Differential regulation of drug transporter expression by all-trans retinoic acid in hepatoma HepaRG cells and human hepatocytes

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Abstract: All-trans retinoic acid (atRA) is the active form of vitamin A, known to activate retinoid receptors, especially the heterodimer retinoid X receptor (RXR):retinoic acid receptor (RAR) that otherwise may play a role in regulation of some drug transporters. The present study was designed to characterize the nature of human hepatic transporters that may be targeted by atRA and the heterodimer RXR:RAR. Exposure of human hepatoma HepaRG cells and primary human hepatocytes to 5 M atRA down-regulated mRNA levels of various sinusoidal solute carrier (SLC) influx transporters, including organic anion transporting polypeptide (OATP) 2B1, OATP1B1, organic cation transporter (OCT) 1 and organic anion transporter (OAT) 2, and induced those of the canalicular breast cancer resistance protein (BCRP). The retinoid concomitantly reduced protein expression of OATP2B1 and OATP1B1 and activity of OATPs and OCT1 and induced BCRP protein expression in HepaRG cells. Some transporters such as OATP1B3 and the bile salt export pump (BSEP) were however down-regulated by atRA in primary human hepatocytes, but induced in HepaRG cells, thus pointing out discrepancies between these two liver cell models in terms of detoxifying protein regulation. atRA-mediated repressions of OATP2B1, OATP1B1, OAT2 and OCT1 mRNA expression were finally shown to be counteracted by knocking-down expression of RAR and RXR through siRNA transfection in HepaRG cells. atRA thus differentially regulated human hepatic drug transporters, mainly in a RXR:RAR-dependent manner, therefore establishing retinoids and retinoid receptors as modulators of liver drug transporter expression.

Polarized expression of drug transporters in differentiated human hepatoma HepaRG cells

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Abbreviations: ABC, ATP-binding cassette; BEI, bile excretion index; BSEP; bile salt export pump; BCRP, breast cancer resistance protein; CF, carboxy-2,7-dichlorofluoresceine; DMSO, dimethylsulfoxide; E3S, estrone-3-sulfate; MATE1, multidrug and toxin extrusion protein 1; MDR1, multidrug resistance gene 1; MRP, multidrug drug resistance-associated protein; NTCP, sodium taurocholate co-transporting polypeptide; OATP, organic anion transporting polypeptide; OAT2, organic anion transporter 2; OCT1, organic cation transporter 1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SLC, solute carrier; TEA, tetra-ethylammonium;

Abstract

The HepaRG cell line is a well-differentiated human hepatoma cell line exhibiting high expression of drug detoxifying proteins, including drug transporters. Polarized status of drug transporters, i.e. coordinated location of transporters at the sinusoidal or canalicular cell membranes, which represents a key hall-mark of hepato-biliary drug transport, remains however very poorly documented in HepaRG cells. In the present study, HepaRG cells were demonstrated to exhibit notable mRNA expression of most of sinusoidal and canalicular hepatic drug transporter, with a global profile of transporter expression significantly correlated to that displayed by freshly isolated human hepatocytes. Immunofluorescence studies next indicated that HepaRG cells, which exhibit bile canaliculi networks, expressed several main hepatic drug transporters at their sinusoidal pole, especially the influx transporters organic anion transporting polypeptide (OATP) 1B1, OATP2B1 and organic cation transporter (OCT) 1 and the efflux transporter multidrug resistance-associated protein (MRP) 3); in the same way, the efflux transporters P-glycoprotein and MRP2 were detected at the canalicular pole of HepaRG cells. This polarized expression of drug transporters was associated with saturable uptake of referent substrates for the sinusoidal transporters sodium-taurocholate cotransporting polypeptide, OATPs and OCT1 and with canalicular secretion of referent substrates for the efflux transporters bile salt export pump and MRP2. This polarized and functional expression of various major sinusoidal and canalicular transporters in HepaRG cells clearly highlights the interest of using these highly-differentiated hepatoma cells as surrogates for human hepatocytes in drug transporter studies.

Introduction

The HepaRG cell line is a recently-characterized hepatic cell line, established from a hepatocholangiocarcinoma of a female patient (Gripon et al., 2002). In contrast to most, if not all, other available hepatic cell lines, the HepaRG cell line exhibits various liver-specific functions and is therefore proposed as a surrogate to the use of primary human hepatocytes, especially for drug metabolism and toxicity studies (Guillouzo et al., 2007; Andersson et al., 2012). Indeed, when cultured in appropriated conditions, i.e., in the presence of 2 % (vol/vol) dimethylsulfoxide (DMSO), HepaRG cells express high levels of hepatic drug detoxifying pathways, including phase 1 enzymes, such as cytochromes P-450 1A2, 2B6 and 3A4, and phase 2 enzymes such as such glutathione S-transferases A1/A2, A4 and M1 and UDP-glucuronosyl transferase 1A1 (Aninat et al., 2006; Antherieu et al., 2010). Moreover, HepaRG cells exhibit notable expression of drug-sensing receptors such as pregnane X receptor and constitutive androstane receptor (Antherieu et al., 2012) and are consequently responsive to inducers of drug metabolism (Kanebratt and Andersson, 2008; Turpeinen et al., 2009).

