

DMD #4762

Functional expression of sinusoidal drug transporters in primary human and rat hepatocytes

Emilie JIGOREL, Marc LE VEE, Claire BOURSIER-NEYRET, Marc BERTAND and Olivier FARDEL

INSERM U620, Faculté de Pharmacie, Rennes, France (E.J., M.L.V., O.F.) and Technologie Servier, Orléans, France (C.B-N., M.B.)

DMD #4762

Running title

Sinusoidal drug transporters in cultured hepatocytes

Corresponding author

Dr Olivier FARDEL, INSERM U620, Faculté de Pharmacie, 2 avenue du Pr. Léon Bernard, 35043
Rennes, France. Tel (33) 2 23 23 48 80; Fax: (33) 2 23 23 47 94; E-mail: olivier.fardel@univ-rennes1.fr

Text: 14 pages
Table: 1 table
Figures: 3 figures
References: 26 references
Abstract: 192 words
Introduction: 315 words
Results and Discussion: 1037 words

Abbreviations

ES: estrone-3-sulfate
MRP: multidrug resistance protein
NTCP: Na⁺-taurocholate transporter
OAT: organic anion transporter
OATP: organic anion transporting polypeptide
OCT: organic cation transporter
PAH: para-aminohippurate
RT-qPCR: reverse transcriptase-quantitative polymerase chain reaction
SLC: solute carrier
TEA: tetra-ethylammonium

ABSTRACT

Primary hepatocyte cultures are considered as an useful *in vitro* system for pharmacological/toxicological studies. Although expression of drug metabolizing enzymes and canalicular drug transporters has been well documented in this cellular model, less information is available about sinusoidal drug transporter activities. This has led us to investigate functional expression of the major sinusoidal transporters in primary human and rat hepatocytes. Using radio-labelled substrates and chemical transporter inhibitors, activities of organic cation transporter 1 (OCT1/Oct1), organic anion transporting polypeptides (OATPs/Oatps), organic anion transporter 2 (OAT2/Oat2) and Na⁺-taurocholate transporter (NTCP/Ntcp) were detected in cultured human and rat hepatocytes. In parallel, mRNA expression of these transporters was demonstrated using RT-quantitative PCR assays. Functional expression of sinusoidal transport proteins markedly decreased with time in primary rat hepatocyte cultures; by contrast, it remained relatively constant in primary human hepatocytes all along the culture, illustrating the fact that liver-specific functions, including drug detoxifying pathways, are usually better preserved in cultured human hepatocytes than in their rodent counterparts. Primary hepatocytes, especially human hepatocytes, thus exhibit a pattern of sinusoidal transporter expression close to that found *in vivo*, highlighting the interest of hepatocyte cultures for drug detoxification studies.

INTRODUCTION

Membrane transport proteins belonging to the solute carrier (SLC) superfamily of transporters play a major role into the initial sinusoidal influx of drugs to hepatocytes (van Montfoort et al., 2003). Organic cation transporters (OCT1/SLC22A1 and Oct1/Slc22a1) thus mediate the transport of small organic cations such as tetra-ethylammonium (TEA) (Jonker and Schinkel, 2004). Organic anion transporting polypeptides (OATP-A/SLCO1A2, OATP-B/SLCO2B1, OATP-C/SLCO1B1 and OATP8/SLCO1B3 in human hepatocytes and Oatp1/Slco1a1, Oatp2/Slco1a4 and Oatp4/Slco1b2 in rat hepatocytes) are implicated in sodium-independent uptake of organic anions such as estrone-3-sulfate (ES), biliary acids, the anti-histamine fexofenadine and the cholesterol-lowering pravastatin (Kim, 2003); some of these OATPs also transport amphipathic compounds like digoxin handled by OATP8 and Oatp4. Organic anion transporters (OAT2/SLC22A7 and Oat2/Slc22a7) mediate the influx of organic anions like para-aminohippurate (PAH) and salicylates (Sekine et al., 1998) whereas the Na⁺-taurocholic acid transporting polypeptides (NTCP/SLC10A1 and Ntcp/Slc10a1) are involved in Na⁺-dependent uptake of biliary acids such as taurocholate (Trauner and Boyer, 2003).

Primary hepatocytes represent a major *in vitro* model for studying activity and regulation of liver detoxifying pathways and can therefore contribute to predict hepatic elimination of xenobiotics (Modriansky et al., 2000; Gebhardt et al., 2003). Such a liver cell culture system has been extensively characterized with respect to expression and activity of drug metabolizing enzymes and canalicular transporters (Fardel et al., 1993; Payen et al., 2000; Gomez-Lechon et al., 2003; Annaert and Brouwer, 2005). By contrast, activity of sinusoidal membrane transport proteins has yet been less studied to our knowledge in cultured hepatocytes, especially in primary human hepatocytes, although these influx transporters play a crucial role in xenobiotic pharmacokinetic and in drug-drug interactions (Chandra and Brouwer, 2004; Shitara et al., 2004). The present study was therefore designed to investigate functional expression of the major sinusoidal transporters in primary hepatocyte cultures. Owing to known inter-species differences in liver detoxifying pathways, this work was conducted with both human and rat hepatocytes.

