 2011 [17]	RA	PO	5–25 mg weekly	MTX + 7-OH-MTX	16	Plasma	Validation
2	Bannwarth 1996 [3] and/or Seideman 1993 [7]	RA	IV and PO	15 mg SD	MTX + 7-OH-MTX	14	Plasma	Training set
3	Carmichael 2002 [18]	HV	IV and PO	15 mg SD	MTX	1	Plasma	Training set
4	Chladek 1997 [19]	Psoriasis	PO	15 mg weekly	MTX + 7-OH-MTX	10	Plasma	Validation
5	Chladek 2002 [20]	Psoriasis	PO	2.5 or 5 mg 3×BID weekly	MTX + 7-OH-MTX	12	Plasma + urine	Validation
6	Chladek 2008 [21]	Psoriasis	PO	10 mg SD	MTX	2×10	Plasma	Validation
7	Cohen 2009 [22]	RA	PO	15–25 mg weekly	MTX	12	Plasma	Validation
8	Fox 2003 [6]	RA	PO	7.5–22.5 mg weekly	MTX + 7-OH-MTX	30	Plasma + urine	Validation
9	Haagsma 1996 [23]	RA	PO	7.5 mg SD	MTX	15	Plasma + urine	Training set
10	Hartmann 2004 [24]	RA	PO	7.5 –15 mg weekly	MTX + 7-OH-MTX	18	Plasma	Validation
11	Herrick 1996 [25]	RA	PO	7.5 mg weekly	MTX	10	Plasma	Validation
12	Hroch 2007 [26]	Psoriasis	PO	10 mg SD	MTX	15	Plasma	Validation
13	Iqbal 1998 [27]	RA	PO	7.5 mg SD	MTX	37	Plasma	Validation
14	Jones 2009 [28]	HV	PO	7.5 mg SD	MTX	13	Plasma	Training set

15	Liu 2014 [29]	RA	PO	10, 12.5, 15, 17.5 or 20 mg SD	MTX + 7-OH-MTX	1–6	Plasma	Validation
16	Namour 2012 [30]	HV	PO	7.5 mg SD	MTX	6	Plasma	Validation
17	Odderskov 2019 [31]	RA	PO	7.5 mg weekly	MTX	14–16	Plasma	Training set
18	Oguey 1992 [32]	RA	PO	15 mg SD, FE study	MTX	10	Plasma	Training set Food effect
19	Pichlmeier 2014 [33]	HV	PO and SC	7.5–30 mg	MTX	12–14	Plasma	Validation
20	Schwartz 2001 [34]	RA	PO	7.5–20 mg weekly	MTX + 7-OH-MTX	18	Plasma	Validation
21	Schwartz 2009 [35]	RA	PO	12.5 mg weekly; 2 studies	MTX	4–25	Plasma	Validation
22	Seideman 1993 [36]	RA	IV, PO, im	15 mg SD	MTX + 7-OH-MTX	9–14	Plasma + urine	IV and PO training set; im evaluation
23	Vakily 2005 [37]	RA	PO	7.5–15 mg weekly	MTX + 7-OH-MTX	27	Plasma	Validation
24	Vergunst 2009 [38]	RA	PO	7.5–25 mg weekly	MTX + 7-OH-MTX	10	Plasma	Validation
25	Yamazaki 2017 [39]	HV	PO	7.5 mg SD	MTX + 7-OH-MTX	24	Plasma	Training set
26	Zhang 2010 [40]	RA	PO	15 mg SD	MTX + 7-OH-MTX	15	Plasma	Training set

BID twice daily, *FE* food effect, *HV* healthy volunteer, *im* intra muscular, *IV* intravenous, *MTX* methotrexate, *PO* orally, *RA* rheumatoid arthritis, *SD* single dose

Table S2 Overview of literature reports contributing data to the development of the clinical physiologically based pharmacokinetic model for CsA in humans

#	Reference	Population	Route	Dose	Anylates	Formulation	Matrix	Use in model development
1	Gupta 1990 [41]	HV	IV infusion, 2.5 h	4 mg/kg	CsA	Unknown	Whole blood, plasma	Training set
2	Hebert 1992 [42]	HV	IV infusion, 2.5 h	3 mg/kg	CsA	Unknown	Whole blood	Training set
3	Reymond 1988 [43]	HV	PO	700 mg	CsA	Solution (Sandimmune) 224 g whole milk	Whole blood	Training set
4	Lim 2012 [44]	HV	PO	2 and 200 mg	CsA	Neoral	Whole blood	Training set
5	Ducharme 1995 [45]	HV	IV infusion, 3 h	2.5 mg/kg	CsA		Whole blood	Validation
6	Ptachcinsky 1987 [13]	HV	IV infusion, 2 h	2.1 mg/kg	CsA	Unknown	Whole blood	Validation
7	Ku 1998 [46]	HV	IV infusion, 3 h; PO	1.5 mg/kg; 5 mg/kg	CsA	Microemulsion	Whole blood	Validation
8	Gupta 1990 [41]	HV	PO	10 mg/kg	CsA	Unknown, low-fat diet	Whole blood, plasma	Validation
9	Ducharme 1995 [45]	HV	PO	7.5 mg/kg	CsA	Sandimmune Soft Gelatin Capsules	Whole blood	Validation
10	Kropeit 2018 [47]	HV	PO	50 mg	CsA	Sandimmune Optoral	Plasma	Validation
11	Reymond 1988 [43]	HV	PO	350/700/1400 mg	CsA	Solution (Sandimmune 224 g whole milk	Whole blood	Validation
12	Chang 1996 [48]	HV	PO	10 mg/kg	CsA	Sandimmune, with chocolate milk	Whole blood	Validation

