Functional Evaluation of SLC Transporters in BD Gentest™ Cryopreserved Human Hepatocytes Using an Oil-Filtration Suspension Assay

[1]Eric Sands, [1]Vina Ketty, [2]Yong-Hae-Han, [1]Joanne Bourgea, [1]Chris J. Patten, [1]Charles L. Crespi, and [1]Guangqing Xiao
[1]**BD Biosciences** – Discovery Labware, Woburn, MA, [2]Bristol-Myers Squibb Co, Princeton, NJ

Abstract
The purpose of this study was to evaluate the function of Sodium Taurocholate Co-transporting Polypeptide (NTCP), Organic Anion Transporting Polypeptide (OATP), and Organic Cation Transporter (OCT) in cryopreserved human hepatocytes, and to examine the impact of various drugs on hepatic transporter-mediated uptake. Cryopreserved human hepatocytes were prepared using the Percoll™ method. Hepatocytes were suspended in Krebs-Henseleit buffer (KHB) and uptake assays were performed using an oil-filtration method. The function of NTCP, OATP, and OCT1 was characterized at both 37˚C and 4˚C by using relatively specific substrates and inhibitors. The transport activity in cryopreserved hepatocytes prepared from different donors was investigated. Time-, temperature-, and concentration-dependent uptake of Taurocholate, Estrone-3-Sulfate, and Tetraethylammonium (TEA) were observed in cryopreserved human hepatocytes. The transporter-mediated uptake was decreased in the presence of various inhibitors. Donor-to-donor variation on the uptake of Taurocholate, Estrone-3-Sulfate, and TEA was found. Our results indicated that significant function of NTCP, OATP, and OCT1 remained in cryopreserved human hepatocytes. Cryopreserved human hepatocytes can be used to study drug transport in the liver by using specific substrates and specific inhibitors. Cryopreserved human hepatocytes can also be useful for studying hepatic transporter involved drug-drug interactions.

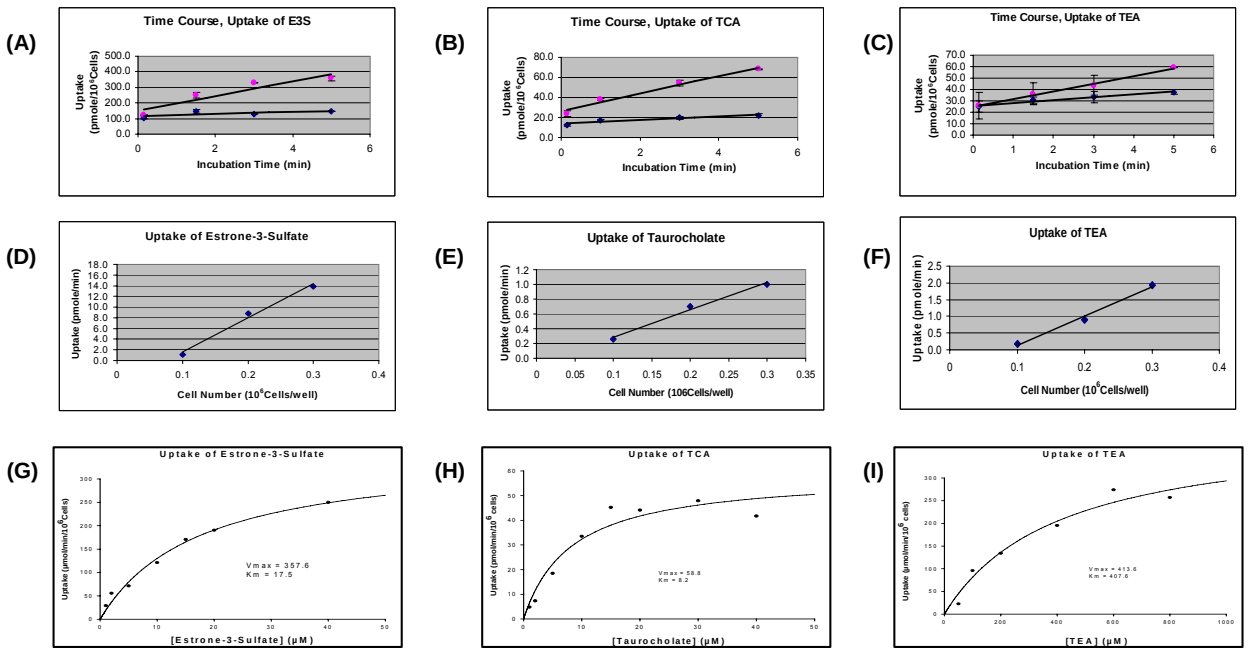
Introduction
Human hepatocytes express many uptake transporters, such as NTCP, OATP1B1 and 1B3, and OCT1.[1-4] These transporters mediate the transport of many drugs into hepatocytes.[5-7] The hepatocyte suspension transport assay has been accepted as an established model for drug uptake studies because the mRNA levels of many transporters remain unchanged in both fresh and cryopreserved human hepatocytes when kept in suspension,[8] and ABC transporter are degraded after isolation.[9] Evaluation of uptake transporter function in cryopreserved human hepatocytes may provide information to predict *in vivo* drug transport and clearance. The uptake of Taurocholate and Estradiol-17-β-D-Glucuronide into suspended human and rat cryopreserved hepatocytes demonstrated that function of human OATP and NTCP transporters remained after cryopreservation and thawing.[10-11] To date, the function of hOCT1 in cryopreserved human hepatocytes has not been extensively investigated. In this study, we evaluated the function of NTCP, OATP, and OCT1 in cryopreserved human hepatocytes using an oil-filtration suspension uptake method. Estrone-3-Sulfate, Taurocholate, and TEA were selected as the relatively specific substrates for OATP, NTCP, and OCT1, respectively. The correlation between the transport activities of the probe substrates was also examined. Our results demonstrated that the oil-filtration suspension assay can be used to evaluate drug transport and clearance in the liver tissue. The activity and contribution of each transporter can be assessed when specific substrates and inhibitors are available. The donor variation in transporting activity can be used to predict individual variation in drug response.

