Glycyrrhizin has a high likelihood to be a victim of drug-drug interactions mediated by hepatic organic anion-transporting polypeptide 1B1/1B3

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- Supporting Information Appendix S1 -

Table S1

Inputs used in GastroPlus-based physiologically based pharmacokinetic (PBPK) modeling for i.v. glycyrrhizin

Item	Input			
	Rat	Source	Human	Source
Compound: glycyrrhizin				
Molecular formula	$C_{42}H_{62}O_{16}$	Filonova *et al.*, 2015	$C_{42}H_{62}O_{16}$	Filonova *et al.*, 2015
MW ($g \cdot mol^{-1}$)	823	Filonova *et al.*, 2015	823	Filonova *et al.*, 2015
Log*P*	2.02	Measured	2.02	Measured
pK_a (acid)	3.39, 5.55, 9.81	Measured	3.39, 5.55, 9.81	Measured
Rat Oatp1b2, liver				
Transporter location	Basolateral/influx		—	
In vitro K_m (μM)	18.2	Measured	—	—
In vitro V_{max} ($pmol \cdot min^{-1} \cdot mg^{-1}$ protein)	35.6	Measured	—	—
In vitro fraction unbound ($f_{u\text{-incubation}}$)	1.0	Measured	—	—
RAF	15	Fitted	—	—
Rat Mrp2, liver				
Transporter location	Apical/efflux		—	
In vitro K_m (μM)	17.2	Measured	—	—
In vitro V_{max} ($pmol \cdot min^{-1} \cdot mg^{-1}$ protein)	25.6	Measured	—	—
In vitro fraction unbound ($f_{u\text{-incubation}}$)	1.0	Measured	—	—
RAF	3	Fitted	—	—
Human OATP1B1, liver				
Transporter location	—		Basolateral/influx	
In vitro K_m (μM)	—	—	21.7	Measured
In vitro V_{max} ($pmol \cdot min^{-1} \cdot mg^{-1}$ protein)	—	—	16.6	Measured
In vitro fraction unbound ($f_{u\text{-incubation}}$)	—	—	1.0	Measured
RAF	—	—	10	Fitted
Human OATP1B3, liver				
Transporter location	—		Basolateral/influx	
In vitro K_m (μM)	—	—	18.4	Measured
In vitro V_{max} ($pmol \cdot min^{-1} \cdot mg^{-1}$ protein)	—	—	12.4	Measured
In vitro fraction unbound ($f_{u\text{-incubation}}$)	—	—	1.0	Measured
RAF	—	—	10	Fitted
Human MRP2, liver				
Transporter location	—		Apical/efflux	
In vitro K_m (μM)	—	—	12.3	Measured
In vitro V_{max} ($pmol \cdot min^{-1} \cdot mg^{-1}$ protein)	—	—	96.3	Measured
In vitro fraction unbound ($f_{u\text{-incubation}}$)	—	—	1.0	Measured
RAF	—	—	1	Fitted
Dosage form	I.v. bolus	From this investigation	I.v. infusion	Yamamura *et al.*, 1992
Initial dose (mg)	0.66	From this investigation	40, 80, 120	Yamamura *et al.*, 1992
Infusion time (h)	—	—	0.2	Yamamura *et al.*, 1992
Pharmacokinetics				
New PBPK: population estimates for age-related (PEAR)				
Species	Rat		Human	
Population	—		Japanese	
Gender	—		Male	
Age (year)	—		30	
Weight (kg)	0.25		50	
Edit PBPK:				
SpecPStc ($mL \cdot s^{-1} \cdot mL^{-1}$)	Used, 2×10^{-3}	Fitted	Used, 2×10^{-4}	Fitted
Liver: K_p	0.47	Measured	0.47	Same as rat liver K_p
Lung: K_p	0.22	Measured	0.22	Same as rat lung K_p
Adipose: K_p	0.06	Measured	0.06	Same as rat adipose K_p