CsA cyclosporin A, *HV* healthy volunteer, *IV* intravenous, *PO* orally

3 Model Structure

3.1 PBPK Model Structure

A whole-body PBPK model (Fig. S1) contains an explicit representation of the organs that are most relevant to the absorption, distribution, excretion, and metabolisation of the drug due to their physiological/pharmacological function or their volume. In PK-Sim, these are heart, lung, brain, stomach, spleen, pancreas, gut (large and small intestine), liver (zonated), kidney, gonads, adipose tissue, muscle, bone, and skin. The tissues are linked by the arterial and venous blood compartments, and each one of them is characterised by an associated blood-flow rate, volume, tissue-partition coefficient, and permeability [50].

Organs are subcompartmentalised into plasma, blood cell, interstitial and intracellular compartments (for proteins this is extended by the endosomal space). Diffusion across the vascular wall from plasma to interstitial space of the organs or diffusion across the cellular membranes of tissue cells or red blood cells determines how fast drug distribution takes place and can be rate-limiting for distribution (permeation-limited kinetics). In general, the diffusion across the vascular wall is assumed to be fast for small molecules. Transport across the membranes can be facilitated with active transport (uptake or efflux, see next section).

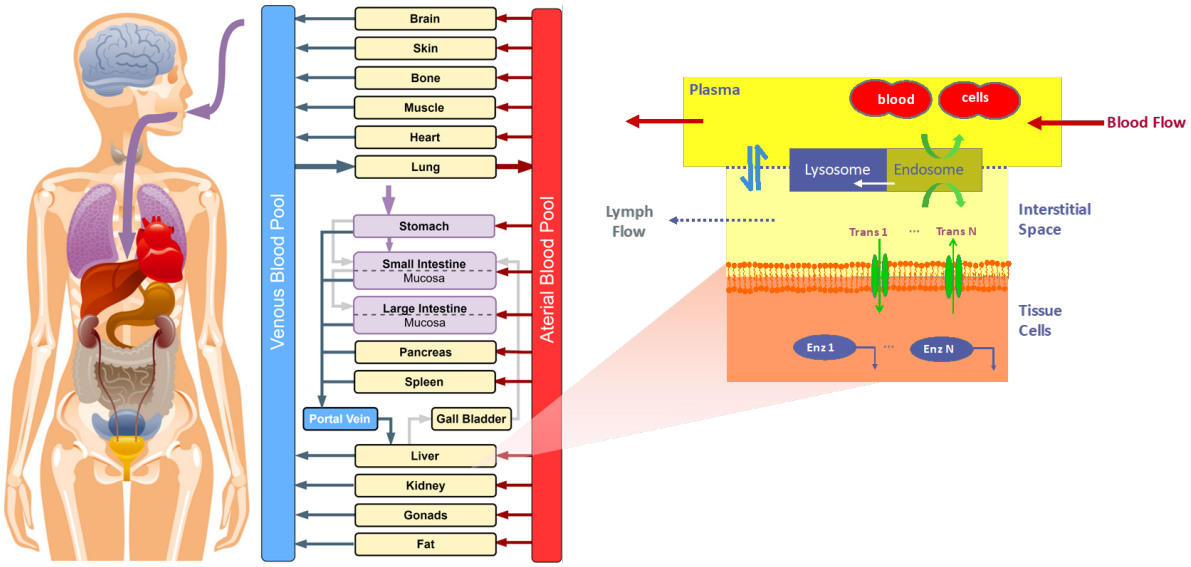

Fig. S1 Physiologically based pharmacokinetic model structure in PK-Sim. A detailed description of how to build physiologically based pharmacokinetic models with PK-Sim can be found in the software documentation [51] and in tutorial literature [50].

3.2 Enzyme Expression

A description of transport protein localisation provided in the PK-Sim manual on expression [52], i.e. localisation of transporters (localisations, directions, and initial concentrations of transport proteins) as depicted in Fig. S2.