MATERIALS AND METHODS

Chemicals

[³H(G)]taurocholic acid (sp. act. 1.19 Ci/mmol), [6, 7-³H(N)]ES (sp. act. 57.30 Ci/mmol), [³H]PAH (sp. act. 4.18 Ci/mmol) and [1-¹⁴C]TEA (sp. act. 2.4 mCi/mmol) were purchased from Perkin Elmer Life Sciences (Boston, MA). Probenecid, verapamil and dexamethasone, were obtained from Sigma-Aldrich (St. Louis, MO).

Cell isolation and culture

Hepatocytes from adult male Sprague–Dawley rats weighing 150-200g were isolated by a perfusion of the liver as previously described (Fardel et al., 1993), except that liberase, a mixture of purified collagenases and proteases, was used for dissociation of liver cells instead of collagenase. Human hepatocytes from adult donors undergoing resection for primary and secondary tumors were obtained by perfusion of histologically-normal liver fragments using a collagenase solution (Payen et al., 2000). All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee. Cells were seeded at a density of 10⁵ cells/cm² in plastic dishes in Williams'E medium supplemented with 2 mM glutamine, 1 mg/mL bovine serum albumin, 1 µg/mL bovin insulin, 10 U/mL penicillin, 10 µg/mL streptomycin and 10 % (v/v) fetal calf serum. The medium was discarded 4 hr (for rat hepatocytes) or 24 hr (for human hepatocytes) after seeding and cells were thereafter maintained in serum-free medium supplemented with 10⁻⁷ M dexamethasone as previously reported (Fardel et al., 1993).

Drug transport assays

Activity of sinusoidal drug transporters was evaluated through determination of cellular accumulation of radio-labelled substrates in the presence or absence of transporter inhibitors as previously described (Lecureur et al., 1998). Briefly, hepatocyte monolayers were washed with transport assay buffer (Payen et al., 2000) consisting of 5.3 mM KCl, 1.1 mM KH₂PO₄, 0.8 mM MgSO₄, 1.8 mM CaCl₂, 11 mM D-Glucose, 10 mM HEPES and 136 mM N-methyl glucamine (Na⁺-free buffer) or 136 mM NaCl (Na⁺-

DMD #4762

containing buffer) and adjusted to pH 7.4. Cells were then incubated with transport assay buffer supplemented with transporter substrates, i.e. 0.2 μM [^3H]taurocholate, 1.7 nM [^3H]ES, 0.2 μM [^3H]PAH or 40 μM [^{14}C]TEA handled by NTCP/Ntcp, OATPs/Oatps, OAT2/Oat2 and OCT1/Oct1, respectively (Faber et al., 2003). These incubations were performed at 37°C in the presence or absence of Na^+ , of the OCT1/Oct1 inhibitor verapamil (Jonker and Schinkel, 2004) or of probenecid known to block organic anion transporters such as OATPs/Oatps and OAT2/Oat2 (Shitara et al., 2004). After 30 min incubation, cells were washed twice with ice-cold phosphate buffer saline, lysed in distilled water and accumulation of radio-labelled substrates was determined through scintillation counting. Data were then normalized to amounts of total protein quantified by Bradford's method (Bradford, 1976) using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

RNA isolation and RT-qPCR analysis

Total RNA was isolated from cells using the TRIzol method. Total RNA (2 μg) was then subjected to reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using the fluorescent dye SYBR Green methodology and an ABI Prism 7000 detector (Applied Biosystem, Foster City, CA) as previously described (Piton et al., 2005). Gene-specific primers, shown in Table I, were designed with the Primer3 software; known intron-exon boundary informations were taken into account for each target gene to avoid detection of genomic DNA. The specificity of each gene amplification was moreover verified at the end of each qPCR reaction by analysis of dissociation curves of the PCR products. The curves of amplification were read with ABI Prism 7000 SDS software using the comparative cycle threshold method. Relative quantification of the steady-state target mRNA levels was calculated after normalization of the total amount of cDNA tested to an 18S RNA endogenous reference.