Methods
BD Gentest™ Cryopreserved Human Hepatocyte Preparation
BD Gentest™ Cryopreserved Human Hepatocytes (Cat. Nos. 456426 and 456427) were prepared using the BD™ CryoHepatocyte Purification Kit (Cat. No. 454500). Hepatocytes were thawed at 37ºC, immediately added to ISOM's media containing 30% Isotonic Percoll™, and centrifuged at 60 x g for 7 minutes at room temperature. The pellet was resuspended in ISOM's media containing 10% FBS and centrifuged again at 60 x g for 3 minutes at room temperature. The hepatocytes were then suspended in KHB at a concentration of $2.0x10^6$ cells/mL.

Hepatocyte Transporter Suspension Uptake Assay
The suspension assays were performed at both 4ºC and 37ºC using BD Gentest™ Hepatocyte Suspension Assay Kit (Cat. No. 454460). Hepatocytes were pre-incubated at the assay temperature for 5 minutes. 50 µL of a substrate solution were added to the well of a 48-well plate, and the uptake was initiated by adding 100 µL of hepatocytes. The mixture was incubated at 4ºC and 37ºC for 3 minutes. The uptake was terminated by separating the hepatocytes from the uptake solution by using the oil-filtration method.[9,10] After overnight incubation, the oil tube was cut, and the radioactivity in both the uptake solution and hepatocytes was determined using a liquid scintillation counter.

Data analysis
The uptake activity was expressed as pmol/10^6 cells/min. Each data point represents mean ± S.D. (n=4). Results with the probability of $p<0.05$ were considered significantly different.

1 Time-, Temperature-, Cell Concentration and Substrate Concentration-Dependent Uptake of Estrone-3-Sulfate, Taurocholate, and TEA