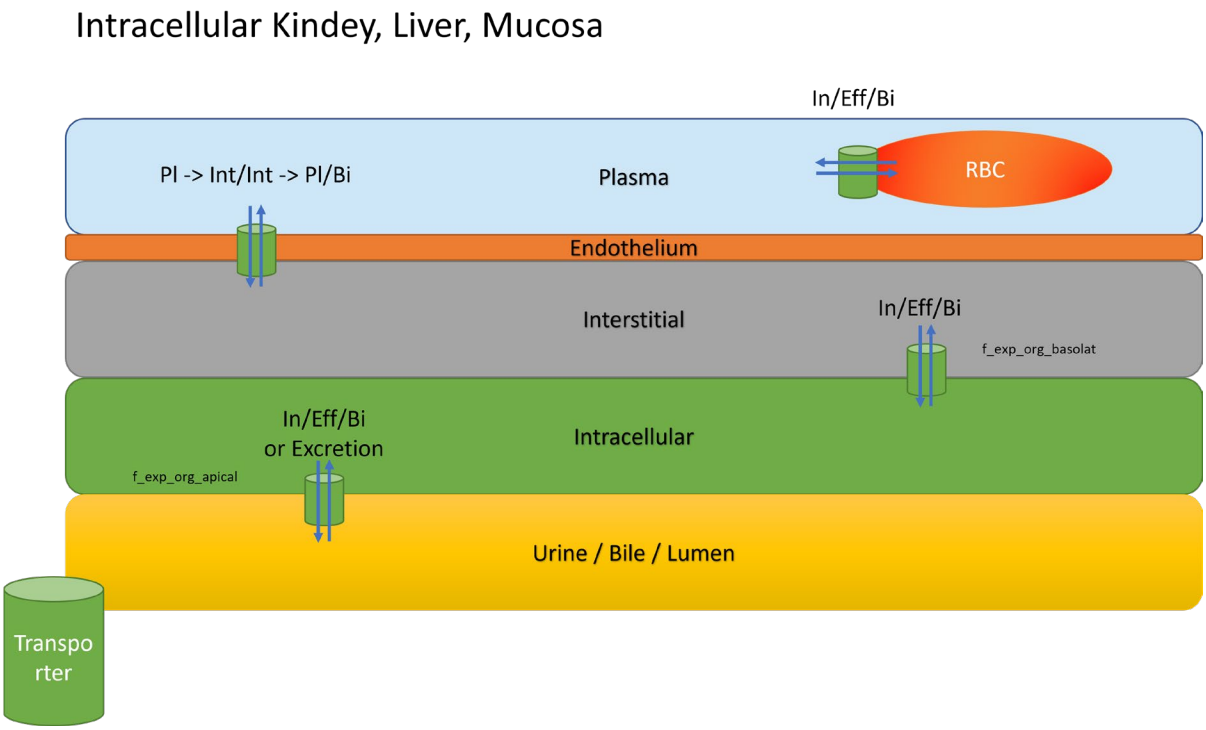

Fig. S2 Localisation of transport proteins in a tissue in the PBPK software PK-Sim.

3.3 Drug-Drug-Interactions

A description of drug interaction kinetics is provided in the PK-Sim manual on compound kinetics [53], e.g. the implementation of competitive inhibition. In a competitive enzyme inhibition, the inhibitor binds reversibly to the enzyme and competes with the substrate for free enzyme. In the case of a reversible inhibition, high substrate concentrations can overcome this inhibition. The apparent Michaelis-Menten constant increases while the apparent maximum reaction velocity remains unchanged.

4 Model evaluation and output

The statistical summary of the pharmacokinetic (PK) properties C_{max} and AUC were used for model performance verification of the PBPK model. The C_{max} and AUC were determined from the averaged profiles with non-compartmental analysis for literature that did not provide

summary statistics of exposure parameters. The simulated PK properties were calculated from the individual simulated curves or the geometric mean of population simulations (*n* = 100), using similar demographical and dosing information from literature, considering the dose, route of administration, age, sex proportionality (male/female ratio), ethnicity and body weight. The (geometric) mean fold error ([G]MFE; the difference between predicted in silico and observed in vivo values from the qualification data set) was used for model evaluation.

$$MFE = \frac{PK\ parameter_{predicted\ mean}}{PK\ parameter_{observed\ mean}} \qquad \text{(Equation S1)}$$

$$GMFE = 10^{\Sigma\left(log_{10}\left(\frac{PK\ parameter_{predicted}}{PK\ parameter_{observed}}\right)\right)/n} \qquad \text{(Equation S2)}$$

The model was accepted when all predicted PK parameters were within 2-fold of the corresponding observed values (e.g. MFE 0.5–2.0).

Predicted drug–drug interaction (DDI) AUC_{last} and C_{max} ratios were evaluated as described in Equations S3 and S4.

$$DDI\ AUC_{last}R = \frac{\text{AUClast victim drug during co} - \text{administration}}{\text{AUClast victim drug control}} \qquad \text{(Equation S3)}$$

$$DDI\ C_{max}R = \frac{\text{C}_{\text{max}}\ \text{victim drug during co} - \text{administration}}{\text{C}_{\text{max}}\ \text{victim drug control}} \qquad \text{(Equation S4)}$$