In addition to drug metabolizing enzymes, hepatic drug transporters are present and functional in HepaRG cells (Le Vee et al., 2006; Kotani et al., 2012). This point is likely noteworthy, because the important role that played hepatic drug transporters in drug-drug interactions and pharmacokinetics, including hepatic clearance of drugs, is now well-recognized (Giacomini et al., 2010). Expression of transporters in HepaRG cells remains however incompletely characterized, especially with respect to the polarized status of these transporters. Polarization into two complementary poles, i.e., a blood sinusoidal/basolateral pole and a biliary apical/canalicular pole, corresponds to a key hell-mark of differentiated hepatocytes and has important functional consequences in terms of hepato-biliary secretion of drugs (Chandra and Brouwer, 2004). Indeed, influx hepatic transporters expressed at the sinusoidal/basolateral pole of hepatocytes mediate uptake of drugs from blood into liver,

whereas canalicular transporters are responsible for secretion of drugs or drug metabolites into bile (Funk, 2008; Kock and Brouwer, 2012); in addition, some efflux transporters expressed at the sinusoidal membrane can secrete some drugs or drug metabolites back into blood, for a secondary renal elimination (Zelcer et al., 2005). The present study was therefore designed to carefully analyze expression and cellular location of both sinusoidal and canalicular hepatic transporters in HepaRG cells. Our data indicate that HepaRG cells express most of these transporters, with an adequate cellular localization for several of them, thus fully supporting the growing interest for using HepaRG cells as surrogates for human hepatocytes.

Materials and methods

Chemicals. Taurocholate, estrone-3-sulfate (E3S) and tetra-ethylammonium (TEA) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France), whereas carboxy-2,7-dichlorofluoresceine (CF) diacetate was provided by Invitrogen/Life Technologies (Villebon sur Yvette, France).. [^{3}H(G)] taurocholic acid (sp. act. 1.19 Ci/mmol), [6, 7-^{3}H(N)] E3S (sp. act. 57.3 Ci/mmol) and [1-^{14}C] TEA (sp. act. 2.4 mCi/mmol), were from Perkin-Elmer (Boston, MA). All other chemicals were commercial products of the highest purity available.

Cell culture. Human highly-differentiated hepatoma HepaRG cells were routinely cultured in Williams' E medium supplemented with 10% (vol/vol) fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin, 2 mM glutamine, and 5 x 10^{-5} M hydrocortisone hemisuccinate; additional culture for two weeks in the same medium added with 2 % (vol/vol) DMSO was performed in order to get a full hepatocytic differentiation of the cells (Gripon et al., 2002).

Preparation of freshly isolated human hepatocytes. Human hepatocytes were obtained from adult donors undergoing hepatic resection for primary and secondary tumors, via the Biological Resource Center (Rennes, France). Cells were prepared by perfusion of histologically-normal liver fragments using a collagenase solution as previously described (Payen et al., 2000).

RNA isolation and analysis. Total RNA was isolated from cells using the TRIzol reagent (Invitrogen/Life Technologies). RNA (20 ng) was then subjected to reverse transcription-quantitative polymerase chain reaction (RT-qPCR), using the fluorescent dye SYBR Green methodology and an ABI Prism 7300 detector (Applied Biosystem, Foster City, CA, USA), as previously reported (Maubon et al., 2007). Gene primers were exactly as previously described (Moreau et al., 2011), except multidrug and toxin extrusion protein 1 (MATE1/SLC47A1) sense, GCAATCGCGGTTATCAATG, MATE1 antisense, AAGCCTGGACACATCTGGG. Amplification curves were next analysed 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 a 18S mRNA endogenous reference using the $2^{(-\Delta Ct)}$ method. This allowed to get a relative value of expression for each gene comparatively to the 18S RNA amount found in the sample, arbitrarily set at 10^6 units (Moreau et al., 2011).

Light and fluorescence microscopy. Light microscopy and CF fluorescence were employed to monitor the presence of functional canalicular networks in HepaRG cells, as previously described (Noel et al., 2013). For light microscopy studies, cells were observed using an Axiovert microscope (Zeiss, Le Pecq, France). For fluorescence studies, HepaRG cells were incubated with 3 µM CF diacetate for 10 min at 37°C; cells were then washed with ice-cold

phosphate-buffered saline and visualized using a Leica DM IRB microscope (Leica Microsystems, Wetzlar, Germany) equipped with a black/white CoolSNAP ES camera (Roper Scientific, Planegg/Martinsried, Germany). Pictures were processed using the Metaview software.