Statistical analysis

Data were analyzed with the Student's *t* test or with the multirange Dunnett's *t* test. The level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

The present study was designed to investigate functional expression of sinusoidal drug transporters in cultured hepatocytes which constitute a useful *in vitro* model for pharmacological and toxicological analyses (Gomez-Lechon et al., 2003). Human and rat primary hepatocytes were first cultured for 3 days and expression of drug transporters was analysed at mRNA levels using RT-qPCR assays. As indicated in Fig. 1, primary human hepatocytes were found to exhibit a pattern of transporter expression close to that found in freshly isolated counterparts. Indeed, they exhibited similar or only moderately-reduced levels of OCT1, OATP-B, NTPC and OAT2 mRNAs; OATP-C and OATP8 expressions, although more importantly decreased when compared to those found in isolated hepatocytes, remained however notable. Only OATP-A mRNAs, present in freshly isolated human hepatocytes, were not detected at all in primary human hepatocytes. Although OATP-A was initially cloned from human liver, it is noteworthy that it is predominantly expressed in cerebral capillary endothelial cells at the blood-brain barrier (Gao et al., 2000) and its substrate specificity overlaps with that of OATP-C (Kullak-Ublick et al., 2001), indicating that OATP-A may contribute in fact in a minor way to drug uptake in the liver. In contrast to primary human hepatocytes, primary rat hepatocytes exhibited a more altered pattern of transporter mRNA expression when compared to freshly isolated counterparts. Indeed, although remaining detectable, expressions of Oct1, Oatp1, Oatp2, Oatp4, Ntcp and Oat2 mRNAs were markedly decreased in cultured hepatocytes when compared to freshly isolated cells (Fig. 1). This reduced expression of sinusoidal drug transporters in cultured rat hepatocytes has also been documented at the protein level for Ntcp and Oatp2 (Rippin et al., 2001). By contrast, mRNA levels of the biliary efflux pump MRP2 remained elevated in rat hepatocyte cultures (Rippin et al., 2001) whereas expression of the canalicular transporter P-glycoprotein has been shown to be induced (Fardel et al., 1992). Taken together, these data suggest opposite regulation of sinusoidal and biliary drug transporters in primary rat hepatocytes.

We next investigated activity of sinusoidal membrane transporters in 3-day-old primary human and rat hepatocytes through measurement of intracellular accumulation of radio-labelled substrates in the absence or presence of transporter inhibitors (for OCT1/Oct1, OATPs/Oatps and OAT2/Oat2) or of Na⁺

DMD #4762

(for NTCP/Ntcp). As indicated in Fig. 2, primary human hepatocytes exhibited activity, i.e. an inhibitor-sensitive or Na^+ -dependent accumulation of substrates, of OCT1, OATPs, OAT2 and NTCP. Activity of Oct1, Oatps and Oat2 were also detected in 3-day-old rat hepatocytes (Fig. 2). However, probenecid-inhibitable uptake of the generic OATP/Oatp substrate ES was about 10-fold lower in rat hepatocytes than in human hepatocytes. Rat hepatocytes similarly displayed reduced probenecid-sensitive accumulation of PAH, a substrate for both OAT2/Oat2, when compared to their human counterparts, and Na^+ -dependent accumulation of the NTCP/Ntcp substrate taurocholate, present in cultured human hepatocytes, was barely, if any, detectable in rat hepatocytes (Fig. 2). These data suggest that 3-day-old cultured rat hepatocytes displayed lower sinusoidal drug transporter activities than their human counterparts, which likely agrees with their reduced expression of transporter mRNAs described above (Fig. 1).

It is noteworthy that culture time has been shown to be a critical parameter for the functional expression of liver specific functions, including drug detoxication pathways such as cytochromes P-450, in primary rat hepatocytes; by contrast, human primary hepatocytes exhibit higher survival and better preservation of differentiated functions (Guillouzo et al., 1993; Mitaka, 1998; Modriansky et al., 2000). This may contribute to the relatively low level of sinusoidal drug transporter activities in 3-day-old cultured rat hepatocytes when compared to that found in their human counterparts. To test this hypothesis, transporter functional assays were performed in rat hepatocytes maintained in culture for 4 hr, 1 day and 3 days whereas human primary cultures were analysed 1, 3 and 5 days after cell seeding (Fig. 3). Verapamil-inhibitable accumulation of the OCT1/Oct1 substrate TEA was found to significantly diminish with time in culture in rat hepatocytes, but not in human counterparts. Probenecid-sensitive accumulation of the OATP/Oatp substrate ES and probenecid-inhibitable accumulation of the OAT2/Oat2 substrate PAH also markedly decreased in 3-day-old cultured rat hepatocytes when compared to ES and PAH uptake in rat hepatocytes maintained in culture for shorter times (4 hr and 1 day); by contrast, they decreased only slightly, although significantly, in primary human hepatocytes along the culture (Fig. 3). Ntcp-mediated uptake of taurocholate was markedly down-regulated during primary culture in rat hepatocytes. On the contrary, Na^+ -related uptake of taurocholate remained relatively high in primary