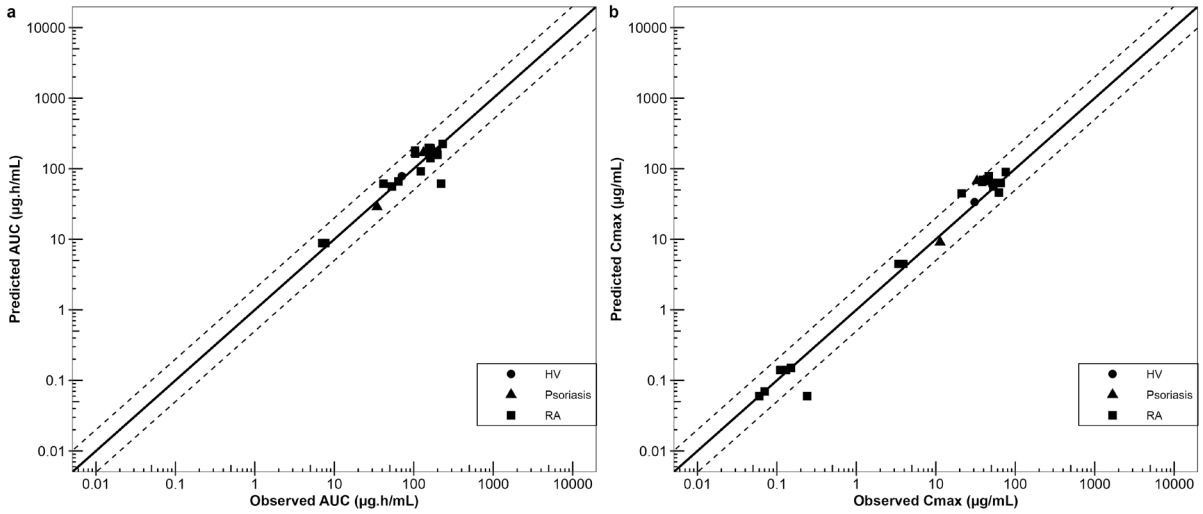

Fig. S3 Comparison between simulated and observed pharmacokinetic parameters of MTX-OH from all studies. *Solid lines* represent line of unity; dashed lines represent 2-fold difference. GMFE 7-OH-MTX C_{max} = 1.16; GMFE 7-OH-MTX AUC = 1.04. *AUC* area under the curve, *C_{max}* peak plasma concentration, *GMFE* geometric mean fold error, *MTX* methotrexate, RA rheumatoid arthritis

5 Sensitivity Analysis

We performed a sensitivity analysis on the final developed PBPK models of MTX and CsA to investigate the effect of changing model parameters on the PK properties C_{max} and AUC_{last}. In a sensitivity analysis, model parameters were deviated (e.g. increased or decreased by 10%), and the effect on PK-related parameters (C_{max}, T_{max}, AUC etc.) was investigated.

The sensitivity for the PK parameter to an input parameter is calculated as the ratio of the relative change of a PK parameter [= (ΔPKj) / PKj] and the relative variation of the input parameter [= (Δpi) / pi].

Thus, the sensitivities are dimensionless quantities. As an example, a sensitivity value of −1.0 implies that a 10% increase of the parameters leads to a 10% decrease of the PK parameter value, and a sensitivity value of +0.5 implies that a 10% increase of the parameters leads to a 5% increase of the PK parameter value.

The outcomes are shown in Fig. S4 and S5 (CsA) and Figs. S6 and Fig. S7 (MTX). For Csa, for both the C_{max} and AUC, the lipophilicity is the most sensitive parameter in the model, followed by protein binding and CYP3A4 metabolism. For MTX, for both, the C_{max} and AUC, parameters associated with intestinal solubility (pH), protein binding and renal clearance are the most sensitive parameters in the model, followed by general distribution parameters (organ volumes) and kinetic parameters of BCRP (*ABCG2*) and AOX.

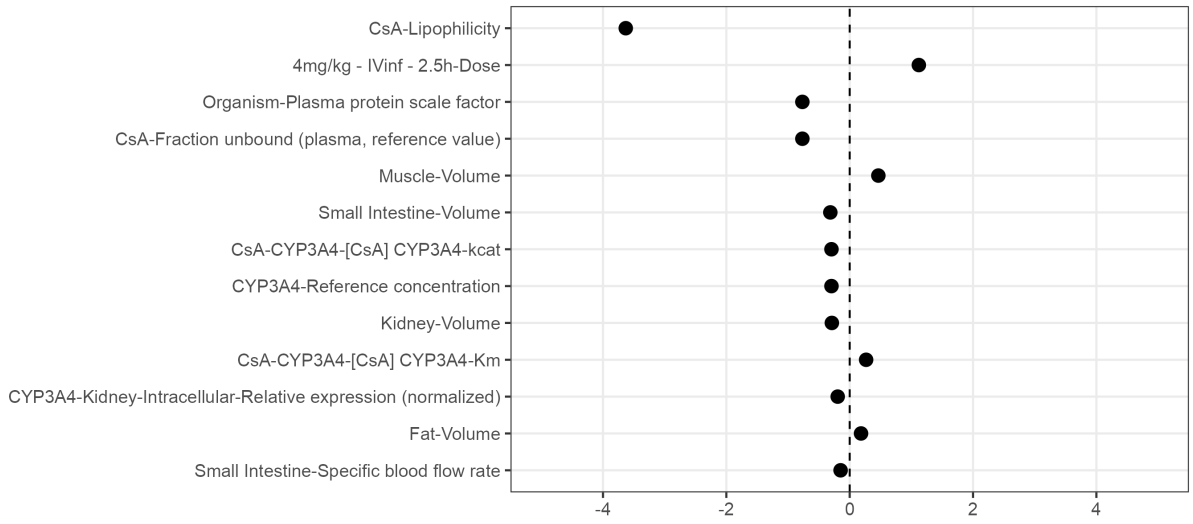

Fig. S4 Results of the sensitivity analysis, which investigated the effect of changing model parameters on pharmacokinetic-related outcome parameters for CsA. In this case, the effects of model parameters on the estimated AUC_{last} in peripheral venous blood are presented. *AUC_{last}* area under the curve, *CsA* cyclosporin A, *IV* intravenous