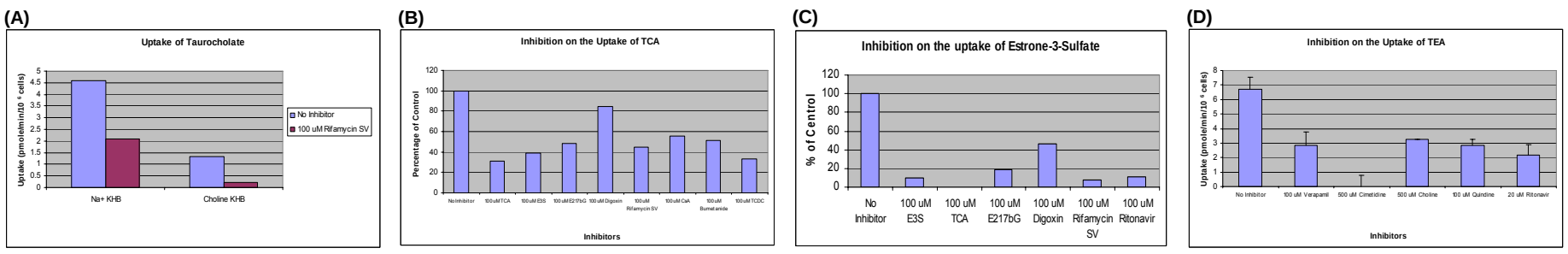


Time-, Temperature-, Cell Concentration and Substrate Concentration-Dependent Uptake of Estrone-3-Sulfate, Taurocholate, and Tetraethylammonium (TEA) in BD Gentest™ Cryopreserved Human Hepatocytes. Time-dependent uptake of Estrone-3-Sulfate (2 µM) **(A)**, Taurocholate (1 µM) **(B)**, and TEA (10 µM) **(C)** at 4˚C and 37˚C in KHB. Cell concentration dependent uptake of Estrone-3-Sulfate (2 µM) **(D)**, Taurocholate (1 µM) **(E)**, and TEA (10 µM) **(F)** in KHB. Substrate concentration-dependent uptake of Estrone-3-sulfate **(G)**, Taurocholate **(H)**, and TEA **(I)**. The activity in D-I was reported as the difference between uptake at 37˚C and 4˚C. The K_m for the uptake of Estrone-3-Sulfate, Taurocholate, and TEA was determined as 17.5 µM, 8.2 µM and 407 µM, respectively.

Based on the results, the uptake assay conditions were chosen as following:

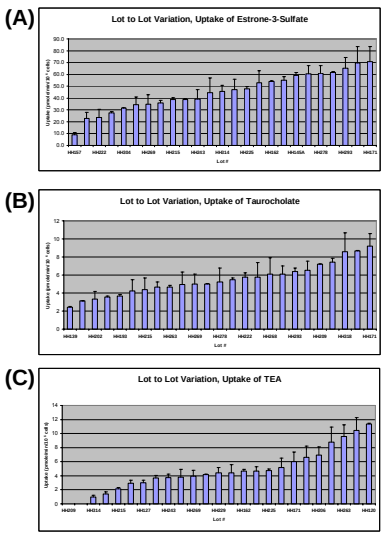
Substrate	Concentration	Cell #/Assay	Incubation
Estrone-3-Sulfate	2 µM	0.2 x 106 Cells	3 min
Taurocholate	1 µM	0.2 x 106 Cells	3 min
TEA	10 µM	0.2 x 106 Cells	3 min

2 Inhibition



Inhibition on the uptake of Taurocholate, Estrone-3-Sulfate, and TEA in BD Gentest™ Cryopreserved Human Hepatocytes. (A) Uptake of Taurocholate (1 µM) in Na^+ or Choline KHB in the presence or absence of Rifamycin SV (100µM). **(B)** Inhibitory effects of Taurocholate, Estrone-3-Sulfate, Estradiol-17-β-D-Glucuronide, Digoxin, Rifamycin SV, CsA, Bumetanide, and TCDC on the uptake of Taurocholate (1 µM). **(C)** Inhibitory effects of Taurocholate, Estrone-3-Sulfate, Estradiol-17-β-D-Glucuronide, Digoxin, Rifamycin SV, and Ritonavir on on the uptake of Estrone-3-Sulfate (2 µM). **(D)** Inhibitory effects of Verapamil, Cimetidine, Choline, Quinidine, and Ritonavir on the uptake of TEA (10 µM).

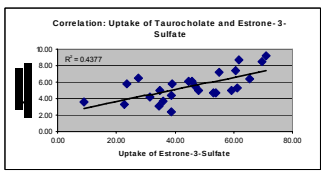
3 Lot variation



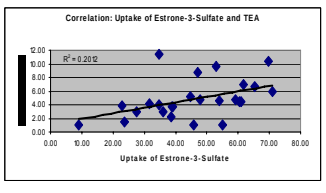
(D)

