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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 μM 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 RARα and RXRα 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.

1. Introduction

Hepatic drug transporters are now well-recognized as important factors contributing to pharmacokinetics and toxicokinetics, through their major implication in hepato-biliary secretion of drugs (DeGorter et al., 2012). They mainly mediate cellular uptake or efflux of xenobiotics at the sinusoidal and canalicular pole of hepatocytes, respectively, and divide up into two superfamilies, i.e., the solute carrier transporter (SLC) and the ATP-binding cassette (ABC) transporter superfamilies (Klaassen and Aleksunes, 2010). They have been implicated in various clinically-demonstrated drug–drug interactions (Giacomini et al., 2010); for example, gemfibrozil-mediated inhibition of hepatic uptake of statins is thought to be involved in the increased risk of myopathy when these drugs are co-administered (Schneck et al., 2004). Moreover, hepatic transporter expression has been shown to be affected by various physiological and pathological factors, including pro-inflammatory cytokines (Fardel and Le Vee, 2009), which is likely to contribute to altered pharmacokinetics in liver diseases.

Factors that govern hepatic drug transporter expression are therefore important to characterize. Some of them have been linked to activation of nuclear receptors, such as pregnane X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AhR) or farnesoid X receptor (FXR) (Jigorel et al., 2006; Klaassen and Aleksunes, 2010). Interestingly, PXR, CAR and FXR, when activated by their ligands, form heterodimers with retinoid X receptor (RXR) α before binding to response elements found in the promoter of responsive genes, thus supporting a role for retinoid receptors in regulation of drug transporters. These retinoid receptors belongs to two subfamilies of nuclear receptors, RXRs and retinoic acid receptors (RARs) (Bastien and Rochette-Egly, 2004). RAR usually combines to RXR to form the heterodimer RXR:RAR that binds to specific elements termed retinoic acid response elements (RAREs) located in the promoter regions of retinoic acid-responsive genes. In the absence of a ligand, RXR:RAR usually functions as a repressor, but when agonists such as all-trans retinoic acid (atRA), that is considered as the active metabo-

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lite of vitamin A, bind to RAR, the heterodimer activates transcription of target genes (Germain et al., 2006).

The exact nature of human hepatic drug transporters that may be regulated by atRA remains yet poorly characterized, but is probably important to determine, owing to the fact that atRA is notably used for treatment of acute promyelocytic leukemias (Mi et al., 2012) and some dermatological affections. The present study was designed to gain insights about this point. For this purpose, we used mainly highly-differentiated human hepatoma HepaRG cells, that are presumed to constitute a convenient model for *in vitro* studying liver detoxifying pathways (Guguen-Guillouzo and Guillouzo, 2010) and that are responsive to atRA (Antoun et al., 2006); some primary cultures of human hepatocytes, which are known to exhibit substantial basal expression of transporters (Jigorel et al., 2005, 2006), were also used in parallel for comparison, but in a rather limited manner owing to the reduced and scarce availability of human hepatocytes. Our data demonstrate that various hepatic drug transporters were regulated in response to atRA treatment, mainly in a RXR:RAR-dependent manner, thus fully establishing retinoids as modulators of hepatic drug transporter expression. Some transporters were however differentially regulated by atRA in HepaRG cells and human hepatocytes, thus pointing out some differences between these two liver cell models with respect to regulation of liver detoxifying pathways; these discrepancies may have to be taken into consideration when using HepaRG cells as surrogates to human hepatocytes in pharmacological and toxicological studies.

2. Materials and methods

2.1. Chemicals and reagents

atRA, verapamil, probenecid and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma–Aldrich (Saint-Quentin Fallavier, France). [1-^{14}C]tetra-ethylammonium (TEA) (sp. act. 2.4 mCi/mmol) and [6,7-^{3}H(N)]estrone 3-sulfate (sp. act. 57.3 Ci/mmol) were purchased from Perkin Elmer Life Sciences (Boston, MA). Antibody against breast cancer resistance protein (BCRP/ABCG2) was provided by Alexis Biochemicals (Lausen, Switzerland). All other compounds and reagents were commercial products of the highest purity available.