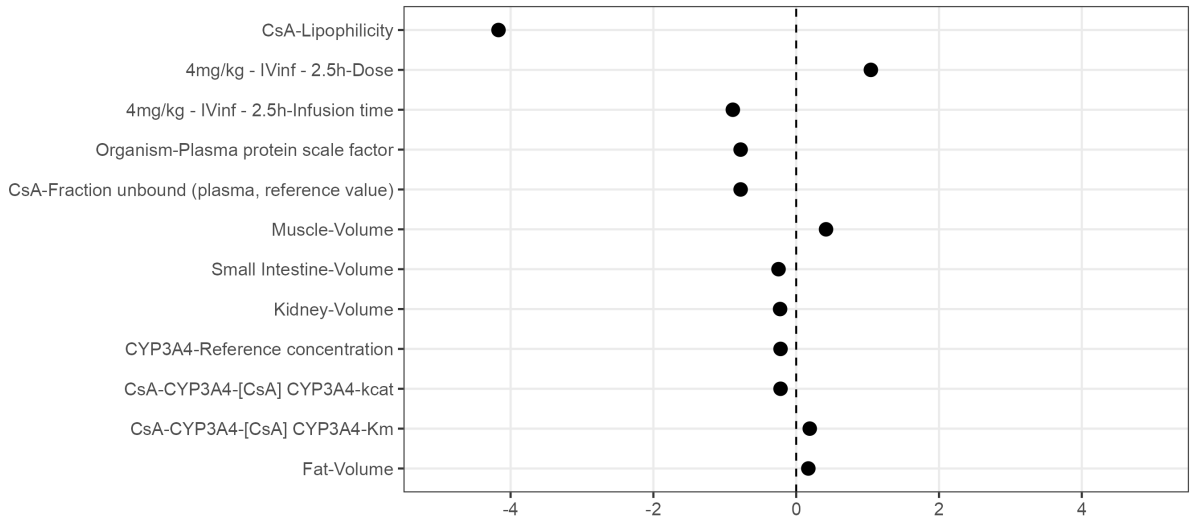

Fig. S5 Results of the sensitivity analysis, which investigated the effect of changing model parameters on pharmacokinetic-related outcome parameters for CsA. In this case, the effects of model parameters on the estimated C_{max} in peripheral venous blood are presented. *C_{max}* peak plasma concentration, *CsA* cyclosporin A, *IV* intravenous

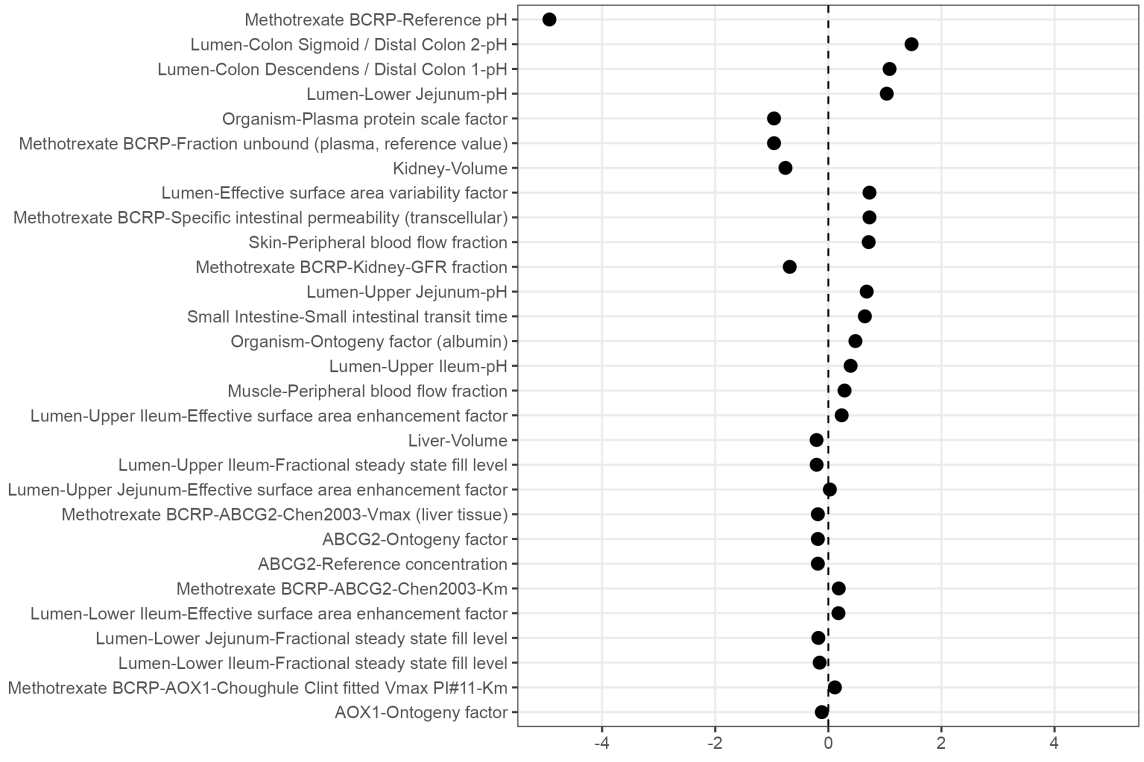

Fig. S6 Results of the sensitivity analysis, which investigated the effect of changing model parameters on pharmacokinetic-related outcome parameters for MTX. In this case, the