Muscle: K_p	0.06	Measured	0.06	Same as rat muscle K_p
Spleen: K_p	0.08	Measured	0.08	Same as rat spleen K_p
Heart: K_p	0.08	Measured	0.08	Same as rat heart K_p
Brain: K_p	0.01	Measured	0.01	Same as rat brain K_p
Kidney: K_p	0.17	Measured	0.17	Same as rat kidney K_p
Renal filtration estimate method	$f_{u\text{-plasma}}$ × GFR		$f_{u\text{-plasma}}$ × GFR	
Skin: K_p	0.09	Measured	0.09	Same as rat skin K_p
Blood/plasma concentration ratio	0.49	Ishida *et al.*, 1990	0.54	Yamamura *et al.*, 1992
Experimental plasma $f_{u\text{-plasma}}$	Used, 0.008	Measured	Used, 0.01	Yu *et al.*, 2012
PBPK settings				
K_p prediction method				
Perfusion limited tissue	—		—	
Permeability limited tissue	User defined (liver, lung, adipose, muscle, spleen, heart, brain, kidney and skin)		User defined (liver, lung, adipose, muscle, spleen, heart, brain, kidney and skin)	
Simulation $f_{u\text{-tissue}}$	Poulin equation	Fitted	Poulin equation	Fitted
Tissue albumin	Rothschild	Fitted	Rothschild	Fitted
Simulation				
Simulation mode	Single simulation		Single simulation	
Simulation length (h)	12		12	

References

Filonova OV, Lekar AV, Borisenko EV, Maksimenko EV, Vetrova EV, Borisenko *et al.* (2015). Synthesis of glycyrrhetinic acid monoglycoside by hydrolysis of glycyrrhizin acid in subcritical water. Russ J Phys Chem 10: 1153–1156.

Ishida S, Sakiya Y, Ichikawa T, Taira Z, Awazu S (1990). Prediction of glycyrrhizin disposition in rat and man by a physiologically based pharmacokinetic model. Chem Pharm Bull 38: 212–218.

Yamamura Y, Kawakami J, Santa T, Kotaki H, Uchino K, Sawada Y *et al.* (1992). Pharmacokinetic profile of glycyrrhizin in healthy volunteers by a new high-performance liquid chromatographic method. J Pharm Sci 81: 1042–1046.

Yu K, Chen F, Li C (2012). Absorption, disposition, and pharmacokinetics of saponins from Chinese medicinal herbs: what do we know and what do we need to know more? Curr Drug Metab 13: 577–598.

Table S2

Input used in GastroPlus-based PBPK modeling for i.v. rifampin in rats

Item	Input	Source
Compound: rifampin		
Molecular formula	$C_{43}H_{58}N_4O_{12}$	Maggi *et al.*,1966
MW ($g \cdot mol^{-1}$)	823	Maggi *et al.*,1966
Log*P*	2.19	Measured
pK_a (acid)	2.27	Measured
pK_a (base)	7.89	Measured
Dosage form	I.v. bolus	Jiang *et al.*, 2015
Initial dose (mg)	2.5, 5	Jiang *et al.*, 2015
Pharmacokinetics		
New PBPK: population estimates for age-related (PEAR)		
Species	Rat	—
Weight (kg)	0.25	—
Edit PBPK:		
SpecPStc ($mL \cdot s^{-1} \cdot mL^{-1}$)	Used, 2.5×10^{-2}	Fitted
Liver: CL_{int} ($L \cdot h^{-1}$)	0.020	Jiang *et al.*, 2015
Kidney: renal filtration estimate method	$f_{u\text{-plasma}}$ × GFR	—
Blood/plasma concentration ratio	0.90	Loos *et al.*, 1985
Experimental plasma $f_{u\text{-plasma}}$	0.231	Jiang *et al.*, 2015
Adjusted plasma $f_{u\text{-plasma}}$	Used	—
PBPK settings		
K_p prediction method		
Perfusion limited tissue	Poulin & Theil - Homogeneous (lung, adipose, muscle, spleen, heart, brain, kidney and skin)	Fitted
Permeability limited tissue	Poulin & Theil - Extracellular (liver)	Fitted
Simulation $f_{u\text{-tissue}}$	S+ equation	Fitted
Tissue albumin	Rothschild	Fitted
Simulation		
Simulation mode	Single simulation	
Simulation length (h)	8	

References

Jiang R-R, Dong J-J, Li X-X, Du F-F, Jia W-W, Xu F *et al.* (2015). Molecular mechanisms governing different pharmacokinetics of ginsenosides and potential for ginsenoside-perpetrated herb-drug interactions on OATP1B3. Br J Pharmacol 172: 1059–1073.

Loos U, Musch E, Jensen JC, Mikus G, Schwabe HK, Eichelbaum M (1985). Pharmacokinetics of oral and intravenous rifampicin during chronic administration. Klin Wochenschr 63:1205–1211.

Maggi N, Pasqualucci CR, Ballotta R, Sensi P (1966). Rifampicin: a new orally active rifamycin. Chemotherapia 11: 285–292.

