Sulfasalazine a BCRP probe substrate: development of a physiologically based pharmacokinetic (PBPK) model

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Background

Breast cancer resistance protein (**BCRP**) **limits Sulfasalazine intestinal absorption** and contributes to its biliary clearance. The aim of this study was to develop a physiologically based pharmacokinetic (PBPK) model for Sulfasalazine to assess the effect **of BCRP genotypes** on Sulfasalazine disposition.

Methods

Model development

Sulfasalazine PBPK model was developed in the Simcyp Simulator V17R1. Table 1 summarizes the input parameters used in the model. Sulfasalazine is a BCS class 4 compound. BCRP affects Sulfasalazine absorption (Wang *et al.* 2017). The solubility and dissolution parameters were estimated by fitting *in-vitro* assays in **S**imcyp **I**n-**v**itro **A**nalysis v2 (SIVA) toolkit (table 1). The membrane passive intrinsic permeability ($P_{trans,0}$) was estimated using a drug drug interaction (DDI) with curcumin assuming that intestinal BCRP was entirely inhibited then BCRP *in-vitro* maximum rate (J_{max}) was optimised against the PK profile in the control arm of this study. Sulfasalazine is extensively metabolised by the anaerobic bacteria present in the colon, therefore an absorption scalar of 0.001 was added to mimic the poor absorption in the colon.

Table 1: Input parameters for Sulfasalazine PBPK model

Parameter	Value	Method/Reference
Molecular weight (g/mol)	398.4	Product information
log P	3.61	Avdeef *et al*. 2012
Compound type	Diprotic Acid	Avdeef *et al*. 2012
pKa	2.44, 8.53	Avdeef *et al.* 2012, McDonell *et al*. 1976, Ol'Kovich *et al*. 2016
B/P	0.58	Matsuda *et al*. 2013
fu	0.007	Product information
Main plasma binding protein	Human serum albumin	Klotz *et al.* 1985
Absorption Model	ADAM Model	
$P_{eff,man}$ (10^{-4}cm/s)	1.31	Predicted
$P_{trans,0}$ (10^{-6}cm/s)	20000	Optimised – Kusuhara *et al*. 2012
Intrinsic solubility (mg/mL)	6.38E-05	SIVA v2 -Da Costa *et al.* 2015, Kusuhara *et al*. 2012, Markopoulos *et al*. 2015
Particle Size Distribution	Monodispersed	
Particle Radius (µm)	73.79	SIVA v2 - Markopoulos *et al*. 2015
LogK m:w Neutral	7.905	SIVA v2 - Markopoulos *et al*. 2015
LogK m:w Ion	3.88	SIVA v2 - Markopoulos *et al*. 2015
Colon Absorption Rate Scalars	0.001	Cf text
Transporter	ABCG2 (BCRP)	
J_{max} (pmol/min/cm²)	450	Optimised using Kusuhara *et al*. 2012
K_m (µM)	11.6	Kondo *et al.* 2004
Distribution Model	Full PBPK Model	
V_{SS} (L/kg)	0.094	Predicted - Method 2 (Rodgers *et al.)*
Elimination		
CL_{int} (HLM) (µL/min/mg protein)	18.14	$CL_{IV} – CL_{bile}$ - CL_{renal}
CL_{int} (Bile) (µL/min/10^6 cells)	1.455	Azad Khan *et al*. 1982
CL_R (L/h)	0.389	Schroeder *et al*. 1972, Azad Khan *et al*. 1982, Product information

BCRP genotype

No difference in the intrinsic activity and in the total protein expression has been shown *in vitro* for the different genotypes, however the expression at the cell surface was higher in wild type cells (WT, 421CC) (Urquhart *et al.* 2008). Similarly, Kondo *et al.* (2004) have shown in vesicles a difference in the protein expression but not in the intrinsic activity. The homozygote mutated cells (BCRP 421AA) had an expression that was 30-40% of the wild type.

The BCRP 421 AA genotype was simulated in the Simcyp simulated by assuming that the intestinal J_{max} and the biliary CL_{int} was **2.8-fold less than the wild type** (Kondo *et al.* 2004). It was assumed that the activity of heterozygotes was an average of the WT and the homozygote mutated (=1.48 fold less active) (figure 2 and table 2).

Simulations

To verify the model, PK profiles were simulated using clinical trial designs that replicate the observed clinical designs by selecting an appropriate Simcyp population library based upon which a representative number of virtual subjects of the selected age range, gender, etc. were generated.

Results

Simulated concentration-time profiles after oral administration of single and multiple doses of Sulfasalazine immediate release formulation were consistent with observed data (figure 1). The predicted mean CL of 40.4 L/h, 25.5 L/h, 20.5 L/h and 18.6 L/h after administration of 4 g (fig1-A), 2 g (Fig1-B), 1 g (Fig1-C) and 500 mg (Fig1-D), respectively. This CL **dose non linearity** was predicted to be mainly due to solubility issues.