2.2. Cell isolation and culture

Human hepatocytes were obtained from adult donors undergoing hepatic resection for primary and secondary tumors or other pathologies, via the Biological Resource Center (Rennes, France); informations related to donors (age, sex and primary pathology) are given in Table 1. Cells were prepared by perfusion of histologically-normal liver fragments using a collagenase solution (Payen et al., 2000). They were primary cultured on plastic dishes in Williams'E medium, as already reported (Le Vee et al., 2009). All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee.

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

Table 1
Donor information.

Hepatocyte population	Sex	Age (year)	Pathology
HL1	F	52	Hematoma
HL2	M	70	Colon carcinoma
HL3	M	77	Colon carcinoma
HL4	M	63	Colon carcinoma
HL5	M	74	Hepatocellular carcinoma

2.3. Cell viability assay

Viability of cultured cells was determined using a colorimetric MTT assay as previously described (Vernhet et al., 2001).

2.4. RNA isolation and analysis

Total RNA was isolated from cells using the TRIzol® reagent (Invitrogen, Cergy-Pontoise, France). RNA was then subjected to reverse transcription-real time quantitative polymerase chain reaction (RT-qPCR) using the fluorescent dye SYBR Green methodology and an ABI Prism 7300 detector (Applied Biosystem, Foster City, CA), as already reported (Jigorel et al., 2006). Gene primers for drug transporters and cytochrome P-450 (CYP) CYP7A1 were exactly as previously described (Le Vee et al., 2009). Other primers were CYP26A1 sense, CCAGTGCAGCCACATCTCT, CYP26A1 antisense, TGGAACTGGGGGATTCAGT, apolipoprotein (apo) A-II sense, GAGCTTTGGTTCGGAGACAG, apo A-II antisense, TGTGTTCCAAGTTCCACGAA, RXRα sense, GCTTCCTTCACCAAGCACA, RXRα antisense, CGCTTGTCAATCAGGCAGT, RARα sense, GGGCATGTCCAAGGAGTCT, RARα antisense, TCCCAGAGGTCAATGTCCA. Relative quantification of the steady-state target mRNA levels was calculated after normalization of the total amount of cDNA tested to a 18S endogenous reference.

2.5. SiRNA transfection

SiRNA transfection was performed in HepaRG cells as previously described (Le Vee et al., 2010). Briefly, HepaRG cells were trypsinized and replated in 24-multiwells with 200 nM chemically synthesized, double-stranded, siRNAs targeting mRNAs of RARα (siRARα) or RXRα (siRXRα) or control non-targeting siRNAs (siNT), provided by Sigma–Aldrich, in the presence of transfection medium, i.e. DharmaFECT-1 transfection reagent (Dharmacon) diluted in DMEM optimum/Williams'E medium supplemented with 1% dimethyl sulfoxide. After 18 h, transfection medium was withdrawn and cells were next maintained for 48 h in Williams'E medium described above, before being treated with atRA.

2.6. Western-blot analysis

Crude membrane extracts were prepared as previously described (Le Vee et al., 2008). Proteins were then separated on polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes. After blocking in Tris-buffered saline containing 4% bovine serum albumin, membranes were incubated overnight at 4 °C with primary antibodies directed against organic anion transporting polypeptide (OATP) 2B1 (SLCO2B1), OATP1B1 (SLCO1B1) (Huber et al., 2007) or BCRP. Peroxidase-conjugated monoclonal antibodies were thereafter used as secondary antibodies. After washing, immuno-labelled proteins were visualized by chemiluminescence. Gel loading and transfer was checked up by staining membranes with Ponceau red. The intensities of stained bands were measured by densitometry using ImageJ 1.40 g software (National Institute of Health, Besthesda, MA).

2.7. Transport assays

Transport activities due to organic cation transporter (OCT) 1 (SLC22A1) and OATPs were analyzed through measuring verapa-