DMD #4762

human hepatocytes along the culture; it was only slightly diminished in 3-day-old cultured human hepatocytes when compared to 1-day or 5-day-old primary hepatocytes (Fig. 3). These data therefore likely add sinusoidal drug transporters to the list of liver-specific functions that are better preserved with culture time in primary human hepatocytes than in their rat counterparts. This may reflect the fact that human hepatocytes are less sensitive than rat cells to the loss of their physiological environment (Mitaka, 1998). In this context, it is noteworthy that culturing rat hepatocytes in conditions mimicking their *in vivo* environment, i.e. in the presence of extracellular matrix components, has been demonstrated to improve expression of drug transporters (Luttringer et al., 2002; Hoffmaster et al., 2004). In the same way, we have found that the addition of dimethylsulfoxide to culture medium, well known to favour the expression of differentiated functions in cultured hepatocytes (Isom et al., 1985), increased Ntcp and Oatp transport activities in primary rat hepatocytes (data not shown).

In addition to the functional expression of major sinusoidal transporters described in the present study, activity of main biliary drug transporters, especially P-glycoprotein and MRP2, has been previously documented in both primary rat and human hepatocytes (Fardel et al., 1993; Payen et al., 2000; Hoffmaster et al., 2004). This demonstrates that primary hepatocytes possess the two kinds of transporters present *in vivo* in hepatocytes, i.e. those responsible for the entry of xenobiotics from blood and those involved in xenobiotic secretion into the bile; they also express phase I and phase II drug metabolizing enzymes. Cultured hepatocytes represent therefore a good model for analysing interactions of drugs and their metabolites with liver transporters. Their use may be may be relevant with respect to drug-drug interactions, as recently demonstrated using sandwich-cultured rat hepatocytes and the P-glycoprotein substrate rhodamine 123 (Annaert and Brouwer, 2005).

ACKNOWLEDGMENTS

We would like to thank Dr. X. Decleves for helpful comments with respect to our study.

REFERENCES

- Annaert PP and Brouwer KL (2005) Assessment of drug interactions in hepatobiliary transport using rhodamine 123 in sandwich-cultured rat hepatocytes. *Drug Metab Dispos* **33**:388-394.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**:248-254.
- Chandra P and Brouwer KL (2004) The complexities of hepatic drug transport: current knowledge and emerging concepts. *Pharm Res* **21**:719-735.
- Faber KN, Muller M and Jansen PL (2003) Drug transport proteins in the liver. *Adv Drug Deliv Rev* **55**:107-124.
- Fardel O, Morel F and Guillouzo A (1993) P-glycoprotein expression in human, mouse, hamster and rat hepatocytes in primary culture. *Carcinogenesis* **14**:781-783.
- Fardel O, Ratanasavanh D, Loyer P, Ketterer B and Guillouzo A (1992) Overexpression of the multidrug resistance gene product in adult rat hepatocytes during primary culture. *Eur J Biochem* **205**:847-852.
- Gao B, Hagenbuch B, Kullak-Ublick GA, Benke D, Aguzzi A and Meier PJ (2000) Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther* **294**:73-79.
- Gebhardt R, Hengstler JG, Muller D, Glockner R, Buening P, Laube B, Schmelzer E, Ullrich M, Utesch D, Hewitt N, Ringel M, Hilz BR, Bader A, Langsch A, Koese T, Burger HJ, Maas J and Oesch F (2003) New hepatocyte in vitro systems for drug metabolism: metabolic capacity and recommendations for application in basic research and drug development, standard operation procedures. *Drug Metab Rev* **35**:145-213.
- Gomez-Lechon MJ, Donato MT, Castell JV and Jover R (2003) Human hepatocytes as a tool for studying toxicity and drug metabolism. *Curr Drug Metab* **4**:292-312.

