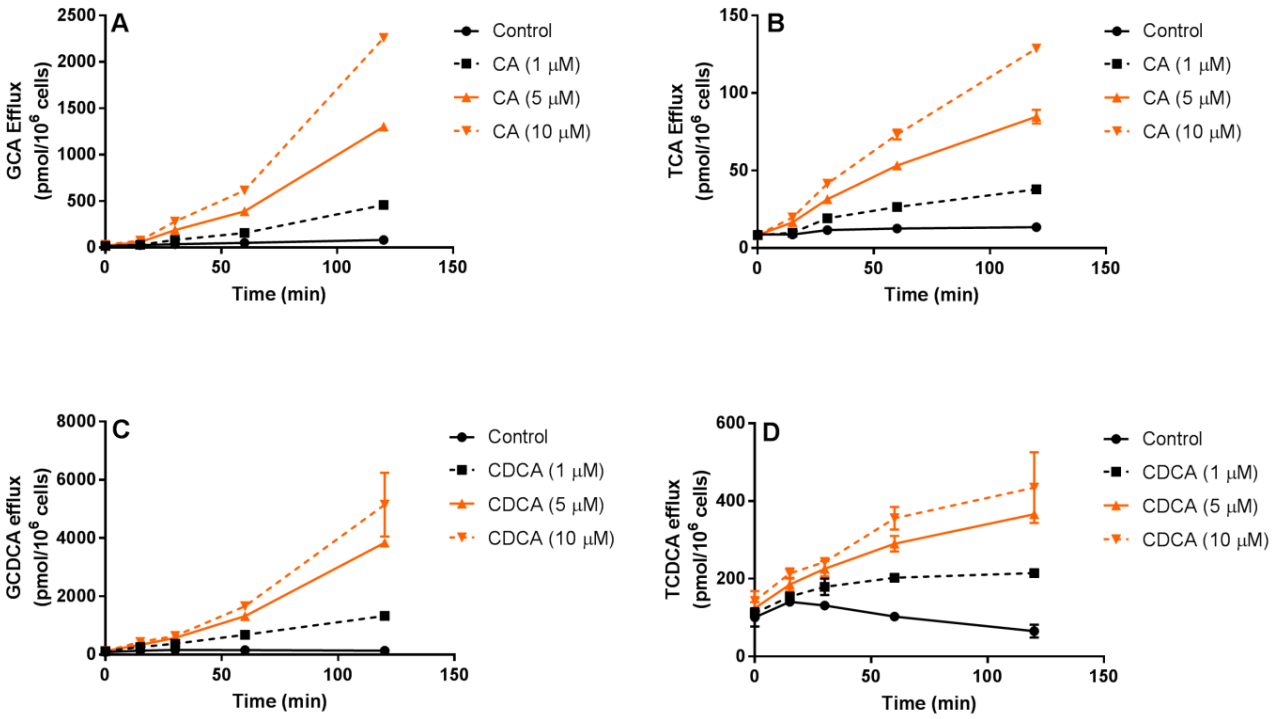
Function and Expression of Bile Salt Export Pump (BSEP) in Suspension Human Hepatocytes

Paresh P. Chothe[*1,2], Rachel Pemberton[1] and Niresh Hariparsad[1,3]