Table S3

Observed and PBPK model-predicted plasma pharmacokinetics of rifampin in rats after an i.v. bolus dose at 10 and 20 mg·kg^{-1}. The observed values were calculated from the rat plasma concentrations of rifampin over time reported by this laboratory (Jiang *et al*., 2015). The PBPK model for i.v. rifampin was developed in this investigation (Table S2 in Supporting Information Appendix S1).

Pharmacokinetic parameter	Observed	Predicted (Accuracy)
Pharmacokinetics of rifampin in rats that received an i.v. bolus dose at 10 mg·kg^{-1}		
C_{5min} (μM)	18.3 ± 0.9	17.9 (98.0%)
$AUC_{0\text{-}8h}$ (μM·h)	48.2 ± 4.0	49.0 (102%)
$t_{1/2}$ (h)	2.58 ± 0.35	3.13 (121%)
$CL_{tot,p}$ (mL·h^{-1}·kg^{-1})	253 ± 20	248 (98.1%)
V_{SS} (mL·kg^{-1})	848 ± 18	904 (107%)
Pharmacokinetics of rifampin in rats that received an i.v. bolus dose at 20 mg·kg^{-1}		
C_{5min} (μM)	41.3 ± 4.7	35.9 (86.9%)
$AUC_{0\text{-}8h}$ (μM·h)	117 ± 16	98.0 (83.6%)
$t_{1/2}$ (h)	2.72 ± 0.32	3.12 (115%)
$CL_{tot,p}$ (mL·h^{-1}·kg^{-1})	256 ± 38	248 (96.8%)
V_{SS} (mL·kg^{-1})	895 ± 121	903 (101%)

References

Jiang R-R, Dong J-J, Li X-X, Du F-F, Jia W-W, Xu F *et al.* (2015). Molecular mechanisms governing different pharmacokinetics of ginsenosides and potential for ginsenoside-perpetrated herb-drug interactions on OATP1B3. Br J Pharmacol 172: 1059–1073.

Table S4

Tissue distribution of glycyrrhizin in rifampin-untreated rats after an i.v. bolus dose at 2.6 mg·kg^{-1}

Tissue	$C_{5\ min}$ (μM)	$AUC_{0\text{-}4\ h}$ (μM·h)	K_P
Plasma	14.7 ± 4.1	15.7 ± 3.0	—
Kidney	2.12 ± 0.28	2.71 ± 0.47	0.17 ± 0.04
Liver	10.8 ± 1.5	7.46 ± 0.95	0.47 ± 0.12
Heart	1.10 ± 0.08	1.28 ± 0.27	0.08 ± 0.02
Lung	2.26 ± 0.62	3.46 ± 0.79	0.22 ± 0.06
Spleen	1.15 ± 0.20	1.23 ± 0.03	0.08 ± 0.01
Brain	0.23 ± 0.06	0.18 ± 0.02	0.01 ± 0.00
Adipose	0.47 ± 0.05	0.86 ± 0.20	0.06 ± 0.06
Muscle	0.54 ± 0.09	0.88 ± 0.17	0.06 ± 0.01
Skin	0.78 ± 0.05	1.30 ± 0.24	0.09 ± 0.02

$C_{5\ min}$, concentration at 5 min after dosing; AUC, area under concentration-time curve; K_P, tissue/plasma partition coefficient. The data for tissue distribution of glycyrrhizin represent means ± SDs (n = 5).

Table S5

Predicted increase of glycyrryhizin's area under concentration-time curve (AUC) in presence of inhibitors of OATP1B1/1B3

