Chan et al.:

Bottom-Up Physiologically-Based Biokinetic Modelling as an Alternative to Animal Testing

Supplementary Data

Results S1: Derivation of Inputs into Mech KiM model for Pitavastatin

In the kidney, pitavastatin is a substrate of OAT3 with K_m = 3.3 µM (Fujino et al., 2005).
To obtain J_{max}, CL_{int} from experiments conducted in kidney slices were used (Watanabe et al., 2011).

$$CL_{int} = \frac{(2.55 + 4.36)}{2}\ ml/g\ kidney/15\ minutes \div 15\ minutes \div (60 \times 10^6)\ PTC/g\ kidney$$
$$= 3.84\ \mu l/min/10^6\ PTC$$

$$J_{max} = CL_{int} \times K_m$$
$$= 3.84\ \mu l/min/10^6\ PTC\ \times 3.3\ \mu M$$
$$= 12.672\ pmol/min/10^6\ PTC$$

Results S2: Derivation of Inputs for OATP1B3 and OATP2B1 Transport of Rosuvastatin

All values used are taken from Bosgra et al. (2014).
1. Determine the average expression of total amount of protein per million HEK-293 (Y):

CL_{int} (l/h) = *in vitro* J_{max} $(pmol/min/mg\ protein) \times Y(mg\ protein/10^6 HEK)$ × REF × HPPGL × liver weight (g) ÷ $K_m(\mu M)$

$Y_{OATP1B1} = \frac{241 \times 13}{202 \times \frac{25.7}{9.8} \times 139 \times 1561} \div 60\ min/h \times 10^6$ = 0.4543 $mg\ protein/10^6 HEK\ cells$

$Y_{OATP1B3} = \frac{145 \times 16.5}{251 \times \frac{2.2}{1.4} \times 139 \times 1561} \div 60\ min/h \times 10^6$ = 0.4659 $mg\ protein/10^6 HEK\ cells$

$Y_{OATP2B1} = \frac{649 \times 26.1}{26.2 \times \frac{24.2}{0.2} \times 139 \times 1561} \div 60\ min/h \times 10^6$ = 0.4104 $mg\ protein/10^6 HEK\ cells$

Average Y = $\frac{0.4543 + 0.4659 + 0.4104}{3}$ = 0.4435 $mg\ protein/10^6 HEK\ cells$

2. J_{max} of OATP1B3 in HEK-293 cells

J_{max} = 251 $pmol/min/mg\ protein \times 0.4435\ mg\ protein/10^6 HEK$
= 111.3 $pmol/min/10^6 HEK\ cells$

3. CL_{int} of OATP2B1 in HEK-293 cells

J_{max} = 26.2 $pmol/min/mg\ protein \times 0.4435\ mg\ protein/10^6 HEK$
= 11.62 $pmol/min/10^6 HEK\ cells$

As the REF value for OATP2B1 (148.830) was greater than the allowed input value of 100, J_{max} was scaled up by 1.4883 times,

J_{max} = 11.62 × 1.4883
= 17.3 $pmol/min/10^6 HEK\ cells$

doi:10.14573/altex.1812051s

Results S3: Derivation of Inputs for Intestinal OATP2B1 Transport of Pitavastatin

From Equation 11:

$$P_{app,trans,n,i}\ (10^{-6} cm/s) = \frac{J_{max}\ (pmol/min)}{A\ (cm^2) \times \left[(K_m\ (\mu M) \times fu_{inc}) + C_{lumen,i}\ (\mu M)\right]} \times REF,$$

where $P_{app,trans,n,I}$ represents apparent permeability contributed by the n^{th} transporter in the i^{th} segment of the intestine, A represents the transwell surface area, fu_{inc} represents the fraction unbound in the incubation system, $C_{lumen,i}$ represents the concentration of substrate in the lumen of the i^{th} segment.

From experiments conducted on Caco-2 cells (Ölander et al., 2016):
J_{max} = 255 ±65 pmol/min/mg protein
K_m = 25 µM
Insert growth area (A) = 0.33 cm^2
fu_{inc} = 1
REF = 0.0625

To use this kinetic data in Simcyp, units of J_{max} must be converted to pmol/min. In a 12-well plate (A = 1.12cm^2), a single filter contains 0.346 ±0.04 mg (mean ±SD, n = 96) of protein[a].
Therefore,
$J_{max} = 255 \pm 65\ pmol/min/mg\ protein \times 0.346\ mg/1.12 cm^2 = 78.777\ \mathrm{pmol/min/cm^2}$
Since A = 0.33 cm^2 was used in our model, the following represents the input for J_{max}:
$J_{max} = 78.777\ pmol/min/cm^2 \times 0.33 = 26.0\ pmol/min$

[a] Personal communication with Magnus Ölander, 2018

Results S4: Derivation of Intersystem Extrapolation Factors (ISEF) for Metabolism