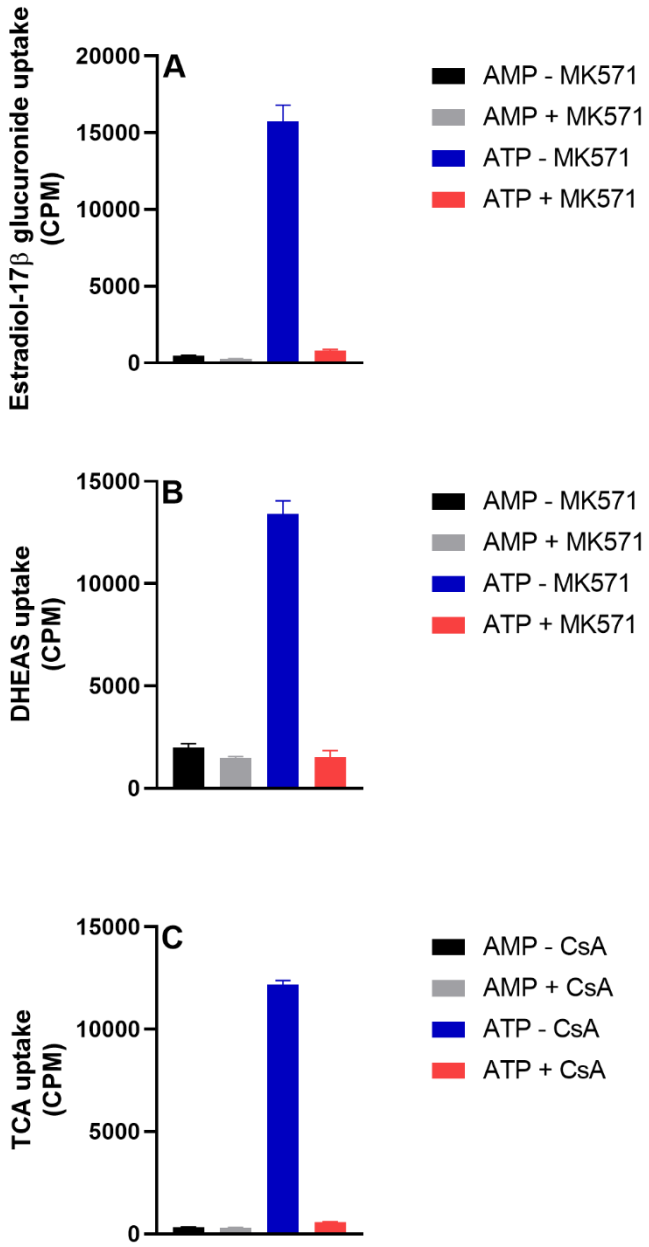
[1]Drug Metabolism and Pharmacokinetics, Vertex Pharmaceuticals Incorporated, Boston, Massachusetts, USA

[2]Current affiliation - Drug Metabolism and Pharmacokinetics, Takeda Pharmaceutical Company Limited, Cambridge, Massachusetts, USA

[3]Current affiliation – Drug Metabolism and Pharmacokinetics, AstraZeneca, 35 Gate House Park, Waltham, Massachusetts, USA

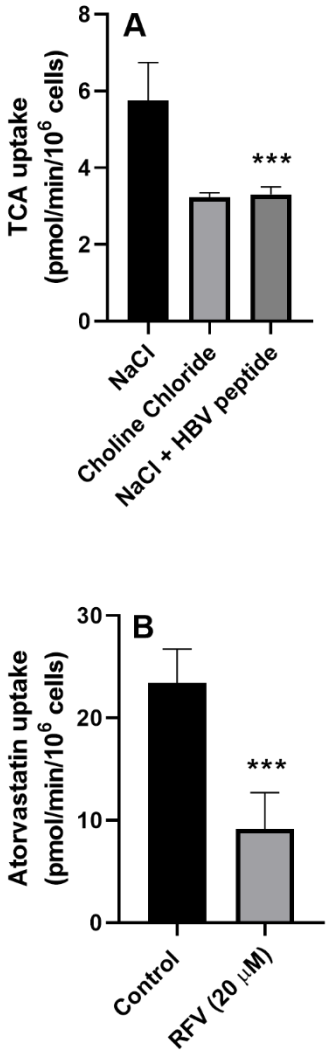


Supplemental Figure 1. Efflux of Glycine- and Taurine-conjugated CA and CDCA in Human Hepatocyte Suspension (WWQ). Cells were incubated with CA and CDCA at 1, 5 and 10 µM. Efflux of GCA (A), TCA (B), GCDCA (C) and TCDCA (D) was measured at 15, 30, 60 and 120 minutes. For control experiment cells were incubated with vehicle (DMSO <0.1%). The experiment was done in triplicate (n=3).