- Guillouzo A, Morel F, Fardel O and Meunier B (1993) Use of human hepatocyte cultures for drug metabolism studies. *Toxicology* **82**:209-219.
- Hoffmaster KA, Turncliff RZ, LeCluyse EL, Kim RB, Meier PJ and Brouwer KL (2004) P-glycoprotein expression, localization, and function in sandwich-cultured primary rat and human hepatocytes: relevance to the hepatobiliary disposition of a model opioid peptide. *Pharm Res* **21**:1294-1302.
- Isom HC, Secott T, Georgoff I, Woodworth C and Mummaw J (1985) Maintenance of differentiated rat hepatocytes in primary culture. *Proc Natl Acad Sci U S A* **82**:3252-3256.
- Jonker JW and Schinkel AH (2004) Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther* **308**:2-9.
- Kim RB (2003) Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur J Clin Invest* **33 Suppl 2**:1-5.
- Kullak-Ublick GA, Ismail MG, Stieger B, Landmann L, Huber R, Pizzagalli F, Fattinger K, Meier PJ and Hagenbuch B (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120**:525-533.
- Lecureur V, Guillouzo A and Fardel O (1998) Differential expression of the polyspecific drug transporter OCT1 in rat hepatocarcinoma cells. *Cancer Lett* **126**:227-233.
- Luttringer O, Theil FP, Lave T, Wernli-Kuratli K, Guentert TW and de Saizieu A (2002) Influence of isolation procedure, extracellular matrix and dexamethasone on the regulation of membrane transporters gene expression in rat hepatocytes. *Biochem Pharmacol* **64**:1637-1650.
- Mitaka T (1998) The current status of primary hepatocyte culture. *Int J Exp Pathol* **79**:393-409.

- Modriansky M, Ulrichova J, Bachleda P, Anzenbacher P, Anzenbacherova E, Walterova D and Simanek V (2000) Human hepatocyte--a model for toxicological studies. Functional and biochemical characterization. *Gen Physiol Biophys* **19**:223-235.
- Payen L, Courtois A, Campion JP, Guillouzo A and Fardel O (2000) Characterization and inhibition by a wide range of xenobiotics of organic anion excretion by primary human hepatocytes. *Biochem Pharmacol* **60**:1967-1975.
- Piton A, Le Ferrec E, Langouet S, Rauch C, Petit E, Le Goff F, Guillouzo A and Morel F (2005) Oltipraz regulates different categories of genes relevant to chemoprevention in human hepatocytes. *Carcinogenesis* **26**:343-351.
- Rippin SJ, Hagenbuch B, Meier PJ and Stieger B (2001) Cholestatic expression pattern of sinusoidal and canalicular organic anion transport systems in primary cultured rat hepatocytes. *Hepatology* **33**:776-782.
- Sekine T, Cha SH, Tsuda M, Apiwattanakul N, Nakajima N, Kanai Y and Endou H (1998) Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett* **429**:179-182.
- Shitara Y, Sato H and Sugiyama Y (2004) Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annu Rev Pharmacol Toxicol*.
- Trauner M and Boyer JL (2003) Bile salt transporters: molecular characterization, function, and regulation. *Physiol Rev* **83**:633-671.
- van Montfoort JE, Hagenbuch B, Groothuis GM, Koepsell H, Meier PJ and Meijer DK (2003) Drug uptake systems in liver and kidney. *Curr Drug Metab* **4**:185-211.

LEGENDS TO FIGURES

Fig. 1: Expression of sinusoidal membrane transporters in 3-day-old primary human and rat hepatocytes.

Total RNAs were prepared from freshly isolated hepatocytes and 3-day-old primary rat and human hepatocytes. mRNA levels of sinusoidal drug transporters were then analyzed using RT-qPCR assays as described in Materials and Methods. Data shown correspond to transporter mRNA levels in 3-day-old primary hepatocytes and are expressed as percentage of those found in freshly isolated hepatocytes, arbitrarily set at 100%. They represent the means \pm SD of three experiments performed on independent hepatocyte preparations from different human liver donors and different Sprague-Dawley rats.

Fig. 2: Sinusoidal membrane transporter activities in 3-day-old primary rat and human hepatocytes.

Primary 3-day-old rat and human hepatocytes were incubated with 40 μ M [14 C]TEA, 1.7 nM [3 H]ES, 0.2 μ M [3 H]PAH and 0.2 μ M [3 H]taurocholate, handled by OCT1/Oct1, OATPs/Oatps, OAT2/Oat2 and NTCP/Ntcp, respectively. These incubations were performed as described in Materials in Methods in the presence or absence of verapamil (an inhibitor of OCT1/Oct1), probenecid (an inhibitor of OATPs/Oatps and OAT2/Oat2) or Na⁺ (for NTCP/Ntcp-related transport activity). Cells were then lysed in distilled water and intracellular accumulation of radio-labelled substrates was determined by scintillation counting and expressed as nmol/mg protein. Data represent the means \pm S.D of four to six experiments realised in triplicates and performed on independent hepatocytes preparations from different human liver donors and different Sprague-Dawley rats. *, $P < 0.05$ when compared to substrate accumulation in the absence of inhibitor or Na⁺.

Fig.3: Time-profile of sinusoidal membrane transporter activities in primary rat and human hepatocytes.