1. The total CL_{int} due to metabolism of fluvastatin in HLM (Watanabe et al., 2010):

$$\begin{aligned} CL_{int,u}(HLM) &= J_{max} \div (K_m \times f_{u,inc}) \\ &= 5.57\ ml/min/g\ liver \div (48.8\ mg\ protein/g\ liver \times 0.308) \\ &= 370.6\ \mu l/min/mg\ protein \end{aligned}$$

2. Using equation (15) from the methods section, ISEF for CYP3A4, 2C9 and 2C8 (Fischer et al., 1999):

$$ISEF = \frac{CL_{int,u}(HLM)\ (\mu L/min/mg\ protein)}{\sum_{j=1}^{n}\left[\sum_{i=1}^{n}\left(CL_{int,i,u}(rhCYP_j)\ (\mu L/min/pmol\ CYP) \times CYP_j\ abundance_{HLM}\ (pmol\ CYP_j/mg\ protein)\right)\right]}$$

$$= 370.6 \div \left[\begin{array}{l} \left(\frac{2\ pmol/min/pmol\ CYP3A4}{7.1\ \mu M \times 0.308} \times 108\ pmol/mg\ protein\right) + \\ \left(\frac{0.078\ pmol/min/pmol\ CYP2C9}{0.9\ \mu M \times 0.308} \times 93\ pmol/mg\ protein\right) + \\ \left(\frac{0.038\ pmol/min/pmol\ CYP2C9}{1.0\ \mu M \times 0.308} \times 93\ pmol/mg\ protein\right) + \\ \left(\frac{0.038\ pmol/min/pmol\ CYP2C9}{1.8\ \mu M \times 0.308} \times 93\ pmol/mg\ protein\right) + \\ \left(\frac{0.132\ pmol/min/pmol\ CYP2C8}{2.8\ \mu M \times 0.308} \times 56\ pmol/mg\ protein\right) + \end{array}\right]$$

$$= 370.6 \div 151.363$$
$$= 2.444$$

Results S5: Derivation of Inputs into Mech KiM for Rosuvastatin

All data is taken from Verhulst et al. (2008)
1. Number of proximal and distal tubular cells (PTC, DTC) per surface area of filter

$$= \frac{Total\ number\ of cells\ seeded}{Surface\ area\ of\ the\ filter} = \frac{50000\ cells}{0.33\ cm^2} = 0.1515 \times 10^6\ cells/cm^2$$

2. Clearance due to passive diffusion across DTC:

$$\begin{aligned} CL_{PD} &= Rate\ of\ entry\ of\ rosuvastatin\ into\ DTC \div Concentration\ of\ drug\ in\ the\ medium \\ &= 460.70\ pmol/cm^2/h \div 10\ \mu mol/L \\ &= 46.07\ \mu l/cm^2/h \div 0.1515 \times 10^6\ cells/cm^2 \div 60\ min/h \\ &= 0.00507\ ml/min/10^6\ cells \end{aligned}$$

3. Converting J_{max} to a function of 10^6 PTC:

$$J_{max} = 4951\ pmol/cm^2/h \div 0.1515 \times 10^6\ cells/cm^2 \div 60\ min/h = 546\ pmol/min/10^6\ PTC$$

Tab. S1: Rosuvastatin input parameters

Parameter	Value	Reference	Comments
Molecular weight (g/mol)	481.54	Jamei et al., 2014	
$logP_{o:w}$	2.4	Jamei et al., 2014	
Compound type	Monoprotic acid	Jamei et al., 2014	
pK_a	4.27	Jamei et al., 2014	
Blood/plasma ratio	0.625	Jamei et al., 2014	
Fraction unbound in plasma	0.107	Jamei et al., 2014	
Absorption			
Absorption model	ADAM		
fu_{gut}	1		Default value
$P_{eff,man}$ (10^{-4} cm/s)	0.1843941		Predicted in Simcyp
Permeability assay	Caco-2	Li et al., 2011	
Apical pH : basolateral pH	7.4 : 7.4	Li et al., 2011	
Activity	Passive & active	Li et al., 2011	
Apparent permeability (10^{-6} cm/s)	0.4	Li et al., 2011	Digitized plot
Reference compound	Propranolol		
Reference compound value (10^{-6} cm/s)	43		Default value in Simcyp
Scalar	1		
Formulation	Solution or tablet	Farshi, 2011; Potur, 2014	Refer to Table S5 and S6
Distribution			
Distribution model	Full PBPK model		
Tissue: plasma coefficient	Modified based on rat distribution data	Nezasa et al., 2002	Refer to Table S14
V_{SS} (L/kg)	0.7002882	Rodgers and Rowland, 2007	Predicted in Simcyp
Elimination			
Metabolism			
Enzyme	CYP3A4		
Metabolic assay	HLM	Fujino et al., 2004b	
CL_{int} (µl/min/mg protein)	1.1	Fujino et al., 2004b	
fu_{mic}	0.937		Predicted in Simcyp
Enzyme	UGT 1A1		
rUGT system	BD Supersomes		
V_{max} (pmol/min/mg protein)	17	Schirris et al., 2015	
K_m (µM)	16	Schirris et al., 2015	
rUGT scalar	0.92	Simcyp Database	
Enzyme	UGT 1A3		
rUGT system	BD Supersomes		
V_{max} (pmol/min/mg protein)	105	Schirris et al., 2015	
K_m (µM)	220	Schirris et al., 2015	
rUGT scalar	1		Simcyp default value
Hepatic Transport			
CL_{PD} (ml/min/million hepatocytes)	0.0025	Jamei et al., 2014	
fu_{IW}	0.9673012		Predicted in Simcyp
fu_{EW}	0.1869325		Predicted in Simcyp
Transporter	NTCP	Bi et al., 2013	