Supplemental Figure 2. Uptake Analysis of Estradiol-17β glucuronide, DHEAS and TCA in human MRP3, MRP4 and BSEP Overexpressed Membrane Vesicles, respectively. (A) Uptake of Estradiol-17β glucuronide (10 µM) was measured in the presence of AMP and ATP with and without MK571 (150 µM) in MRP3 overexpressed membrane vesicles. (B) Uptake of DHEAS (0.5 µM) was measured in the presence of

AMP and ATP with and without MK571 (150 µM) in MRP4 overexpressed membrane vesicles. (C) Uptake of TCA (0.2 µM) was measured in the presence of AMP and ATP with and without CsA (10 µM) in BSEP overexpressed membrane vesicles as described under method section. The experiment was done in triplicate (n=3).



Supplemental Figure 3. Uptake Analysis of Taurocholate and Atorvastatin in Pooled Suspension Human Hepatocytes (YJG). (A) The uptake of taurocholate (2 μM) was measured in the presence of sodium-containing buffer (KHB buffer), sodium-free buffer (sodium chloride was replaced with choline chloride) and KHB buffer containing HBV peptide (0.1 μM) for 5 min. (B) The uptake of atorvastatin (0.5 μM) was measured in the absence and presence of 20 μM RFV for 3 min. The experiment was performed in triplicates (n=3). *** $p < 0.001$.

Supplemental Table 1. Demographic Information of Human Hepatocyte Donors

Donor ID	Donor	Sex	Age (year)	Race	Cause of death	BMI
WWQ	Single	Male	22	Caucasian	Head Trauma second to Blunt Injury	26.9
YZG	Pooled (5)	Mixed	NA	NA	NA	NA

Supplemental Table 2: The multiple Reaction Monitoring (MRM) Transitions of Compounds

Compound	Electrospray Ionization (ESI) mode	Parent Ion m/z	Fragment ion m/z	DP (V)	CE (V)
Atorvastatin	Positive	559.336	250.139	100	60
Bosentan	Positive	552.281	552.281	125	50
Estradiol 17β-glucuronide	Negative	447.3	113.1	-35	-36
Rifampicin	Positive	823.6	791.44	70	25
Troglitazone	Negative	440.203	397.197	-90	-30
Troglitazone sulfate	Negative	520	440	-49	-30
CA[1]	Negative	407.446	407.446	-145	-40
CDCA[2]	Negative	391.2	391.2	-170	-40
G-CA[3]	Positive	466.508	412.317	105	30
T-CA[4]	Positive	516.4	462.2	100	20
G-CDCA[5]	Positive	450.462	414.314	60	20
T-CDCA[6]	Positive	500.464	464.342	85	20

[1]cholic acid, [2]chenodeoxycholic acid, [3]glycocholic acid, [4]taurocholic acid, [5]glycochenodeoxycholic acid, [6]taurochenodeoxycholic acid.

Supplemental Table 3: Human transporter proteotypic heavy labeled tryptic peptide standards