Rat and human hepatocytes, cultured for various lengths of time (4 hr, 1 and 3 days for rat cells and 1, 3 and 5 days for human cells), were incubated with 40 μ M [14 C]TEA, 1.7 nM [3 H]ES, 0.2 μ M [3 H]PAH and 0.2 μ M [3 H]taurocholate, substrates for OCT1/Oct1, OATPs/Oatps, OAT2/Oat2 and NTCP/Ntcp, respectively. These incubations were performed as described in Materials in Methods in the presence or

DMD #4762

absence of verapamil (an inhibitor of OCT1/Oct1), probenecid (an inhibitor of OATPs/Oatps and OAT2/Oat2) or Na⁺ (for NTCP/Ntcp-related transport activity). Cells were then lysed in distilled water and intracellular accumulation of radio-labelled substrates was determined by scintillation counting. Data are expressed as inhibitor- or Na⁺-sensitive accumulation of substrates, i.e. accumulation values in the absence of inhibitor or Na⁺ minus accumulation values in the presence of inhibitor or Na⁺; they correspond to the means \pm S.D of four to six experiments realised in triplicates and performed on independent hepatocyte preparations from different human liver donors and different Sprague-Dawley rats. *, $P < 0.05$ when compared to substrate accumulation found in 4 hr-cultured rat hepatocytes or 1 day-cultured human hepatocytes.

DMD #4762

TABLE 1		
<i>Primer sequences for RT-qPCR assays</i>		
Gene	Forward Primer (5'- 3')	Reverse Primer (5'- 3')
Human		
OATP-A/SLCO1A2	TGGGGAAC TTTGAAATGTGG	AAGGCTGGAACAAAGCTTGA
OATP-B/SLCO2B1	TGATTGGCTATGGGGCTATC	CATATCCTCAGGGCTGGTGT
OATP-C/SLCO1B1	GCCCAAGAGATGATGCTTGT	ATTGAGTGGAAACCCAGTGC
OATP8/SLCO1B3	GGGTGAATGCCCAAGAGATA	ATTGACTGGAAACCCATTGC
NTCP/SLC10A1	GGGACATGAACCTCAGCATT	CGTTTGGATTTGAGGACGAT
OAT2/SLC22A7	GAGGATGAACCTGCCACAGT	TCTGCTCACACACCAGATCC
OCT1/SLC22A1	TAATGGACCACATCGCTCAA	AGCCCCTGATAGAGCACAGA
18S	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTTCGCTC
Rat		
Oatp1/Slco1a1	AGGTCGAGAATGACGGAGAA	ATTCCGAGGAAGGGAAGTGT
Oatp2/Slco1a4	ATCTCGCTACCCCATTTTCCT	TGGGATCTGTTTTCCACACA
Oatp4/Slco1b2	GACATCACCCACTGGACCTT	AGCATGTTCCCCATCAAGAC
Ntcp/Slc10a1	CAAGTCCAAAAGGCCACACT	TGATGACAGAGAGGGCTGTG
Oat2/Slc22a7	CGCTCAGAATTCTCCTCCAC	GCACACCAATGAAGAAGCAG
Oct1/Slc22a1	GGTTGCCAACAGGAGTCAGT	AGACAGAGCTTACGGCCAAA
18S	CGAAAGCATTTGCCAAGAAT	AGTCGGCATCGTTTATGGTC

