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Comparison of In Vitro to In Vivo Extrapolation Approaches for Predicting Transporter-Mediated Hepatic Uptake Clearance Using Suspended Rat Hepatocytes

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The material includes Supplemental Table S1, Supplemental Methods and References.

Supplemental Materials:

Table S1: Summary of the in vitro and in vivo evidence of OATP-mediated hepatic uptake of the selected compounds

	Transporters involved in hepatic uptake			Preclinical and clinical evidence of OATP involvement		
Compound	**Approach**	**Hepatic Transporters**	**Reference**	**Approach**	**PK Change**	**Reference**
Rosuvastatin	RAF and REF	OATP1B1 (M) and OATP1B3 (total 96% and 80%)	(Kunze et al., 2014) [a]	oatp1a/1b KO mice	i.v. : AUC↑ 1.7-fold PO: Cmax ↑ 3-fold; AUC↑ 7.1-fold	(Iusuf et al., 2013)
	RAF	OATP1B1(>95%) and OATP1B3 (<5%)	(Izumi et al., 2018) [b]	oatp1a/1b KO mice	i.v. : AUC↑ 2.3-fold PO: Cmax ↑ 35-fold; AUC↑ 48-fold	(Salphati et al., 2014)
	Hepatocytes with inhibitor	OATP1B1/1B3 (71%), 2B1 (21%) and NTCP (6%)	(Bi et al., 2019)	Clinical DDI with rifampicin (RIF)	+ 600 mg RIF: Cmax ↑ 5.2-fold; AUC↑ 2.5-fold	(Mori et al., 2020)
Pravastatin	RAF and REF	OATP1B1 (M) and OATP1B3 (total 88% and 70%)	(Kunze et al., 2014) [a]	oatp1a/1b KO mice	AUC↑ 4.3-fold	(Higgins et al., 2014)
	RAF	OATP1B1 (>95%) and OATP1B3 (<5%)	(Izumi et al., 2018) [b]	oatp1a/1b KO mice	i.v. AUC↑ 4-fold PO: Cmax ↑ 9.7-fold; AUC↑ 18-fold	(Salphati et al., 2014)
	Hepatocytes with inhibitor	OATP1B1/1B3 (98%)	(Bi et al., 2019)	Clinical DDI with cyclosporin A (CsA)	+ 2x 100 mg CsA: Cmax ↑ 3.2-fold; AUC↑ 3.4-fold	(Yee et al., 2019)
Valsartan	RAF	OATP1B1 (20-70%) and 1B3 (30-80%)	(Yamashiro et al., 2006)	Clinical DDI with RIF	+ 600 mg RIF: Cmax ↑ 3.7-fold; AUC↑ 5.5-fold	(Mori et al., 2020)
	RAF	OATP1B1 (>75%) and 1B3 (<25%)	(Izumi et al., 2018)[b]			
Pitavastatin	RAF	OATP1B1 (90%) and OATP1B3 (10%)	(Hirano et al., 2004)	oatp1a/1b KO mice	PO: Cmax ↑ 13.5-fold; AUC↑ 11.7-fold	(Salphati et al., 2014)
	RAF and REF	OATP1B1 (M) and OATP1B3 (total 43% and 35%)	(Kunze et al., 2014) [a]	oatp1a/1b KO mice	i.v.: AUC↑ 3.7-fold	(Chang et al., 2019)
	RAF	OATP1B1 (>90%) and OATP1B3 (<10%)	(Izumi et al., 2018) [b]	Clinical DDI with RIF	+ 600 mg RIF: Cmax ↑ 3.7-fold; AUC↑ 4.0-fold	(Mori et al., 2020)
	Hepatocytes: inhibitor	OATP1B1/1B3 (81%), 2B1 (12%)	(Bi et al., 2019)			
Fluvastatin	RAF and REF	OATP1B1 (M) and OATP1B3 (total 50% and 42%)	(Kunze et al., 2014) [a]	oatp1a/1b KO mice	i.v.: AUC↑ 1.7-fold	(Chang et al., 2019)
	RAF	OATP1B1 (>93%) and OATP1B3 (<7%)	(Izumi et al., 2018) [b]			
	Hepatocytes: inhibitor	OATP1B1/1B3 (48%), 2B1 (12%) and NTCP (16%)	(Bi et al., 2019)			
Cerivastatin	RAF and REF	OATP1B1 (M) and OATP1B3 (total 19% and 16%)	(Kunze et al., 2014) [a]	Clinical DDI with CsA	AUC↑ 3.8-fold	(Shitara et al., 2013)
	RAF	OATP1B1 (>95%) and OATP1B3 (<5%)	(Izumi et al., 2018) [b]			
Nateglinide	RAF	OATP1B1(>85%) and 1B3 (<15%)	(Izumi et al., 2018) [b]	Clinical DDI with fluconazole	AUC↑ 1.5-fold	(Niemi et al., 2003)
Atorvastatin	RAF and REF	OATP1B1 (M) and OATP1B3 (total 73% and 63%)	(Kunze et al., 2014) [a]	oatp1a/1b KO mice	AUC↑ 19-fold	(Higgins et al., 2014)
	RAF	OATP1B1 (>85%) and 1B3(<15%)	(Izumi et al., 2018) [b]	Clinical DDI with RIF	+ 600 mg RIF: Cmax ↑ 13.3-fold; AUC↑ 7.3-fold	(Mori et al., 2020)
Bosentan	RAF	OATP1B1 (>90%) and 1B3 (<10%)	(Izumi et al., 2018) [b]	Clinical DDI with RIF	+ 600 mg RIF: AUC↑ 3.2-fold	(Yoshikado et al., 2017)
Glyburide	RAF	OATP1B1 (>95%) and 1B3 (<5%)	(Izumi et al., 2018) [b]	Clinical DDI with RIF	+ 600 mg RIF: AUC↑ 2.2-fold	(Shitara et al., 2013)
Asunaprevir	Hepatocytes	Saturable Uptake	(Eley et al., 2015)	Clinical DDI with rifampin	PO: Cmax ↑ 21-fold; AUC↑ 15-fold	(Eley et al., 2015)
Telmisartan	RAF	OATP1B3 only	(Ishiguro et al., 2006)	Clinical DDI with Nisoldipine	AUC↑ 2.3-fold	(Bajcetic et al., 2007)
	RAF	OATP1B3 only	(Izumi et al., 2018) [b]			