Transporter assay	Suspension hepatocyte		Difference in uptake with and without sodium
$CL_{int,T}$ (µl/min/million)	3.4	Bi et al., 2013	
REF	1.353	Bi et al., 2013; Vildhede et al., 2015	Refer to Table S11
Transporter	OATP1B1		
Transporter assay	HEK-293 cells	Izumi et al., 2018	Gene transfected cell
J_{max} (pmol/min/million)	103	Izumi et al., 2018	
K_m (µM)	9.31	Izumi et al., 2018	
REF	8.656	Vildhede et al., 2015; Izumi et al., 2018	Refer to Table S11
Transporter	OATP1B3		
Transporter assay	HEK-293 cells	Bosgra et al., 2014	Gene transfected cell
J_{max} (pmol/min/million)	111.3	Bosgra et al., 2014	Refer to Results S2
K_m (µM)	16.5	Bosgra et al., 2014	
REF	8.036	Bosgra et al., 2014; Vildhede et al., 2015	Refer to Table S11
Transporter	OATP2B1		
Transporter assay	HEK-293 cells	Bosgra et al., 2014	Gene transfected cell
J_{max} (pmol/min/million)	17.3	Bosgra et al., 2014	Refer to Results S2
K_m (µM)	26.1	Bosgra et al., 2014	
REF	100	Bosgra et al., 2014; Vildhede et al., 2015	Refer to Table S11
Transporter	MRP4		
Transporter assay	Membrane vesicles	Pfeifer et al., 2013	
J_{max} (pmol/min/million)	1140	Pfeifer et al., 2013	
K_m (µM)	21	Pfeifer et al., 2013	
REF	0.028	Pfeifer et al., 2013; Vildhede et al., 2015	Refer to Table S11
Transporter	Canalicular efflux		
Transporter assay	SCHH		
$CL_{int,T}$ (µl/min/million)	1.5	Jones et al., 2012	
REF	1.611	Vildhede et al., 2015	Refer to Table S11
Renal Transporters			
$CL_{PD,basal}$ (ml/min/million PTC)	0.00507	Verhulst et al., 2008	Digitized and calculated Refer to Results S5
$CL_{PD,apical}$ (ml/min/million PTC)	0.00507	Verhulst et al., 2008	Assumed to be equal to $CL_{PD,apical}$
$fu_{Kidney,cell}$	0.985129		Predicted in Simcyp
fu_{Urine}	1		Default value in Simcyp
Transporter	OAT3		
Function	Uptake		
Transporter assay	PTC and DTC mixed monolayer	Verhulst et al., 2008	
J_{max} (pmol/min/million cells)	546	Verhulst et al., 2008	Refer to Results S5
K_m (µM)	20.4	Verhulst et al., 2008	

Transporter	MRP4		
Function	Efflux		
J_{max} (pmol/min/million cells)	546		Assumed to be equal to uptake kinetics
K_m (μM)	20.4		

Caco-2, colorectal adenocarcinoma cells; CL_{int}, intrinsic clearance; CL_{PD}, passive diffusion clearance; $CL_{PD,apical}$, passive diffusion clearance for the kidney between the renal cell and the tubule; $CL_{PD,basal}$, passive diffusion clearance for the kidney between the blood and renal cell; DTC, distal tubular cells; fu_{gut}, fraction unbound within the enterocyte; fu_{mic}, fraction unbound in microsomal system; HEK-293, human embryonic kidney cells; HLM, human liver microsome; J_{max}, maximum flux; K_m, Michaelis–Menten constant; K_p, partition coefficient; MRP4, multidrug resistance protein 4; NTCP, sodium-taurocholate co-transporting polypeptide; OAT3, organic anion transporter 3; OATP, organic anion-transporting polypeptide; $P_{eff,man}$, Effective human jejunum permeability; pK_a, acid dissociation constant; PTC, proximal tubular cells; REF, relative expression factor; SCHH, sandwich-cultured human hepatocyte; V_{ss}, apparent volume of distribution at steady state