Immunolocalization studies. Immunofluorescence analyses were performed as previously described (Vee et al., 2009). HepaRG cells cultured on glass coverslips were first fixed in ice-cold methanol for 10 min. Cells were next incubated for 2 h with mouse monoclonal antibodies directed against organic cation transporter 1 (OCT1)/SLC22A1 (Abcam, Cambridge, UK), multidrug resistance gene 1 (MDR1)/ABCB1/P-glycoprotein (Alexis Corporation, Lausen, Switzerland), multidrug drug resistance-associated protein (MRP) 2/ABCC2 or MRP3/ABCC3 (Millipore Bioscience Research Reagents, Temecula, CA), with rabbit polyclonal antibodies directed against organic anion transporting polypeptide (OATP) 1B1/SLCO1B1 or OATP2B1/SLCO2B1 (Huber et al., 2007), or with corresponding mouse or rabbit isotypic Ig controls. After washing, AlexaFluor 488- or tetramethyl rhodamine isothiocyanate-labeled secondary antibodies (Invitrogen/Life Technologies) were added for 1 h, and nuclei were subsequently stained with 4,6-diamidino-2-phenylindole. Immunofluorescence images were finally detected with a DMRXA Leica microscope (Leica Microsystems) and a COHU high performance CCD camera (CohuHD, Poway, CA), using the Metaview software.

Drug transport assays. For characterizing sinusoidal influx of taurocholate (a referent substrate for sodium taurocholate co-transporting polypeptide (NTCP/SLC10A1)), of E3S (a referent substrate for OATPs) and of TEA (a referent substrate for OCT1), HepaRG cells were incubated at 37°C with 43.4 nM [^{3}H] taurocholate, 3.4 nM [^{3}H] E3S or 40 µM [^{14}C]

TEA in the absence or presence of various concentrations of corresponding unlabelled substrates, using a defined sodium- and calcium-containing transport assay medium (Jigorel et al., 2005). Incubation times were 3 min (for taurocholate and E3S) or 5 min (for TEA); preliminary experiments indicated that uptakes of substrates were linear over these periods (data not shown). After washing in phosphate-buffered saline, cells were lyzed and accumulation of radiolabelled substrates was determined through scintillation counting. Kinetic parameters (K_m and V_{max}) of taurocholate, E3S and TEA uptake in HepaRG cells were next estimated though fitting data with Prism software (GraphPad software, La Jolla, CA) to Michaelis-Menten plots based on the following equation:

$$v = (V_{max} \text{ x } [S])/(K_m + |S|)$$

where v is the initial uptake rate of substrate, [S] is the substrate concentration in the medium, K_m is the Michaelis–Menten constant, and V_{max} is the maximum uptake rate.

Biliary secretion of taurocholate and CF was analyzed through determination of drug accumulation into bile canaliculi-like structures as previously reported (Annaert et al., 2001). Briefly, HepaRG cells were first incubated for 10 min at 37°C with transport assay buffer containing Ca^{2+} or with the same buffer, except that 1.8 mM $CaCl_2$ was withdrawed and 100 μM EGTA was added, knowing that incubation with this Ca^{2+}-free buffer promotes disruption of tight junctions and opening of bile canaliculi networks. Buffers were then removed and HepaRG cells were further incubated for 10 min at 37°C in transport assay medium containing 43.4 nM [^{3}H] taurocholate or 3 μM CF diacetate. After washing with ice-cold phosphate-buffered saline, accumulations of taurocholate and CF into cells + bile canaliculi (Ca^{2+}-containing conditions) and into cells only (Ca^{2+}-free conditions) were determined by scintillation counting (for taurocholate) or spectrofluorimetry (for CF) using a SpectraMax Gemini SX spectrofluorometer (Molecular Devices, Sunnyvale, CA) (excitation and emission

wavelengths were 485 and 535 nm, respectively). Biliary excretion index (BEI) was finally

calculated using the following equation (Liu et al., 1999):

$$BEI = \frac{\text{Accumulation (Cells+Bile canaliculi)} - \text{Accumulation (Cells)}}{\text{Accumulation (Cells+Bile canaliculi)}} \times 100$$

Statistical analysis. Quantitative data were statistically analyzed using Student's t-test paired

or nonparametric Spearman's rank correlation method. The criterion of significance

was $p < 0.05$.

Results and Discussion

Expression of sinusoidal and canalicular drug transporters in HepaRG cells was first

determined by RT-qPCR and compared to that found in freshly isolated human hepatocytes.