effects of model parameters on the estimated AUC_{last} concentration in peripheral venous blood are presented. *AOX* aldehyde oxidase, *AUC_{last}* area under the curve, *BCRP* breast cancer resistance protein transporter, *GFR* glomerular filtration rate, *MTX* methotrexate

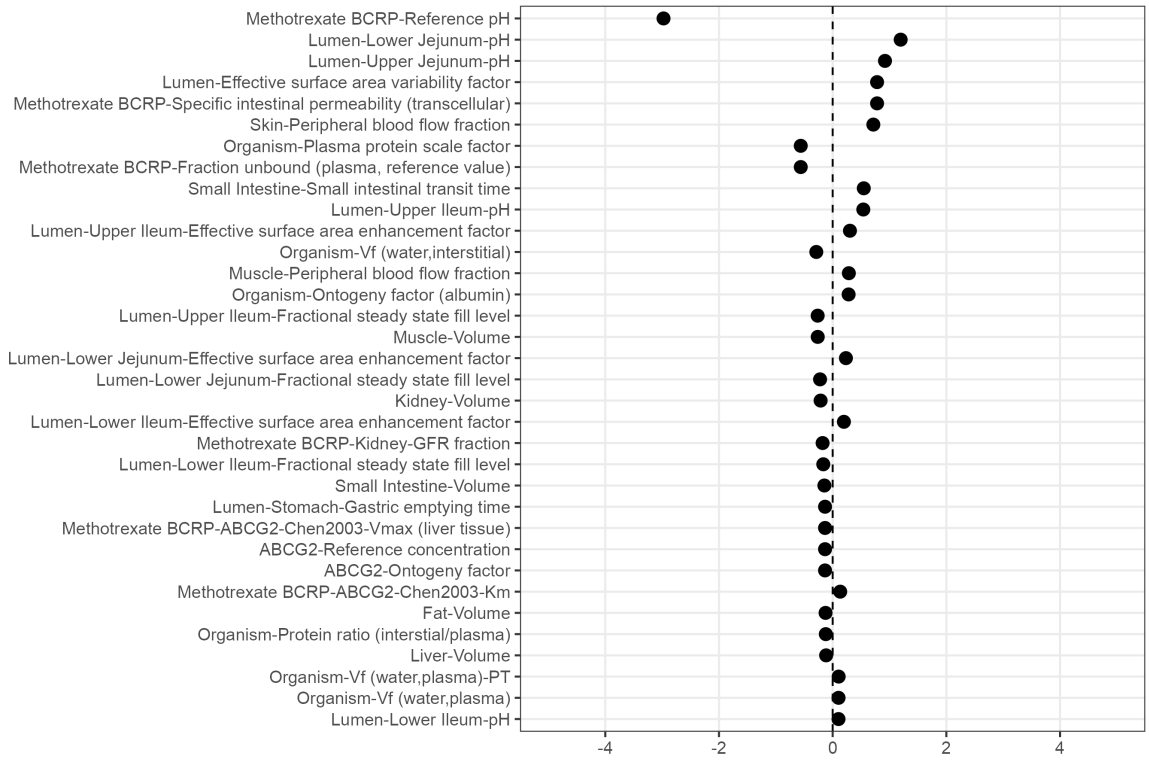

Fig. S7 Results of the sensitivity analysis, which investigated the effect of changing model parameters on pharmacokinetic-related outcome parameters for MTX. In this case, the effects of model parameters on the estimated C_{max} in peripheral venous blood are presented. *BCRP* breast cancer resistance protein transporter, *C_{max}* peak plasma concentration, *GFR* glomerular filtration rate, *MTX* methotrexate, *Vf* volume fraction

6 BCRP effect size evaluation for MTX PBPK model

As a secondary evaluation of the total effect size of BCRP transport on MTX pharmacokinetics, a BCRP “knock-out” scenario was simulated. This evaluation was compared with a pharmacogenomic analysis of several single-nucleotide polymorphisms in BCRP by Liu et al. 2018 [49], which showed a maximal reduction of 16% of total clearance after IV dosing.

Switching off BCRP in the model results in a reduction in total body clearance of 45% both after IV and after PO dosing, and an 8% increase in C_{max} and a 71% increase in AUC. This indicates a sufficient maximum effect size of BCRP in the model to predict the outcome of the reported DDI on the AUC (Fox et al. 2003), which was reported as a 32% increase, not for C_{max}, which was reported as a 28% increase.