Tab. S2: Fluvastatin input parameters

Parameter	Value	Reference	Comments
Molecular weight (g/mol)	411.5	Winiwarter et al., 1998	
$logP_{o:w}$	4.17	Winiwarter et al., 1998	
Compound type	Monoprotic acid	Winiwarter et al., 1998	
pK_a	4.31	Winiwarter et al., 1998	
Blood/plasma ratio	0.55	Tse et al., 1993	
Fraction unbound in plasma	0.009	Tse et al., 1993	
Absorption			
Absorption model	ADAM		
fu_{gut}	1		Default value
$P_{eff,man}$ (10^{-4} cm/s)	3.069295		Predicted in Simcyp
Permeability assay	Caco-2	Lindahl et al., 2004	
Apical pH : basolateral pH	6.5 : 7.4	Lindahl et al., 2004	
Activity	Passive & active	Lindahl et al., 2004	
Apparent permeability (10^{-6} cm/s)	16.237	Lindahl et al., 2004	Digitized data
Reference compound	Verapamil		
Reference compound value (10^{-6} cm/s)	20.6		Default value in Simcyp
Scalar	1		
Formulation	Solution		
Distribution			
Distribution model	Full PBPK model		
V_{SS} (l/kg)	0.09742813	Rodgers and Rowland, 2007	Predicted in Simcyp
Elimination			
Metabolism			
Enzyme	CYP3A4		
Metabolism assay	rhCYP	Fischer et al., 1999	
Metabolic pathway	Pathway 2	Fischer et al., 1999	5-hydroxylation
V_{max} (pmol/min/pmol)	2	Fischer et al., 1999	
K_m (µM)	7.1	Fischer et al., 1999	
fu_{mic}	0.308	Watanabe et al., 2010	
ISEF	2.444	Watanabe et al., 2010; Nakamura et al., 2016	Refer to Results S4
Enzyme	CYP2C9		
Metabolism assay	rhCYP	Fischer et al., 1999	
Metabolic pathway	Pathway 1	Fischer et al., 1999	6-hydroxylation
V_{max} (pmol/min/pmol)	0.0783	Fischer et al., 1999	
K_m (µM)	0.9	Fischer et al., 1999	
fu_{mic}	0.308	Watanabe et al., 2010	
ISEF	2.444	Watanabe et al., 2010; Nakamura et al., 2016	Refer to Results S4
Enzyme	CYP2C9		
Metabolism assay	rhCYP	Fischer et al., 1999	
Metabolic pathway	Pathway 2	Fischer et al., 1999	5-hydroxylation
V_{max} (pmol/min/pmol)	0.0383	Fischer et al., 1999	
K_m (µM)	1	Fischer et al., 1999	
fu_{mic}	0.308	Watanabe et al., 2010	
ISEF	2.444	Watanabe et al., 2010; Nakamura et al., 2016	Refer to Results S4

Enzyme	CYP2C9		
Metabolism assay	rhCYP	Fischer et al., 1999	
Metabolic pathway	Pathway 3	Fischer et al., 1999	N-deisopropyl
V_{max} (pmol/min/pmol)	0.0383	Fischer et al., 1999	
K_m (µM)	1.8	Fischer et al., 1999	
fu_{mic}	0.308	Watanabe et al., 2010	
ISEF	2.444	Watanabe et al., 2010; Nakamura et al., 2016	Refer to Results S4
Enzyme	CYP2C8		
Metabolism assay	rhCYP	Fischer et al., 1999	
Metabolic pathway	Pathway 2	Fischer et al., 1999	5-hydroxylation
V_{max} (pmol/min/pmol)	0.1317	Fischer et al., 1999	
K_m (µM)	2.8	Fischer et al., 1999	
fu_{mic}	0.308	Watanabe et al., 2010	
ISEF	2.444	Watanabe et al., 2010; Nakamura et al., 2016	Refer to Results S4
CL_R (l/h)	0		Default value in Simcyp
Hepatic transport			
CL_{PD} (ml/min/million hepatocytes)	0.025	Bi et al., 2013	
fu_{IW}	0.3146003		Predicted in Simcyp
fu_{EW}	0.0171335		Predicted in Simcyp
Transporter	Sinusoidal uptake		
Transporter assay	SCHH		
$CL_{int,T}$ (µl/min/million hepatocytes)	158.1	Izumi et al., 2018	
REF	3.55		Refer to Table S12
Transporter	Canalicular efflux		
Transporter assay	SCHH		
$CL_{int,T}$ (µl/min/million hepatocytes)	17	Jones et al., 2012	
RAF/REF	1.627		Refer to Table S12