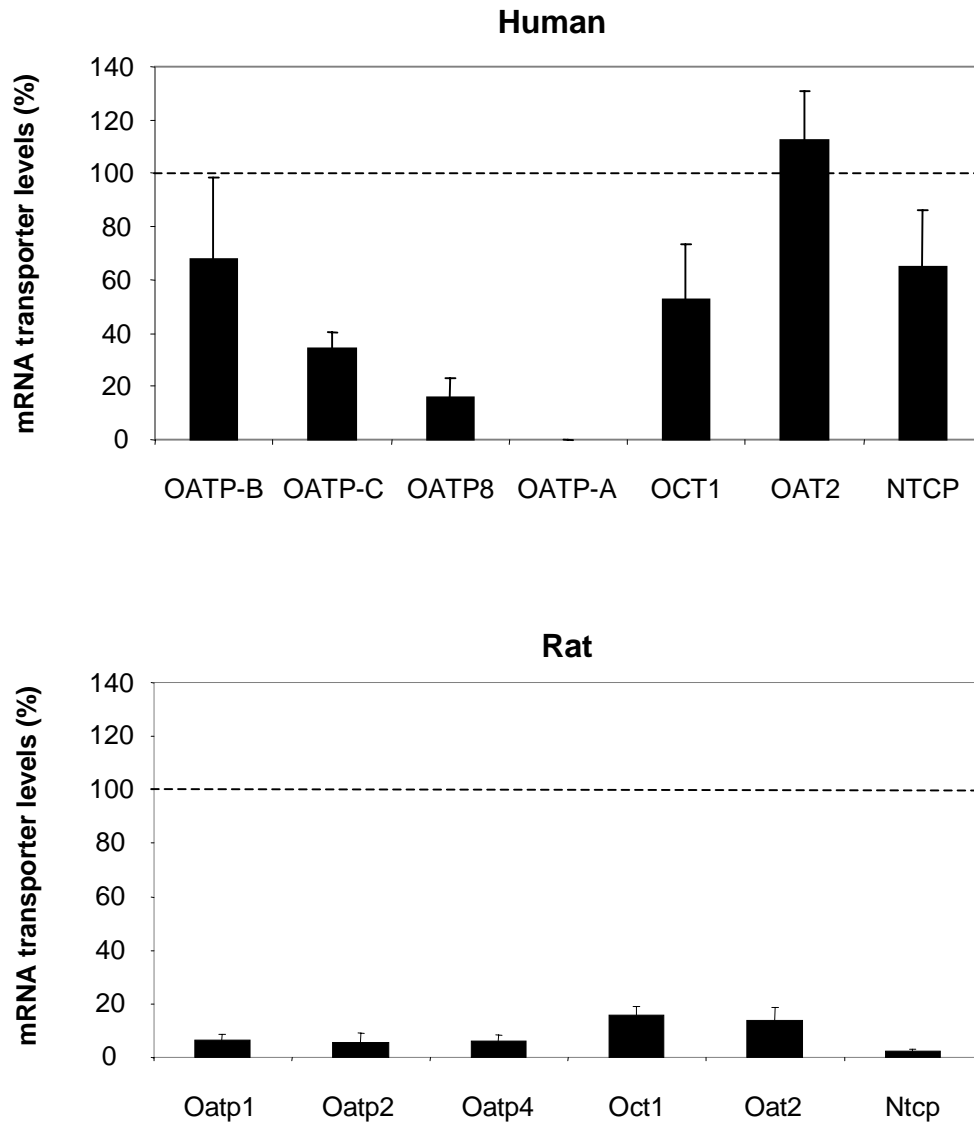


Figure 1

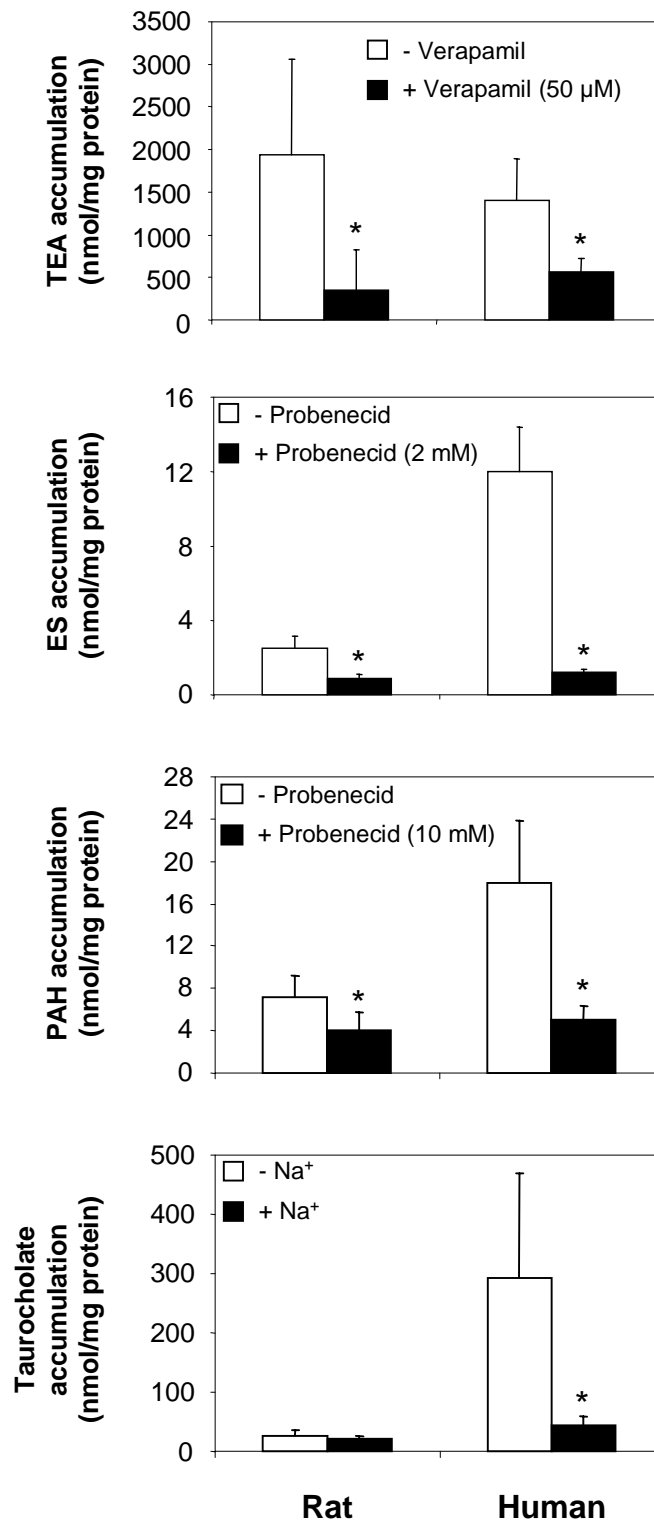