These freshly isolated human hepatocytes were chosen as referent cells, in contrast to

previous studies using primary hepatocytes or cryopreserved hepatocytes as references (Le

Vee et al., 2006; Antherieu et al., 2010; Kotani et al., 2012), because placing hepatocytes in

culture or cryopreservation are susceptible to alter drug detoxifying protein expression and

activity, including those of transporters (Badolo et al., 2011; Ramboer et al., 2013). Moreover,

freshly isolated human hepatocytes were obtained from 8 donors, in order to take into

consideration potential major inter-individual differences in drug transporter mRNA levels.

As shown in Fig 1A, expressions of sinusoidal influx solute carrier (SLC) transporters such as

NTCP, OATPs, OCT1 and organic anion transporter 2 (OAT2/SLC22A7), were reduced in

HepaRG cells when compared to hepatocytes. Nevertheless, when considering mRNA levels

expressed in arbitrary units, substantial expressions (> 5 arbitrary units) of NTCP, OATP1B1,

OATP2B1, OCT1 and OAT2 were detected in HepaRG cells, knowing that these transporters

were otherwise highly expressed in freshly isolated human hepatocytes (Fig. 1A). Only

OATP1B3, which is markedly down-regulated in HepaRG cells when compared to hepatocytes, was finally very poorly expressed in HepaRG cells (expression < 1 arbitrary unit), in agreement with a recent report (Kotani et al., 2012). In contrast to SLC transporters, sinusoidal ATP-binding cassette (ABC) efflux transporters, except MRP6 (ABCC6), were up-regulated in HepaRG cells when compared to human hepatocytes (Fig. 1A); levels of expressions of MRP1, MRP4 and MRP5 remained however low in HepaRG cells (expression < 5 arbitrary units), unlike that of MRP3 (expression = 45.0 arbitrary units). With respect to canalicular transporters, some of them, such as MRP2 and especially MDR1, were induced in HepaRG cells, when compared to human hepatocytes (Fig. 1B). By contrast, the canalicular SLC transporter MATE1 remained unchanged, whereas breast cancer resistance protein (BCRP/ABCG2) and especially bile salt export pump (BSEP/ABCB11) were down-regulated (Fig. 1B).

To more globally characterize drug transporter expression in HepaRG cells and human hepatocytes, sinusoidal and canalicular drug transporters were next ranked from the most expressed to the less expressed according to mRNA level of expression and the resulting expression profiles were compared. As shown in Fig. 1C, expression profile of drug transporters in HepaRG cells was significantly correlated with that exhibited by freshly isolated human hepatocytes. Therefore, even if some discrepancies in mRNA levels of drug transporters exist between HepaRG cells and human hepatocytes, as reported above, this does not result in an altered profile of transporter expression in HepaRG cells when compared to that found in normal hepatocytes.

DMSO-treated differentiated HepaRG cells are well-known to be polarized (Antherieu et al., 2012) and consequently exhibit bile canaliculi-like structures, which are fully functional because they accumulated the fluorescent dye CF (Fig. 2A). Immunofluorescence studies next indicated that various hepatic transporters such as OATP1B1, OATP2B1, OCT1 and MRP3,

known to be physiologically present at the sinusoidal pole of hepatocytes in the liver (Funk, 2008), were similarly expressed at the sinusoidal-like pole of cultured HepaRG cells (Fig. 2B). In the same way, the canalicular ABC transporters MDR1/P-glycoprotein and MRP2 were located to the bile canaliculi-like structures of HepaRG cultures (Fig. 2B). Taken together, these data suggest that transporter expression is correctly polarized in HepaRG cells; this point is noteworthy, because polarized localization of transporter is crucial for *in vitro* models of hepatic drug transport, and is well-exhibited by the present gold standard of these models, i.e., sandwich-cultured hepatocytes (Swift et al., 2010).

HepaRG cells have previously been shown to exhibit sinusoidal transport activity, especially NTCP-mediated uptake of taurocholate, OATP-mediated uptake of E3S, estradiol-17β glucuronide and pitavastatin, and OCT1-mediated uptake of TEA (Le Vee et al., 2006; Kotani et al., 2012). In order to better characterize some of these sinusoidal transport activities, we determined their kinetic parameters. As shown in Fig 3A, uptake of taurocholate in HepaRG cells was found to be saturable, with a K_m value (6.3 µM) closed to that described for NTCP-mediated taurocholate in NTCP-transfected cells (K_m=7.9 µM) (Kim et al., 1999). E3S uptake was also saturable in HepaRG cells (Fig. 3A), with a K_m value (44.0 µM) nearly identical to that corresponding to the low-affinity binding site for OATP1B1-mediated E3S transport in OATP1B1-transfected cells (K_m=45.0 µM) (Noe et al., 2007). A contribution of the high-affinity binding site of OATP1B1 for E3S (K_m=0.09 µM) (Tamai et al., 2001) and of OATP2B1, which is well known to transport E3S (K_m=10.2 µM) (Noe et al., 2007) and is expressed in HepaRG cells at substantial level (Fig. 1A), has however likely to be additionally taken into consideration for E3S influx in HepaRG cells. By contrast, a role for OATP1B3 may be discarded, since this transporter is only present at a very low level in HepaRG cells (Fig. 1A). TEA uptake was also found to be saturable in HepaRG cells (Fig. 3A), with however a K_m value (1.44 mM) higher than those reported for TEA uptake in OCT1-