CL_{int}, intrinsic hepatic clearance; CL_{PD}, passive diffusion clearance; CL_R, renal clearance; fu_{gut}, fraction unbound within the enterocyte; fu_{mic}, fraction unbound in microsomal system; J_{max}, maximum flux; K_m, Michaelis–Menten constant; K_p, partition coefficient; $P_{eff,man}$, Effective human jejunum permeability; pK_a, acid dissociation constant; PTC, proximal tubular cells; REF, relative expression factor; rhCYP, recombinant human CYP450; SCHH, sandwich-cultured human hepatocyte; V_{ss}, apparent volume of distribution at steady state

Tab. S3: Pitavastatin input parameters

Parameter	Value	Reference	Comment
Molecular weight (g/mol)	421.49	FDA, 2012	
$logP_{o:w}$	1.49	Kajinami et al., 2003	
Compound type	Monoprotic acid		
pK_a	5.31	FDA, 2012	
Blood:plasma ratio	0.55	FDA, 2012	
Fraction unbound in plasma	0.0052	Watanabe et al., 2011	
Absorption			
Absorption model	ADAM model		
fu_{gut}	1		Default value in Simcyp
$P_{eff,man}$ (10^{-4} cm/s)	1.023977		Predicted in Simcyp
Permeability assay	LLC-PK1	Shirasaka et al., 2011	
Value	4.16	Shirasaka et al., 2011	
Reference compound	Propranolol		
Reference compound value (10^{-6} cm/s)	36		Default value in Simcyp
Scalar	1		
Formulation	Solution		Assumption due to lack of dissolution data
Intestinal Transporter			
Transporter	OATP2B1		
J_{max} (pmol/min/cm^2)	78.777	Ölander et al., 2016	Refer to Results S3
K_m (µM)	25	Ölander et al., 2016	
fu_{inc}	1		
Insert growth area of the Transwell (cm^2)	0.33		
System	User		
RAF/REF	0.0625	Ölander et al., 2016	
Distribution			
Distribution model	Full PBPK model		
Tissue:plasma coefficient	Modified based on rat distribution data	Kimata et al., 1998	Table S15 and S16
V_{SS} (L/kg)	0.5103121	Rodgers and Rowland, 2007	Predicted in Simcyp
Elimination			
Enzyme	CYP2C9		
Metabolic assay	HLM	Fujino et al., 2004b	Allocated to CYP3A4
CL_{int} (µl/min/mg protein)	2.5	Fujino et al., 2004b	
fu_{mic}	0.962		Predicted in Simcyp
Enzyme	UGT 1A3		
Metabolic assay	BD Supersomes	Schirris et al., 2015	

V_{max} (pmol/min/mg protein)	880	Schirris et al., 2015	
K_m (µM)	10	Schirris et al., 2015	
rUGT scaler	1.0		Simcyp default value
Enzyme	UGT 2B7		
Metabolic assay	BD Supersomes	Schirris et al., 2015	
V_{max} (pmol/min/mg protein)	12900	Schirris et al., 2015	
K_m (µM)	220	Schirris et al., 2015	
rUGT scalar	2.81	Simcyp Database	
Hepatic Transporters			
CL_{PD} (ml/min/million hepatocytes)	0.00773	Fujino et al., 2004a	
fu_{IW}	0.9566636		Predicted in Simcyp
fu_{EW}	0.009929368		Predicted in Simcyp
Transporter	NTCP		
Transporter assay	Suspension hepatocyte	Bi et al., 2013	Difference in uptake with and without sodium
$CL_{int,T}$ (µl/min/million)	20	Bi et al., 2013	
RAF/REF	1.353	Bi et al., 2013; Vildhede et al., 2015	Refer to Table S13
Transporter	OATP1B1		
Transporter assay	HEK-293 cells	Hirano, 2004	Gene transfected cells
J_{max} (pmol/min/million)	230	Hirano, 2004	
K_m (µM)	3	Hirano, 2004	
RAF/REF	5.936	Hirano, 2004; Vildhede et al., 2015	Refer to Table S13
Transporter	OATP1B3		
Transporter assay	HEK-293 cells	Hirano, 2004	Gene transfected cells
J_{max} (pmol/min/million)	100	Hirano, 2004	
K_m (µM)	3.25	Hirano, 2004	
RAF/REF	4.904	Hirano, 2004; Vildhede et al., 2015	Refer to Table S13
Transporter	OATP2B1		
Transporter assay	HEK-293 cells	Hirano et al., 2006	Gene transfected cells
J_{max} (pmol/min/million)	7.36	Hirano et al., 2006	
K_m (µM)	1.17	Hirano et al., 2006	
RAF/REF	0.19	Hirano et al., 2006; Vildhede et al., 2015	Refer to Table S13
Transporter	MRP3		
Transporter assay	Membrane vesicles	Vildhede et al., 2016	
J_{max} (pmol/min/million)	2380	Vildhede et al., 2016	
K_m (µM)	448	Vildhede et al., 2016	
RAF/REF	0.025	Vildhede et al., 2015, 2016	Refer to Table S13