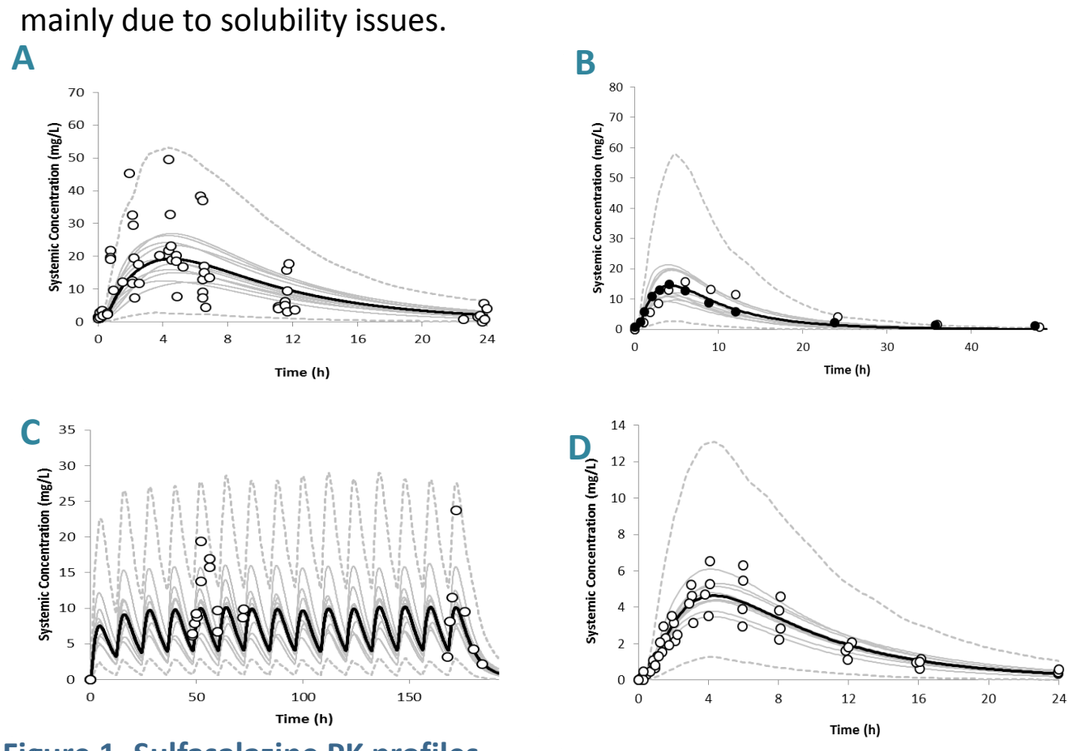

Figure 1. Sulfasalazine PK profiles
Simulated (black line) and observed (data points) plasma concentration-time profile of sulfasalazine after an oral dose of 4 g (**A** – individual data), 2 g (**B**-mean PK profile), 1 g (**C**-mean PK profile) and 0.5 g (**D**-mean PK profile) immediate release in a Caucasian (A and C) or a Japanese (B and D) population. Observed data were extracted from Schroeder *et al.* 1972 (A),Yamasaki *et al.* 2008 (B), Gotanda *et al.* 2015 (B), Awni *et al.* 1995 (C) and Wang *et al.* 2018 (D). The grey lines represent the predictions from individual trials Dashed lines represent the 5th and 95th percentile of the total virtual population.

The BCRP genotype effect is shown in the figure 2 and table 2. the results are in reasonable agreement with observed data however the genotype effect was underestimated.

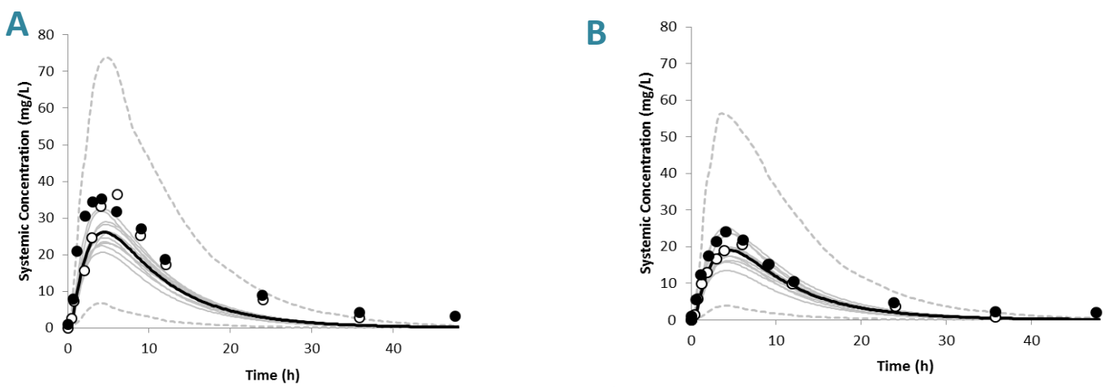

Figure 2. BCRP genotype effect
Simulated (black line) and observed (data points) mean plasma concentration-time profile of sulfasalazine after an oral dose of 2 g immediate release. **A** 421 AA, **B** 421 CC. Observed data were extracted from Yamasaki *et al.* 2008 (black dots) and Gotanda *et al.* 2015 (white dots). The grey lines represent the predictions from individual trials (10 trials x 10 subjects; 20 – 24 years; male; SIM-Japanese population). Dashed lines represent the 5th and 95th percentile of the total virtual population.

Table 2. BCRP genotype effect

	421 CC (WT)		421 CA		421 AA	
	observed	**predicted**	**observed**	**predicted**	**observed**	**predicted**
AUC (mg.h/L)	171±85	175±190	330±194	235.78±215	592±275	328±253
C_{max} (mg/L)	15.4±5.2	15.25±14.4	26.4±16.1	20±17	40.7±13.5	27.4±19

Observed values: Yamasaki *et al.* 2008, all results are mean ± SD

Conclusion

The sulfasalazine PBPK model was able to describe its PK profile for several dosing regimens and in different populations. Simulations in subjects with decreased BCRP activity showed an increase in sulfasalazine C_{max} and AUC when compared with subjects expressing the wild type 421 homozygote.

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