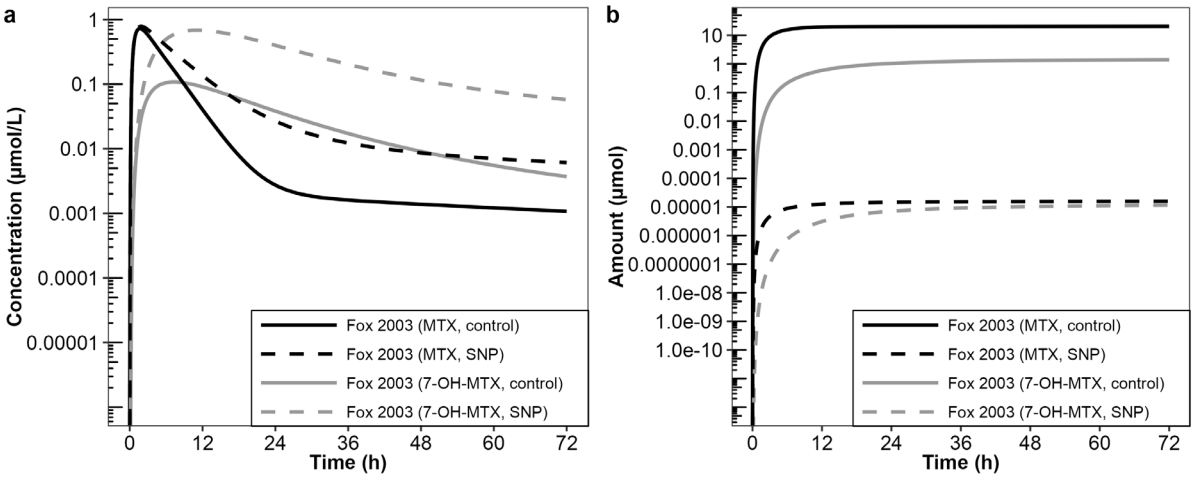

Fig. S8 Outcomes for the MTX physiologically based pharmacokinetic model for the effect-size evaluation of BCRP transport simulating a BCRP "knock-out" scenario (tagged "SNP") for a dose of 15 mg PO. *Black curves* represent simulated data for MTX, while *grey curves* represent simulated data for MTX metabolite 7-OH-MTX. Panel a corresponds to the plasma concentration–time curves, while panel b depicts the amount excreted in urine of the respective compounds over time. *Solid lines* represent the original model parameterisation, while *dashed lines* represent outcomes for a full BCRP "knock-out". Without BCRP, an 8% increase in C_{max} and a 71% increase in AUC were predicted by the model for MTX, and a 600% increase in C_{max} and a 470% increase in AUC were predicted for 7-OH-MTX. *AUC* area under the curve. *BCRP* breast cancer resistance protein transporter, C_{max} peak plasma concentration, *MTX* methotrexate, *PO* orally, *SNP* single-nucleotide polymorphism