Transporter (Gene)	**Peptide Sequence**	**MRMs 1 and 2 (product ion) [mass spectrometer specific]**
P-gp	I_{368}IDNKPSIDSYSK$_{380}$	496.60/631.32 (y11), 496.60/904.46 (y8)
MRP2	Y_{514}FAWEPSFR$_{522}$	606.79/902.44 (y7), 606.79/516.28 (y4)
MRP3	G_{654}ALVAVVGPVGCGK$_{667}$	646.37/682.35 (y7), 646.37/781.42 (y8)
OATP1B1	N_{321}VTGFFQSFK$_{330}$	591.81/969.50 (y8), 591.81/868.46 (y7)
OATP1B3	I_{615}YNSVFFGR$_{623}$	556.79/836.43 (y7), 556.79/722.34 (y6)
OATP2B1	Y_{641}YNNDLLR$_{648}$	540.77/754.41 (y6), 540.77/917.47 (y7)
OAT2	N_{20}VALLALPR$_{28}$	488.81/763.51 (y7), 488.81/579.39 (y5)
OAT7	D_{313}TLTLEILK$_{321}$	527.32/724.48 (y6), 527.32/837.56 (y7)
BSEP	S_{462}TALQLIQR$_{470}$	520.31/539.35 (y4), 520.31/667.41 (y5)
NTCP	G_{144}IYDGDLK$_{151}$	444.73/718.35 (y6), 444.73/555.29 (y5)
NaK ATPase	V_{213}DNSSLTGESEPQTR$_{227}$	543.92/511.29 (y4), 815.38/511.29 (y4)
GammaGTP	L_{156}FQPSIQLAR$_{165}$	591.85/794.48 (y7), 591.85/389.22 (b3)
OCT1	L_{330}SPSFADLFR$_{339}$	581.81/481.75 (y8), 581.81/865.44 (y7)
NaK ATPase	V_{213}DNSSLTGESEPQTR$_{227}$	543.92/511.29 (y4), 815.38/511.29 (y4)

Supplemental Table 4: Uptake data of Cholic Acid (CA) and Chenodeoxycholic Acid (CDCA) in Pooled Human Suspension Hepatocytes

Compound	Uptake (pmol/min/10^6 cells)		
	Control	**RFV (20 µM)**	**HBV peptide (0.1 µM)**
CA	1.22 ± 0.1	0.76** ± 0.08	0.69** ± 0.13
CDCA	6.84 ± 1.1	5.33 ± 0.74	5.60 ± 1.1

The data is presented as mean ± SD

** $p < 0.005$

Supplemental Table 5: Permeability of Cholic Acid (CA) and Chenodeoxycholic Acid (CDCA) and Their Glycine- and Taurine-conjugated Salts

	CA	CDCA	GCA	TCA	GCDCA	TCDCA
MDCK, Papp ($\times 10^{-6}$ cm/s)	0.1	3.7	0.1	0.3	0.3	0.4

Supplemental Table 6: Summary of Uptake Data of Bile Salts in MRP3 Membrane Vesicles

Bile salt	Uptake (pmol/mg)								fold change
	ATP				AMP				
	ATP - MK571		ATP + MK571		AMP - MK571		AMP + MK571		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
GCA	64.69	2.23	5.26	0.87	11.86	0.92	5.86	0.06	5.45
TCA	26.35	0.29	5.8	0.49	12.04	0.35	5.28	0.21	2.18
GCDCA	195.52	4.56	17.6	0.42	36.56	1.92	15.55	0.43	5.35
TCDCA	80.96	3.15	18.71	0.53	32.99	0.81	16.9	1.87	2.45

Supplemental Table 7: Summary of Uptake Data of Bile Salts in MRP4 Membrane Vesicles

Bile Salt	Uptake (pmol/mg)								fold change
	ATP				AMP				
	ATP - MK571		ATP + MK571		AMP - MK571		AMP + MK571		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
GCA	9.81	0.98	4.29	0.76	8.39	0.96	4.24	0.16	1.17
TCA	13.59	1.88	8.25	0.7	11.08	0.63	8.62	0.84	1.23
GCDCA	33.61	4.76	19.08	1.35	24.97	3.23	17.48	1.05	1.35
TCDCA	34.64	6.65	15.1	2.07	22.18	3.33	17.3	2.04	1.56

Supplemental Table 8: Summary of Uptake Data of Bile Salts in BSEP Membrane Vesicles

Bile salt	Uptake (pmol/mg)								fold change
	ATP				AMP				
	ATP - CsA		ATP + CsA		AMP - CsA		AMP + CsA		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
GCA	299.08	9.71	15.81	0.85	7.17	1.15	7.08	0.67	41.72
TCA	617.48	27.6	44.66	2.57	20.05	4.68	17.85	3.33	30.80
GCDCA	255.08	22.38	84.17	32	22.01	1.25	16.58	2.67	11.59
TCDCA	323.71	22.15	94.69	15.42	27.79	2.60	29.37	5.72	11.65