[a]In Kunze, et al, the percent contribution of both OATP1B1 and OATP1B3 to the total active hepatic uptake clearance determined using RAF and REF respectively were listed in the parentheses. M in the parentheses indicated the major OATP involved in hepatic uptake.

[b]The percent contribution (%) cited from Izumi, et al and others represents the relative contribution of OATP1B1 and OATP1B3 to hepatic uptake.

RIF: rifampicin

CsA: cyclosporin A

Supplemental methods:

Bioanalysis using RapidFire

Analysis of specimens for intrinsic metabolic CL in rat hepatocytes was performed using the RapidFire 365 high-throughput SPE system interfaced with a 6550 QTOF mass spectrometer with dual Agilent Jet (AJS) ESI source (Agilent Technologies, Santa Clara, CA, USA). The instrument settings were gas temperature at 200°C, drying gas at 18 l/min, nebulizer 40 psig, sheath gas temperature at 350°C, sheath gas flow at 12 l/min and Vcap at 5000 V. The acquisition rate/time was 5 spectra/s and Mass range was 100 to 700 m/z. Specimens were analyzed in positive mode. The load/wash solvent (solvent A) was water containing 0.1% (v/v) formic acid. The elution solvent (solvent B) was acetonitrile containing 0.1% (v/v) formic acid. Specimens were aspirated serially, under vacuum, directly from multi-well assay plates. In each case, a 10 µL aliquot was loaded onto a C18 SPE cartridge (cartridge type A) to remove buffer salts, using solvent A at a flow rate of 1.5 ml/min for 3500 ms. The retained and purified analytes were eluted to the mass spectrometer by washing the cartridge with solvent B at 0.60 to 0.8 ml/min for 3500 ms. The cartridge was re-equilibrated with solvent A for 500 ms at 1.5 ml/min. The entire sampling cycle was around 8 s per well, enabling the analysis of a 96-well plate in approximately 13 min.

LC-MS/MS Quantification

The Shimadzu LC system included two LC-20ADXR pumps, a SIL-30ACMP auto sampler, a CBM-20A controller and a CTO-20A column oven (Shimadzu, USA). The chromatography was performed using C18 column (Cadenza 5CD - C18, 5 µM, 2 X 30mm, Imtakt, USA). Sample injection volume was 5µL and the LC flow rate was 1.2 mL/min. Mobile phase (A) was water with 0.1% formic acid and mobile phase (B) was acetonitrile with 0.1% formic acid. The gradient elution started with a 0.1 min hold at 5% mobile phase (B), followed by a 0.8 min ramp to 95% mobile phase (B) and hold for 0.5 min, followed by a 0.1 min ramp to 5% mobile phase (B) and a hold for 0.4 min re-equilibration. Integrated valco valve was used and the injected liquid was directed into Mass Spectrometer from 0.3 min to 1.8 min. The triple-quadruple instrument is an AB Sciex 5500 QTrap. The mass spectrometer and peripherals were all controlled by Analyst™ (version 1.6.3; AB Sciex, Ontario, Canada) and DiscoveryQuant™

software (version 3.0.1; AB Sciex, Ontario, Canada). Positive ionizations were used in selected reaction monitoring (MRM) scan mode. All the transitions of twelve compounds are listed below.

Compound	MW	Q1	Q3	DP	CE	CPX	EP
Glyburide	494	495.2	370.1	73	10	9	8
Nateglinide	317	318.2	69.1	120	25	13	12
Valsartan	435	436.2	291.1	43	10	13	12
Telmisartan	514	515.0	276.1	162	50	13	2
Atorvastatin	558	559.4	440.3	81	10	16	10
Asunaprevir	747	748.3	648.3	60	27	17	5
Pitavastatin	421	422.5	290.2	96	39	10	10
Bosentan	551	551.8	202.0	154	25	15	2
Rosuvastatin	481	482.0	258.1	76	28	20	10
Cerivastatin	459	460.2	356.3	86	20	12	10
Fluvastatin	411	412.3	224.1	71	30	22	10
Pravastatin	446	447.1	327.4	50	23	30	10

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