transfected cells, that ranged from 69.2 μM to 229.0 μM (Zhang et al., 1998; Umehara et al., 2007). The reason for such a discrepancy between K_m values for TEA uptake in OCT1-expressing HepaRG cells versus OCT1-transfected cells remains to be clarified; it could be linked to post-transcriptional processes specifically targeting OCT1 in HepaRG cells, such as phosphorylation or glycosylation, or, alternatively, to the artificially elevated levels of OCT1 in OCT1-transfected cells.

Canalicular secretion of taurocholate and CF was finally quantified in HepaRG cells using the BEI approach based on Ca^{2+} withdrawal-mediated disruption of canaliculi (Liu et al., 1999). As shown in Fig. 3B, HepaRG cells pre-incubated in Ca^{2+}-free conditions exhibited decreased retention of taurocholate and CF comparatively to counterparts maintained in the presence of Ca^{2+}, which indicates that HepaRG cells secreted taurocholate and CF in bile canaliculi-like structures and agrees, for CF, with Fig. 2A. BSEP and MRP2, that handle taurocholate and CF, respectively (Meier and Stieger, 2002; Zamek-Gliszczynski et al., 2003), at the canalicular pole of hepatocytes, appear therefore to be most likely functional in HepaRG cells. Interestingly, BEI value for CF in HepaRG cells (34.4 %) was nearly identical to CF BEI value reported in sandwich-cultured human hepatocytes (BEI=34%) (Hoffmaster et al., 2004), which suggests that MRP2 is similarly active in these primary human hepatocytes and in HepaRG cells. By contrast, taurocholate BEI values in sandwich-cultured human hepatocytes (around 70-75%) (Marion et al., 2007) are rather higher than that found in HepaRG cells (BEI=29.2%), which likely indicates a lower BSEP activity in HepaRG cells, consistent with the down-regulation of BSEP mRNA expression observed in these hepatoma cells (Fig. 1B).

In summary, HepaRG cells were demonstrated to exhibit a polarized and functional expression of various major sinusoidal and canalicular transporters, with a global drug transporter mRNA level profile correlated to that displayed by freshly isolated human

hepatocytes. Otherwise, HepaRG cells have been shown to be fully responsive to physiological, pharmacological or toxicological stimuli regulating hepatic transporter expression (Fardel and Le Vee, 2009; Lambert et al., 2009). Taken together, these data clearly highlight the interest of using HepaRG cells in drug transporter studies as surrogates for human hepatocytes, even if some quantitative differences between HepaRG cells and human hepatocytes with respect to transporter expression levels and transport parameters exist and have likely to be kept in mind.

Authorship Contributions

Participated in research design: Le Vee and Fardel.

Conducted experiments: Le Vee, Noel and Jouan.

Contributed new reagents: Stieger.

Performed data analysis: Le Vee and Fardel.

Wrote or contributed to the writing of the manuscript: Fardel

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Legends to figures

Fig. 1. Sinusoidal and canalicular drug transporter mRNA expression in HepaRG cells.

(A, B) Expression of sinusoidal (A) and canalicular (B) drug transporter expression was determined in HepaRG cells and freshly isolated human hepatocytes (FIHH) by RT-qPCR as described in Materials and Methods. Data from 8 independent HepaRG cultures and from 8 independent hepatocyte populations are expressed as boxplots. Transporter expression in HepaRG cells expressed as % of that found in human hepatocytes (arbitrarily set at 100 %) are indicated by numbers in brackets.*, $p < 0.05$ when compared to hepatocytes. (C) Drug transporters were ranked according to their level of mRNA expression; correlation between transporter expression profiles was next analyzed using the Spearman's rank correlation method. Spearman's rank coefficient (ρ) and p values are indicated at the top of the correlation graph.

Fig. 2. Drug transporter localization in HepaRG cells.

(A) HepaRG cell morphology was examined by light phase contrast microscopy whereas CF-labeled functional bile canaliculi were detected through fluorescence microscopy; lile canaliculi are indicated by arrows. Bar = 30 μM. (B) Immunolocalization of sinusoidal and canalicular drug transporters was performed as described in Materials and Methods. Pictures correspond to single immunolabeling, with membrane transporter-related green fluorescence, except bottom right picture, for which double immunolabeling was performed (red fluorescence for MRP2 and green fluorescence for OATP1B1); blue fluorescence corresponds to 4,6-diamidino-2-phenylindole-stained nuclei. Bar = 10 μM.