Figure 2

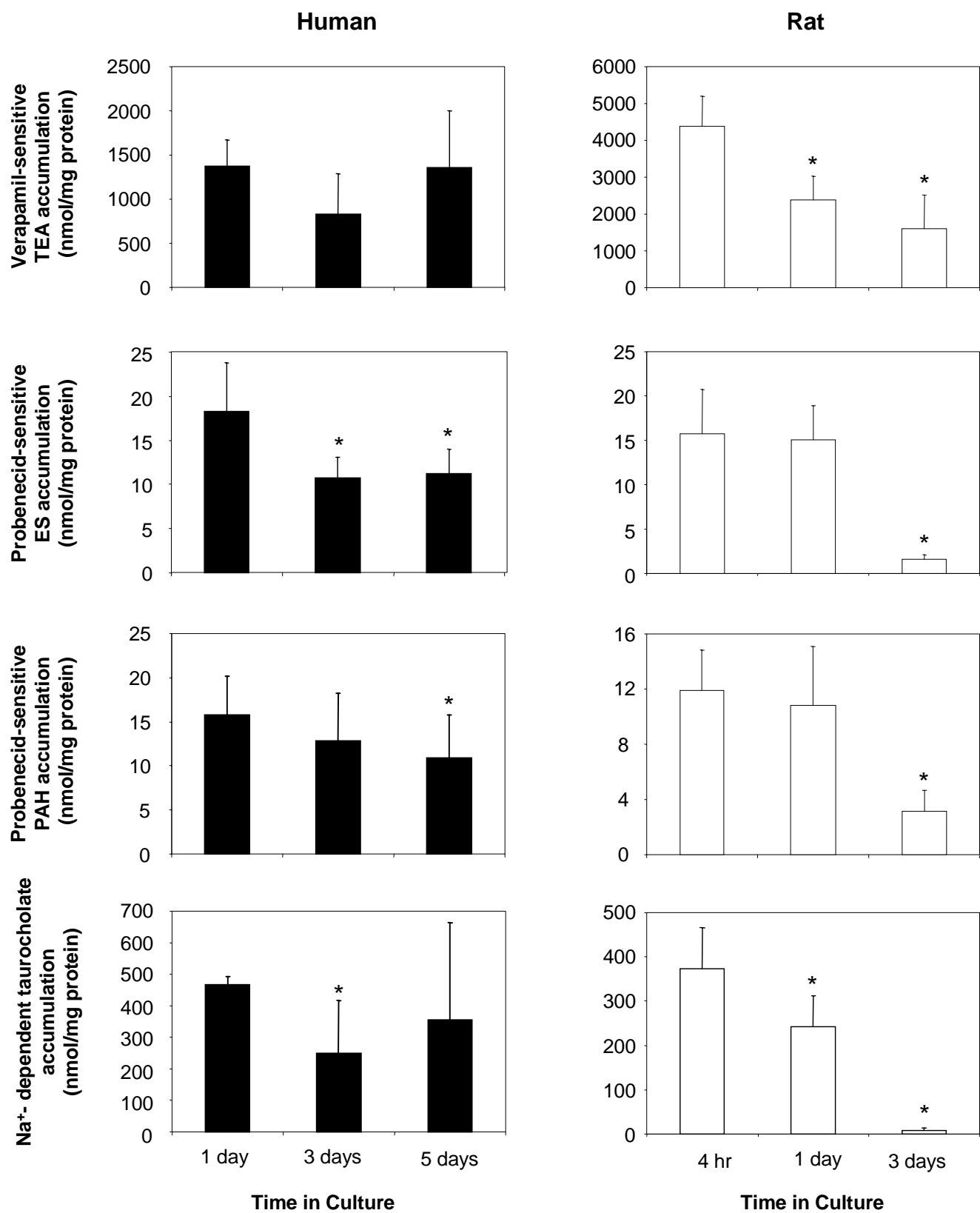


Figure 3