Transporter	P-gp/MDR1		
Transporter assay	Membrane vesicles	Vildhede et al., 2016	
J_{max} (pmol/min/million)	433	Vildhede et al., 2016	
K_m (µM)	83.7	Vildhede et al., 2016	
RAF/REF	0.00181	Vildhede et al., 2015, 2016	Refer to Table S13
Transporter	BCRP		
Transporter assay	Membrane vesicles	Vildhede et al., 2016	
J_{max} (pmol/min/million)	95.3	Vildhede et al., 2016	
K_m (µM)	1.2	Vildhede et al., 2016	
RAF/REF	0.00052	Vildhede et al., 2015, 2016	Refer to Table S13
Renal Transporters			
$CL_{PD,basal}$ (ml/min/PTC)	0.0151	Watanabe et al., 2011	Calculated. Refer to Results S1
$CL_{PD,apical}$ (ml/min/million PTC)	0.0151		Assumed to be equal to $CL_{PD,basal}$
$fu_{Kidney,cell}$	0.9800262		Predicted in Simcyp
fu_{Urine}	1		Default value in Simcyp
Transporter	OAT3		
Transporter Assay	Human kidney slices		
Function	Uptake		
J_{max} (pmol/min/million cells)	12.672	Watanabe et al., 2011	Refer to Results S1
K_m (µM)	3.3	Fujino et al., 2005	BD Supersomes
Transporter	MRP4		
Function	Efflux		
J_{max} (pmol/min/million cells)	12.672		Assumed to be equal to uptake kinetics
K_m (µM)	3.3		

BCRP, breast cancer resistant protein; Caco-2, colorectal adenocarcinoma cells; CL_{int}, intrinsic hepatic clearance; CL_{PD}, passive diffusion clearance; $CL_{PD,apical}$, passive diffusion clearance for the kidney between the renal cell and the tubule; $CL_{PD,basal}$, passive diffusion clearance for the kidney between the blood and renal cell; DTC, distal tubular cells; fu_{gut}, fraction unbound within the enterocyte; fu_{mic}, fraction unbound in microsomal system; HEK-293, human embryonic kidney cells; HLM, human liver microsome; J_{max}, maximum flux; K_m, Michaelis-Menten constant; K_p, partition coefficient; MRP4, multidrug resistance protein 4; NTCP, sodium-taurocholate co-transporting polypeptide; OAT3, organic anion transporter 3; OATP, organic anion-transporting polypeptide; $P_{eff,man}$, Effective human jejunum permeability; P-gp, p-glycoprotein; pK_a, acid dissociation constant; PTC, proximal tubular cells; REF, relative expression factor; SCHH, sandwich-cultured human hepatocyte; V_{ss}, apparent volume of distribution at steady state

Tab. S4: Referenced clinical data

Drug	Route of Administration	Dose (mg)	Dosing Frequency	Number of participants	Proportion of females	Reference
Rosuvastatin	IV infusion	8	Over 4 hours	10	0	Martin et al., 2003
	Oral	20	Single dose	7	0.43	Wu et al., 2017
	Oral	10	Once daily for 14 days	21	0.083	Martin et al., 2002
Fluvastatin	IV infusion	2	Over 20 minutes	6	0	Tse et al., 1992
	Oral	10	Single dose	6	0	Tse et al., 1992
	Oral	40	Once daily for 6 days	6	0	Tse et al., 1992
Pitavastatin	IV	2	Over 1 hour	18	0	FDA, 2012
	Oral	2	Single dose	18	0	FDA, 2012
	Oral	4	Once daily for 5 days	18	0	FDA, 2012

Tab. S5: Dissolution rate for rosuvastatin 20 mg

At pH = 1.2				
Time (h)	0.25	0.5	0.75	1
% Dissolved	52.3	56.85	57.5	57.5
At pH = 6.8				
Time (h)	0.25	0.5	0.75	1
% Dissolved	96.45	96.95	97.32	97.39

Dissolution data of Crestor® 20 mg in pH 6.8 and 1.2 were used (Potur, 2014).

Tab. S6: Dissolution rate for rosuvastatin 10 mg

At pH = 1.2							
Time (h)	0.0833	0.167	0.25	0.333	0.5	0.75	1
% Dissolved	18.1	41.6	55.1	64.1	75.9	85.2	89.8
At pH = 6.8							
Time (h)	0.25	0.5	0.75	1			
% Dissolved	100	100	100	100			

Dissolution data of Crestor® 10 mg (pH = 8.6) and 40 mg (pH = 1.2) were used (Farshi, 2011; Potur, 2014).