Fig. 3. Sinusoidal (A) and canalicular (B) drug transport activity in HepaRG cells.

(A) Saturable uptakes of referent substrates for sinusoidal transporters (Taurocholate for NTCP, E3S for OATPs and TEA for OCT1) were analyzed as described in Materials and Methods. Data are the means $\pm$ SEM of three independent experiments. Kinetic parameters

(K_m, V_{max}) are indicated on the top of each graph. (B) Canalicular secretions of the BSEP substrate taurocholate and the MRP2 substrate CF were determined as described in Material and Methods. Data are the means $\pm$ SEM of five (Taurocholate) or four (CF) independent experiments. BEI values are indicated on the top of each graph. *, $p < 0.05$.
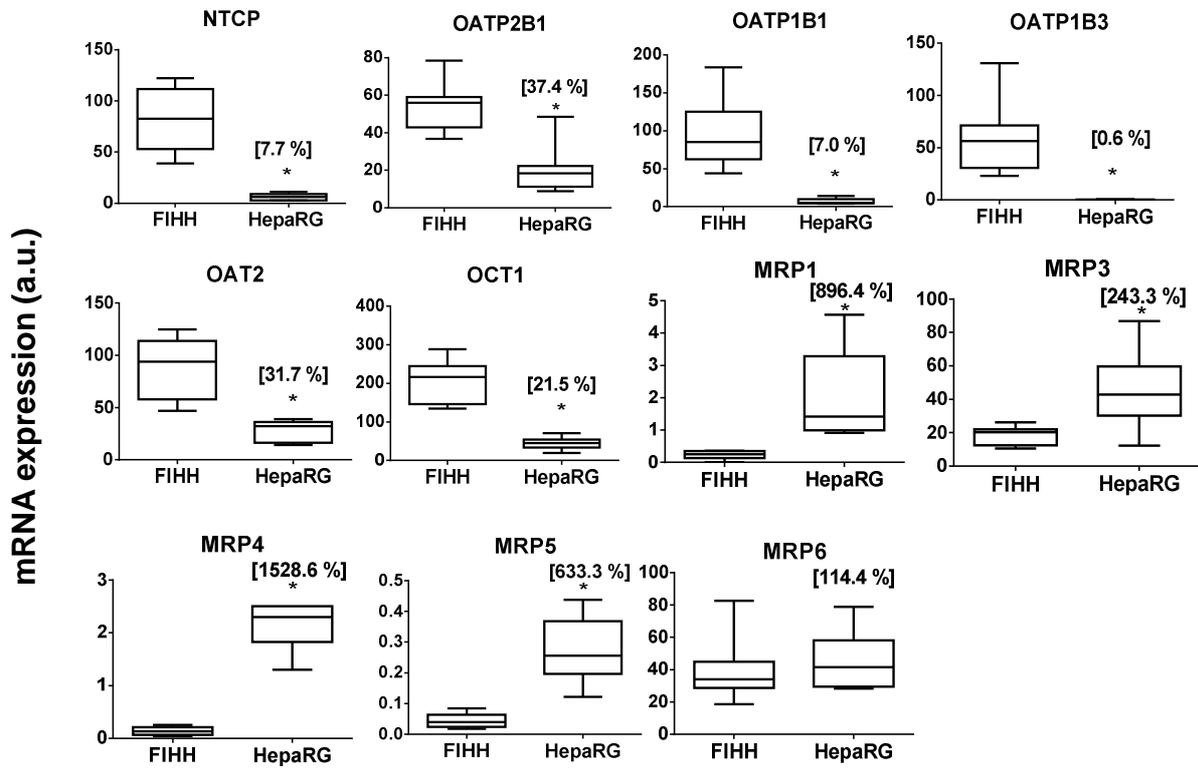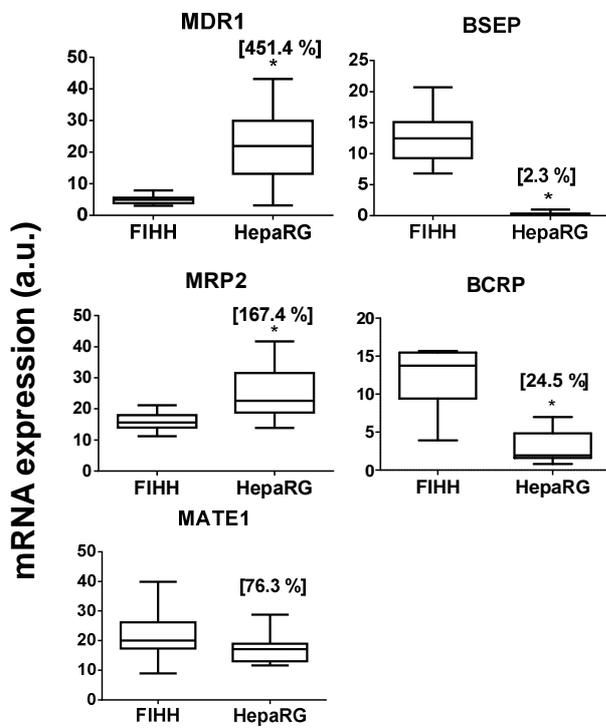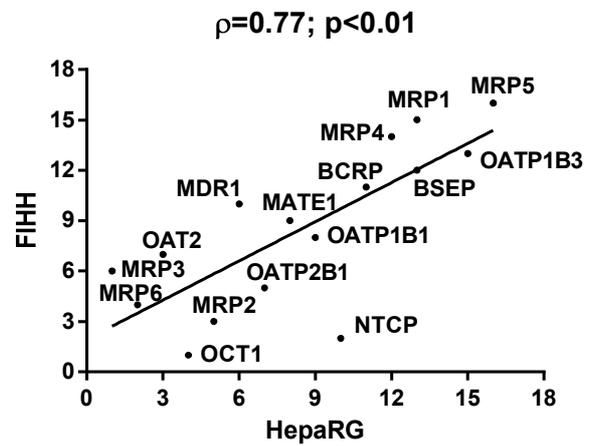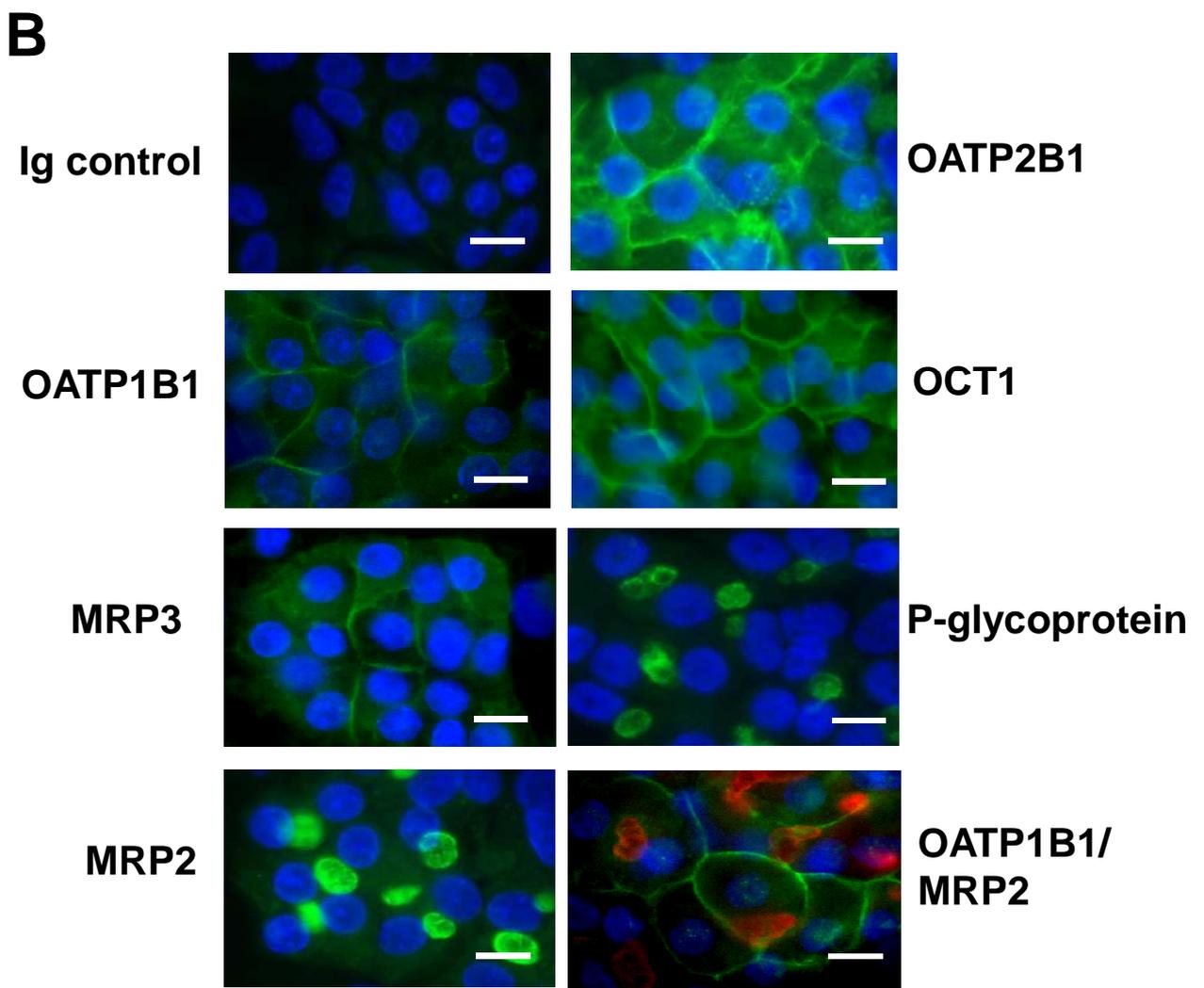
Figure 1