Inhibitor	K_i or IC_{50} (μM)		Dose (mg)	C_{max} (μM)	$t_{1/2}$ (h)	K_a (min)	$f_{u\text{-plasma}}$	R	R_{AUC}	Note
	OATP1B1	OATP1B3								
Paritaprevir (oral; qd)	IC_{50}, 0.03	IC_{50}, 0.02	150	1.9	2.7	0.004	0.02	1.0	2.6	Antiviral agent; (Menon *et al.*, 2016; Shebley *et al.*, 2017)
Telaprevir (oral; tid)	IC_{50}, 1.4	IC_{50}, 9.7	750	5.5	4.7	0.1	0.37	1.4	4.1	Antiviral agent; (Furihata *et al.*, 2004)
Simeprevir (oral; qd)	IC_{50}, 0.3	IC_{50}, 0.2	150	5.9	11.5	0.1	0.01	1.3	1.7	Antiviral agent; (Furihata *et al.*, 2004; Sanford, 2015)
Sofosbuvir (oral; qd)	IC_{50}, 16.5	IC_{50}, 61.9	400	1.1	0.5	0.1	0.37	1.0	1.3	Antiviral agent; (Furihata *et al.*, 2004; Lawitz *et al.*, 2013)
Ritonavir (oral; bid)	IC_{50}, 0.5	IC_{50}, 0.6	600	15.5	3.2	0.003	0.01	1.1	1.3	Antiviral agent; (Danner *et al.*, 1995; Shebley *et al.*, 2017)
Atazanavir (oral; qd)	IC_{50}, 1.4	IC_{50}, 0.4	300	0.9	6.5	0.1	0.04	1.1	1.8	Antiviral agent; (Karlgren *et al.*, 2012; Metsu *et al.*, 2017)
Indinavir (oral; tid)	K_i, 10.8	K_i, 8.5	800	11.4	1.05	0.1	0.4	1.0	4.6	Antiviral agent; (Acosta *et al.*, 1999; Anderson *et al.*; 2000; Annaert *et al.*, 2010)
Rifampin (oral; qd)	K_i, 0.2	K_i, 0.3	450	10.4	3.6	0.1	0.20	1.0	28.7	Antibacterial agent; (Pargal *et al.*, 2001; Karlgren *et al.*, 2012)
Erythromycin ethylsuccinate (oral; tid)	IC_{50}, 7.5	IC_{50}, 1.6	600	1.7	2.7	0.07	0.20	1.2	4.9	Antibacterial agent; (Croteau *et al.*, 1988; De Bruyn *et al.*, 2013)
Dicloxacillin (oral; qid)	K_i, 0.4	K_i, 1.6	250	29.0	1.4	0.1	0.04	1.1	2.5	Antibacterial agent; (Roder *et al.*, 1995; De Bruyn *et al.*, 2013; Wu *et al.*, 2015)
Silibinin (oral; qd)	K_i, 3.4	K_i, 3.3	240	1.3	6.0	0.1	0.25	1.1	3.6	Adjuvant in chronic hepatitis and cirrhosis; (Lorenz *et al.*, 1984; Weyhenmeyer *et al.*, 1992; De Bruyn *et al.*, 2013)

C_{max}, maximum plasma concentration of inhibitor after oral dosing in humans; dose, inhibitor dose; $f_{u\text{-plasma}}$, unbound fraction of inhibitor in plasma; K_i, inhibition constant; IC_{50}, half maximal inhibitory concentration of inhibitor; K_a, absorption rate constant of inhibitor; R, accumulative factor for inhibitor; $t_{1/2}$, terminal half-life of inhibitor; R_{AUC}, ratio of glycyrrhizin's AUC in presence and absence of an inhibitor of OATP1B1/1B3. R_{AUC} was calculated using the following equation (Tweedie *et al.*, 2013):

$$R_{AUC} = 1 + I_{in,max,u}/K_i \text{ or } R_{AUC} = 1 + I_{in,max,u}/IC_{50} \quad (1)$$

where $I_{in,max,u}$ is the estimated maximum unbound concentration of the inhibitor at the inlet to the liver and is equal to $f_{u\text{-plasma}}\cdot[C_{max} \times R + (F_aF_g \times K_a \times \text{Dose})/Q_h]$. R is equal to $1/(1-e^{-k\tau})$, where k is the elimination rate constant ($0.693/t_{1/2}$) and τ is the dosing interval (h). F_aF_g is the inhibitor's fraction of dose absorbed and was set at 1. Q_h is the human hepatic blood flow (estimated as1500 mL·min^{-1}). If K_a value is unknown, K_a was set at 0.1 min^{-1}. For $I_{in,max,u}$ estimation, blood-to-plasma concentration ratios of the inhibitors were assumed to be 1. Glycyrrhizin is a dual substrate of OATP1B1 and OATP1B3, which exhibit comparable transport capabilities based on the *in vitro* data in Table 2. Because OATP1B1 and OATP1B3 normally can compensate for the lack of each other, R_{AUC} is calculated using the higher of the K_i (or IC_{50}) values for these two transporters; genetic polymorphisms, which may result in poor hepatic expression or negligible transport activity of either of these transporters, are not considered here.

References

Acosta EP, Henry K, Baken L, Page LM, Fletcher CV (1999). Indinavir concentrations and antiviral effect. Pharmacotherapy 19(6): 708–712.