Tab. S7: Derivation of SF1 values for rosuvastatin

Hepatic Transporter	*In vitro* system		Isolated hepatocyte system		SF1
	Type of system	Activity/expression	Type of system	Activity/expression	
NTCP	-	-	Hepatocyte suspension	3.4[a] µl/min/10^6 cells	-
OATP1B1	HEK-293	127[b] µl/min/mg protein	Hepatocyte suspension	332[b] µl/min/10^6 cells	2.610 mg protein/10^6 cells
OATP1B3	HEK-293	1.4[c] pmol/10^6 cells	Primary hepatocyte	2.2[c] pmol/10^6 cells	1.571
OATP2B1	HEK-293	0.2[c] pmol/10^6 cells	Primary hepatocyte	24.2[c] pmol/10^6 cells	121
MRP4	Membrane vesicles	54.3[d] µl/min/mg protein	SCHH	0.85[d,e] µl/min/10^6 cells	0.0157 mg protein/10^6 cells
Canalicular efflux	-	-	SCHH	17[f] µl/min/10^6 cells	-

[a] Bi et al. (2013); [b] Izumi et al. (2018); [c] Bosgra et al. (2014); [d] Pfeifer et al. (2013); [e] Basolateral efflux clearance in SCHH was scaled by the number of hepatocyte per gram of liver obtained from Simcyp (118 x 10^6 HHEP/g); [f] Jones et al. (2012)

Tab. S8: Derivation of SF1 values for pitavastatin

Hepatic transporter	*In vitro* system		Isolated hepatocyte system		SF1
	Type of system	Activity/expression	Type of system	Activity/expression	
NTCP	-	-	Hepatocyte suspension	20[a] µl/min/10^6 cells	-
OATP1B1	HEK-293	-	Primary hepatocyte	-	1.790[b] mg protein/10^6 cells
OATP1B3	HEK-293	-	Primary hepatocyte	-	0.959[b] mg protein/10^6 cells
OATP2B1	HEK-293	-	Primary hepatocyte	-	0.155[b] mg protein/10^6 cells
MRP3	Membrane vesicles	40.0[c] pmol/mg vesicular protein	SCHH	0.417[c,d] pmol/10^6 cells	0.0105 mg protein/10^6 cells
BCRP	Membrane vesicles	260[c] pmol/mg vesicular protein	SCHH	0.0841[c,d] pmol/10^6 cells	0.000327 mg protein/10^6 cells
P-gp	Membrane vesicles	280[c] pmol/mg vesicular protein	SCHH	0.3167[c,d] pmol/10^6 cells	0.00113 mg protein/10^6 cells

[a] Bi et al. (2013); [b] Hirano (2004); [c] Vildhede et al. (2016); [d] Expression of transporters in SCHH was scaled by 0.3235 mg protein/10^6 HHEP obtained by dividing amount of protein per well (0.1214 mg) with number of HHEP per well (0.375 x 10^6 HHEP)

Tab. S9: Derivation of SF2 from uptake transporters

Hepatic uptake transporter	Absolute abundance in (pmol/mg membrane protein)		Ratio of abundance in liver to hepatocyte, SF2
	Liver membrane	Hepatocyte membrane	
OATP1B1	19.600	5.910	3.316
OATP1B3	2.700	0.528	5.114
NTCP	1.880	0.414	4.541
OATP2B1	1.550	1.260	1.230

Values are obtained from Vildhede et al. unless otherwise stated (Vildhede et al., 2015).

Tab. S10: Derivation of SF2 from efflux transporters

Hepatic efflux transporter	Absolute abundance in (pmol/mg membrane protein)		Ratio of abundance in liver to hepatocyte, SF2
	Liver membrane	Hepatocyte membrane	
BSEP	3.050	1.580	1.930
MRP6	1.370	0.844	1.623
MRP2	0.815	0.501	1.627
P-gp	0.576	0.360	1.600
MRP3	0.407	0.171	2.380
BCRP	0.419[a]	0.263[b]	1.593
Average	1.106	0.646	1.793

Values are obtained from Vildhede et al. unless otherwise stated.
[a] Ohtsuki et al. (2011); [b] Vildhede et al. (2016)

Tab. S11: Derivation of REF for rosuvastatin

Hepatic transporter	Scaling factor 1 (SF1)	Scaling factor 2 (SF2)	REF
NTCP	-	1.353[a]	1.353
OATP1B1	2.610	3.316	8.656
OATP1B3	1.571	5.114	8.036
OATP2B1	121	1.230	148.830
MRP4	0.0157	1.793[b]	0.028
Canalicular efflux	-	1.610[c]	1.610

SF1 and SF2 are obtained from Table S7 – S10 unless otherwise stated.
[a] Obtained from transporter expression in Bi et al. (2013) and Vildhede et al. (2015); [b] Average value in Table S10 used; [c] Average value of BCRP and MRP2 ratio in Table S10 used

Tab. S12: Derivation of REF for fluvastatin

Hepatic transporter	Scaling factor 1 (SF1)	Scaling factor 2 (SF2)	REF
Sinusoidal uptake	-	3.55[a]	3.55
Canalicular efflux	-	1.627[b]	1.627