A

| Phase-contrast | Fluorescence | Merged |

B

Ig control

OATP2B1

OATP1B1

OCT1

MRP3

P-glycoprotein

MRP2

OATP1B1/ MRP2
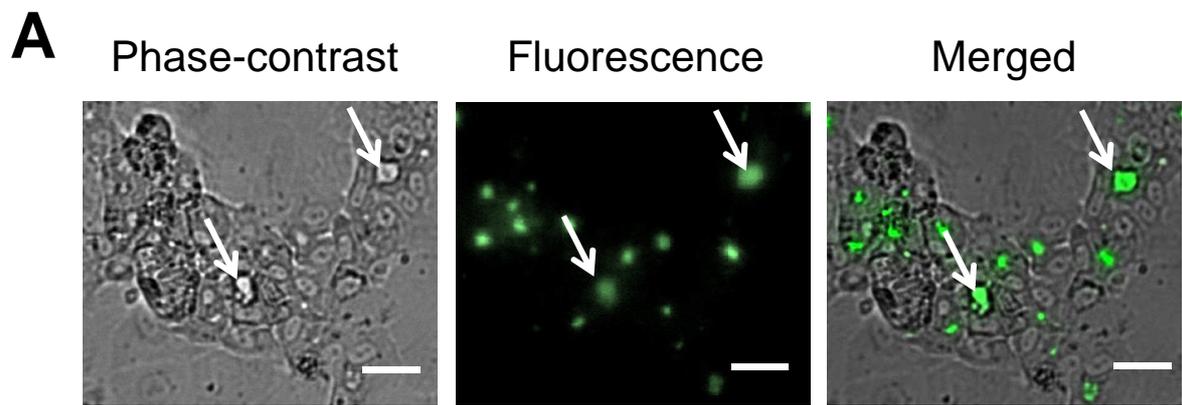
Figure 2

A

NTCP activity
Km=6.3 ± 3.7 µM
Vmax=86.1 ± 19.2 pmol/mg prot.min
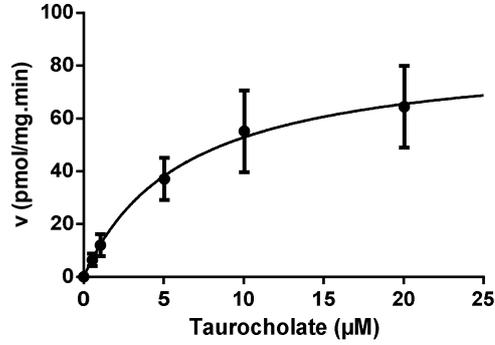
OATP activity
Km=44.0 ± 20.9 µM
Vmax=647.5 ± 176.0 pmol/mg prot.min
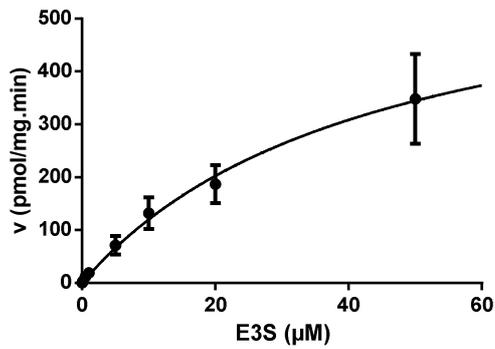
OCT1 activity
Km=1.44 ± 0.42 mM
Vmax=8.20 ± 1.05 nmol/mg prot.min
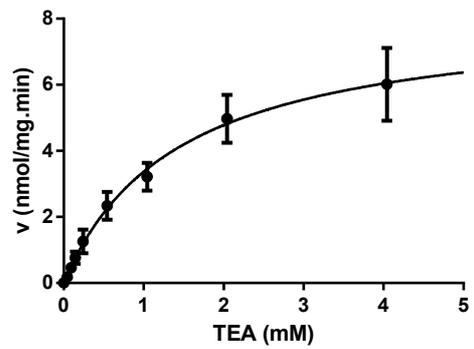
B

Taurocholate
BEI=29.2±6.1%

CF
BEI=34.4±6.9%

Figure 3