7 Additional simulation results

7.1 MTX

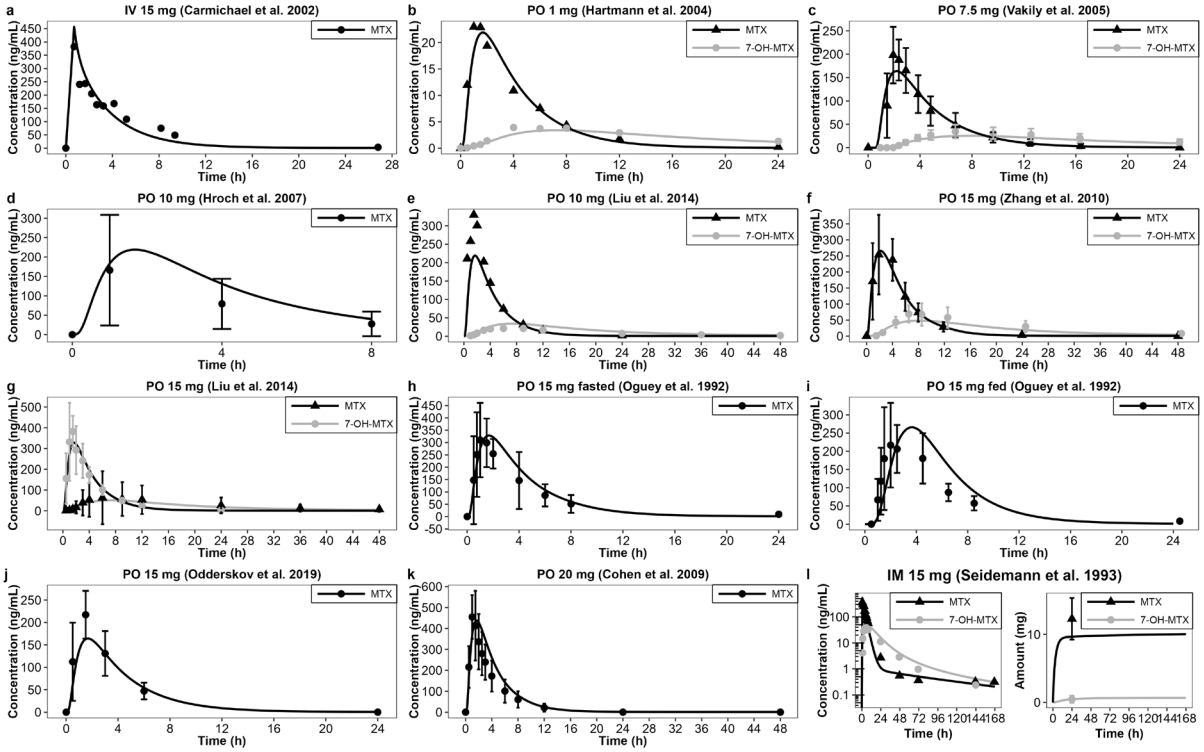

Fig. S9 Outcomes for the MTX physiologically based pharmacokinetic model for both training and validation data sets (Table S1) for doses ranging from 1 to 20 mg IV, IM, and PO. *Black dots*, *triangles*, and *curves* represent observed and simulated data for MTX, while *grey dots* and *curves* represent observed and simulated data for MTX metabolite 7-OH-MTX. The right plot from panel l depicts the amount excreted in urine of the respective compounds over time. Plots are on linear and logarithmic scales. Observed data is referenced in Table S1. *IM* intramuscular, *IV* intravenous, *MTX* methotrexate, *PO* orally

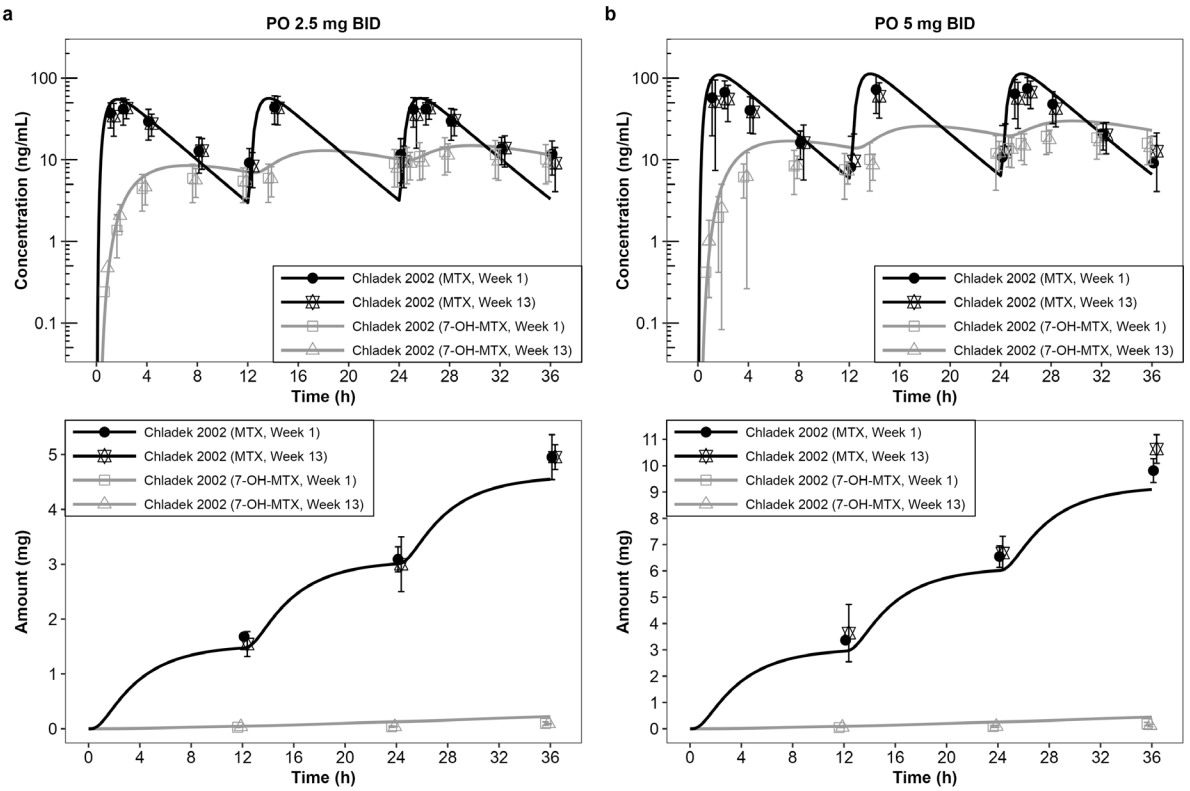

Fig. S10 Outcomes for prediction of multiple-dose scenarios with the MTX physiologically based pharmacokinetic model (for validation data sets; Table S1) for doses ranging from 2.5 to 5 mg PO. *Black dots* and *curves* represent observed and simulated data for MTX, while *grey dots* and *curves* represent observed and simulated data for MTX metabolite 7-OH-MTX. Top panels correspond to the plasma concentration–time curves, while the bottom panels depict the amount excreted in urine of the respective compounds over time. As there is no accumulation, the simulation outcomes for week 1 were also compared against week 13 data (week 13 data use same respective colours with a different symbol). Plots are on a log scale. Observed data is referenced in Table S1. *BID* twice daily, *MTX* methotrexate, *PO* per os

8 References

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