[a] Average of OATP1B1, OATP1B3, OATP2B1 and NTCP from Table S9; [b] Values for MRP2 in Table S10

Tab. S13: Derivation of REF for pitavastatin

Hepatic transporter	Scaling factor 1 (SF1)	Scaling factor 2 (SF2)	REF
NTCP	-	1.353[a]	1.353
OATP1B1	1.790	3.316	5.936
OATP1B3	0.959	5.114	4.904
OATP2B1	0.155	1.230	0.190
MRP3	0.0105	2.380	0.0249
P-gp	0.00113	1.600	0.00181
BCRP	0.000327	1.593	0.00052

SF1 and SF2 are obtained from Table S7-S10 unless otherwise stated.
[a] Obtained from transporter expression in Bi et al. (2013) and Vildhede et al. (2015)

Tab. S14: Tissue concentration of radioactivity and tissue-to-plasma equilibrium distribution ratio (K_p) for rosuvastatin
Tissue concentration of radioactivity (ng.eq/g or ng.eq/ml) and K_p after single oral administration of ^{14}C-rosuvastatin at 5 mg/kg in male Sprague-Dawley rats at various time points (Nezasa et al., 2002).

	Time at									
	15 minute		1.5 hours		4 hours		8 hours		24 hours	
Organs	Radioactivity	K_p	Radioactivity	K_p	Radioactivity	K_p	Radioactivity	K_p	Radioactivity	K_p
Plasma	38.600	1.000	79.400	1.000	68.100	1.000	44.800	1.000	28.900	1.000
Heart	17.500	**0.453**	31.500	0.397	27.700	0.407	16.600	0.371	9.000	0.311
Lung	16.500	0.427	31.700	0.399	31.600	**0.464**	16.700	0.373	n.d.	-
Spleen	11.300	**0.293**	18.000	0.227	14.400	0.211	8.800	0.196	7.000	0.242
Pancreas	26.100	**0.676**	34.300	0.432	26.400	0.388	18.500	0.413	n.d.	-
Fat	n.d.	-	41.500	0.523	69.500	**1.021**	27.700	0.618	15.700	0.543
Brown fat	33.700	0.873	65.500	0.825	57.200	0.840	30.000	0.670	25.300	**0.875**
Skeletal muscle	n.d.	-	15.800	0.199	14.100	**0.207**	n.d.	-	n.d.	-
Skin	11.800	0.306	30.100	0.379	34.800	0.511	23.000	**0.513**	10.000	0.346

n.d., not detected
Values in bold represent the largest K_p value observed and are used in our model.

Tab. S15: Tissue concentration of unchanged drug and K_p for unchanged pitavastatin
Concentration of unchanged pitavastatin (µg/g) and K_p after single oral administration of ^{14}C-pitavastatin at 1 mg/kg in male Sprague-Dawley rats at various time points (Kimata et al., 1998).

	Time at							
	0.5 hour		**1 hour**		**3 hours**		**6 hours**	
Organs	Concentration	K_p	Concentration	K_p	Concentration	K_p	Concentration	K_p
Plasma	0.2	1	0.079	1	0.033	1	0.013	1
Heart	0.101	0.505	0.041	**0.519**	0.007	0.212	n.d.	-
Lung	0.131	0.655	0.034	0.430	0.017	0.515	0.011	**0.846**
Skeletal muscle	0.019	0.095	0.011	**0.139**	0.003	0.091	n.d.	-

n.d., not detected
Values in bold represent the largest K_p value observed and are used in our model.

Tab. S16: Tissue concentration of total radioactivity and K_p for pitavastatin
Tissue concentration of radioactivity (ng.eq/g or ng.eq/ml) and K_p after single oral administration of ^{14}C-pitavastatin at 1 mg/kg in male Sprague-Dawley rats at various time points (Kimata et al., 1998).

	Time at							
	15 minute		**1 hour**		**6 hours**		**24 hours**	
Organs	Radioactivity	K_p	Radioactivity	K_p	Radioactivity	K_p	Radioactivity	K_p
Plasma	0.143	1.000	0.103	1.000	0.04	1.000	0.012	1.000
Brain	0.009	**0.063**	0.006	0.058	n.d.	-	n.d.	-
Spleen	0.026	0.182	0.021	**0.204**	0.008	0.200	n.d.	-
Pancreas	0.051	0.357	0.044	0.427	0.02	**0.500**	n.d.	-
Abdominal fat	0.025	0.175	0.030	0.291	0.023	0.575	0.016	**1.333**
Skin	0.015	0.105	0.020	0.194	0.008	0.200	0.004	**0.333**
Bone marrow	0.032	**0.224**	0.022	0.214	0.007	0.175	n.d.	-

n.d., not detected
Values in bold represents the largest K_p value observed and are used in our model.

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