Lot	Estrone-3-Sulfate		Taurocholate		TEA	
	Ave	Stdev	Ave	Stdev	Ave	Stdev
HH266	47.00	8.82	5.50	0.18	8.76	2.18
HH269	34.79	7.93	4.98	1.13	3.94	0.84
HH206	61.66	0.58	8.68	0.02	6.92	1.19
HH209	55.14	2.87	7.17	0.08	ND	
HH268	44.76	12.26	6.09	1.83	5.15	1.38
HH157	8.92	1.93	3.59	0.08	ND	
HH225	47.96	1.69	4.97	1.37	4.72	0.24
HH127	27.41	1.24	6.51	1.03	3.01	0.38
HH304	31.57	0.53	4.22	1.26	4.17	0.08
HH293	65.44	8.87	6.36	0.41	6.61	1.60
HH263	52.98	10.06	4.68	0.17	9.59	1.67
HH318	69.62	13.99	8.55	2.14	10.41	1.89
HH120	34.60	6.44	3.14	0.01	11.34	0.12
HH162	54.01	0.90	4.66	0.57	4.66	0.24
HH171	70.94	12.40	9.17	1.39	6.00	1.38
HH193	35.94	2.03	3.68	0.17	2.93	0.44
HH202	22.82	5.05	3.32	0.89	3.80	1.10
HH215	38.65	1.81	4.40	1.27	2.15	0.20
HH222	23.63	6.90	5.77	0.46	1.43	0.28
HH229	60.54	6.95	7.41	0.44	4.40	0.77
HH243	39.12	7.95	5.78	1.59	3.77	0.48
HH145A	59.23	2.64	5.01	0.05	4.68	0.63
HH139	38.77	0.82	2.43	0.09	3.67	0.39
HH278	61.06	6.28	5.26	1.51	4.40	1.16
HH314	45.76	5.16	6.09	0.93	0.97	0.27

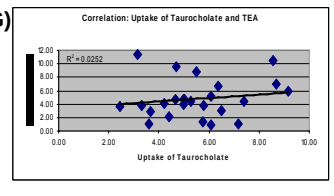
(E)



(F)



(G)



Lot Variation on the Uptake of Estrone-3-Sulfate, Taurocholate, and TEA in BD Gentest™ Cryopreserved Human Hepatocytes. The uptake activities of Estrone-3-Sulfate (2 µM) **(A)**, Taurocholate (1 µM) **(B),** and TEA (10 µM) **(C)** of 25 lots of BD Gentest™ Cryopreserved Human Hepatocytes were measured at 4ºC and 37ºC in KHB. The lot variation was summarized in Table **(D)**. Correlation between the uptake activity of each substrate **(E-G)**. The uptake activity was reported as the difference between 37ºC and 4ºC.

Summary and Conclusions:
1. Significant function on the uptake of Estrone-3-Sulfate, Taurocholate, and TEA remained in BD Gentest™ Cryopreserved Human Hepatocytes. The uptake was time-, temperature-, cell concentration, and substrate concentration-dependent.
2. Moderate to large lot-to-lot variation observed on the uptake of Estrone-3-Sulfate,Taurocholate, and TEA.
3. Good correlation between the uptake activities of Estrone-3-Sulfate and Taurocholate suggested that both hNTCP and hOATP contributed to the uptake of Estrone-3-Sulfate and TCA
4. Na^+ independent uptake of Taurocholate was found in human hepatocytes. Similar results were also reported in human and rat hepatocytes.[11,12] Inhibition and kinetic studies suggested Na^+ independent uptake was mediated by OATP transporters.
5. It has been reported that the uptake of Estrone-3-Sulfate into human hepatocytes was predominately mediated by hOATP1B1.[13] Our kinetics studies also suggested hOATP1B1 is the major transporter of Estrone-3-Sulfate. hOATP1B3 and hNTCP may also contribute to the transport of Estrone-3-Sulfate.

References
1. Abe, T., et al., *J. Biol. Chem.* **274**:17159 (1999).
2. Konig, J., et al., *J. Biol. Chem.* **275**:23136 (2000).
3. Hagenbuch, B. and Meier, P., *J. Clin. Invest.* **93**:1326 (1994).
4. Zhang, L., et al., *Mol Pharmacol.* **51**:912 (1997).
5. Noë, J., et al., *Drug Metab. Dispos.* **35** :1308 (2007).
6. McRae, M.P., et al., *J. Pharmacol. Exp. Ther.* **318**:1368 (2006).
7. Shu, Y., et al., *J. Clin. Invest.* **117**:1422 (2007).
8. Richart, L., et al., *Drug Metab. Dispos.* **34**:870 (2006).
9. Hoffmaster, K.A., *Pharm. Res.* **21**:1294 (2004).
10. Hirano, M., et al., *J. Pharmacol. Exp. Ther.* **311**:139 (2004).
11. Shitara, Y., et al., *Drug Metab. Pharmacokin.* **18**:33 (2003).
12. Kouzuki, H., et al., *J. Pharmacol. Exp. Ther.* **288**:27 (1999).
13. Sujiyama, Y., et al., *J. Pharmacol. Exp. Ther.* **311**:139 (2004).

Presented at the 8th Int'l Society Study of Xenobiotics October 9-12, 2007
Sendai Japan. Abstract No. 477