Anderson PL, Brundage RC, Bushman L, Kakuda TN, Remmel RP, Fletcher CV (2000). Indinavir plasma protein binding in HIV-1-infected adults. AIDS 14(15): 2293–2297.

Annaert P, Ye ZW, Stieger B, Augustijns P (2010). Interaction of HIV protease inhibitors with OATP1B1, 1B3, and 2B1. Xenobiotica 40(3): 163–176.

Croteau D, Bergeron MG, LeBel M (1988). Pharmacokinetic advantages of erythromycin estolate over ethylsuccinate as determined by high-pressure liquid chromatography. Antimicrob Agents Chemother 32(4): 561–565.

Danner SA, Carr A, Leonard JM, Lehman LM, Gudiol F, Gonzales J *et al.* (1995). A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. N Engl J Med 333(23): 1528–1533.

De Bruyn T, van Westen GJ, Ijzerman AP, Stieger B, de Witte P, Augustijns PF *et al.* (2013). Structure-Based Identification of OATP1B1/3 Inhibitors. Mol Pharmacol 83:1257–1267.

Furihata T, Matsumoto S, Fu Z-G, Tsubota A, Sun Y, Matsumoto S *et al.* (2004). Different Interaction Profiles of Direct-Acting Anti-Hepatitis C Virus Agents with Human Organic Anion Transporting Polypeptides. Am J Gastroenterol Antimicrob Agents Ch 58: 4555–4564.

Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E *et al.* (2012). Classification of Inhibitors of Hepatic Organic Anion Transporting Polypeptides (OATPs): Influence of Protein Expression on Drug-Drug Interactions. J Med Chem 55: 4740–4763.

Lawitz EJ, Rodriguez-Torres M, Denning J, Mathias A, Mo H, Gao B *et al.* (2013). All-oral therapy with nucleotide inhibitors sofosbuvir and GS-0938 for 14 days in treatment-naive genotype 1 hepatitis C (NUCLEAR). J Viral Hepat 20(10):699–707.

Lorenz D, Lücker PW, Mennicke WH, Wetzelsberger N (1984). Pharmacokinetic studies with silymarin in human serum and bile. Methods Find Exp Clin Pharmacol 6(10):655–661.

Menon RM, Klein CE, Podsadecki TJ, Chiu YL, Dutta S, Awni WM (2016). Pharmacokinetics and tolerability of paritaprevir, a direct acting antiviral agent for hepatitis C virus treatment, with and without ritonavir in healthy volunteers. Br J Clin Pharmacol 81(5):929–940.

Metsu D, Seraissol P, Delobel P, Cinq-Frais C, Cuzin L, Izopet J *et al.* (2017). Is the unbound concentration of atazanavir of interest in therapeutic drug monitoring? Fund Clin Pharmacol 31: 245–253.

Pargal A, Rani S (2001). Non-linear pharmacokinetics of rifampicin in healthy Asian Indian volunteers. Int J Tuberc Lung Dis 5(1):70–79.

Roder BL, Frimodt-Moller N, Espersen E, Rasmussen SN (1995). Dicloxacillin and flucloxacillin: Pharmacokinetics, protein binding and serum bactericidal titers in healthy subjects after oral administration. Infection 23(2): 107–112.

Sanford M (2015). Simeprevir: a review of its use in patients with chronic hepatitis C virus infection. Drugs 75(2):183–196.

Shebley M, Liu J-R, Kavetskaia O, Sydor J, de Morais SM *et al.* (2017). Mechanisms and Predictions of Drug-Drug Interactions of the Hepatitis C Virus Three Direct-Acting Antiviral Regimen: Paritaprevir/Ritonavir, Ombitasvir, and Dasabuvir. Drug Metab Dispos 45: 755–764.

Tweedie D, Polli JW, Berglund EG, Huang SM, Zhang L, Poirier A, *et al.* (2013). Transporter studies in drug development: experience to date and follow-up on decision trees from the international transporter consortium. Clin Pharmacol Ther 94: 113–125.

Weyhenmeyer R, Mascher H, Birkmayer J (1992). Study on dose-linearity of the pharmacokinetics of silibinin diastereomers using a new stereospecific assay. Int J Clin Pharmacol Ther Toxicol 30(4):134–138.

Wu G, Zheng Y, Zhou H, Hu X, Liu J, Zhai Y *et al.* (2015). Safety and pharmacokinetics of dicloxacillin in healthy Chinese volunteers following single and multiple oral doses. Drug Design Dev Ther 9: 5687–5695.