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Regulation of drug transporter expression by oncostatin M in human hepatocytes

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ABSTRACT

The cytokine oncostatin M (OSM) is a member of the interleukin (IL)-6 family, known to down-regulate expression of drug metabolizing cytochromes P-450 in human hepatocytes. The present study was designed to determine whether OSM may also impair expression of sinusoidal and canalicular drug transporters, which constitute important determinants of drug hepatic clearance. Exposure of primary human hepatocytes to OSM down-regulated mRNA levels of major sinusoidal solute carrier (SLC) influx transporters, including sodium-taurocholate co-transporting polypeptide (NTCP), organic anion transporting polypeptide (OATP) 1B1, OATP1B3, OATP2B1, organic cation transporter 1 and organic anion transporter 2. OSM also repressed mRNA expressions of ATP binding cassette (ABC) efflux transporters such as multidrug resistance protein (MRP) 2/ABCC2 and breast cancer resistance protein/ABCG2, without however impairing those of multidrug resistance gene 1/P-glycoprotein/ABCB1, MRP3/ABCC3, MRP4/ABCC4 and bile salt export pump/ABCB11. The cytokine concomitantly reduced NTCP, OATP1B1, OATP2B1 and ABCG2 protein expression and NTCP and OATP transport activities. OSM effects towards transporters were found to be dose-dependent and highly correlated with those of IL-6, but not with those of other inflammatory cytokines such as tumor necrosis factor-α or interferon-γ. In addition, OSM-mediated repression of some transporters such as NTCP, OATP1B1 and OATP2B1, was counteracted by knocking-down expression of the type II OSM receptor subunits through siRNA transfection. This OSM-mediated down-regulation of drug SLC transporters and ABCG2 in human hepatocytes may contribute to alterations of pharmacokinetics in patients suffering from diseases associated with increased production of OSM.

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1. Introduction

Oncostatin M (OSM) is a member of the interleukin (IL)-6 family, secreted mainly by monocytes, macrophages, T cells and polymorphonuclear neutrophils [1,2]. Numerous biological activities have been ascribed to OSM, including for example differentiation of megakaryocytes, inhibition of tumor cell growth, induction of neurotrophic peptides and bone remodeling [2]. In the liver, OSM has been involved in various physiological processes including development and regeneration [3,4]; this has been hypothesized to occur through activation of the type II OSM receptor expressed by hepatocytes and formed by the association of glycoprotein 130 (gp130) and OSM receptor β (OSMRβ) subunits [5,6].

A well-recognized effect of OSM towards hepatocytes is the induction of acute-phase proteins such as C-reactive protein (CRP), thus supporting a role for OSM in liver inflammation [7,8]. In addition, like other inflammatory cytokines such as IL-1β, tumor necrosis factor (TNF)-α, IL-6 and interferon (IFN)-γ [9,10], OSM has been shown to alter expression of drug metabolizing enzymes; OSM-treated human hepatocytes thus exhibit reduced expression and activity of various cytochromes P-450 (CYPs) such as CYP1A2, CYP2B6 and CYP3A4 [11]. By contrast, whether OSM may concomitantly alter expression of other hepatic detoxifying proteins such as drug transporters, which constitute major actors of the drug hepatobiliary secretion pathway [12,13] and are well-established targets for inflammatory cytokines [14–17], remains unknown. The present study was therefore designed to gain insight about this point, through investigating the expression of main solute carrier (SLC) transporters, involved in drug uptake at the sinusoidal pole of hepatocytes, and ATP-binding cassette (ABC) transporters, usually acting as drug efflux pumps at the canalicular pole of hepatocytes, in OSM-treated primary human hepatocytes. Our data indicate that these OSM-treated hepatocytes exhibit marked reduced expressions of SLC transporters and of some ABC transporters, with a global pattern of transporter changes close to that triggered by IL-6. These effects of OSM towards drug

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transporters, associated with known effects towards CYPs, may support the idea that up-regulation of OSM secretion in various physiological and pathological states may contribute to potential alteration of pharmacokinetics.

2. Materials and methods

2.1. Chemicals and reagents

Recombinant human OSM was provided by R&D Systems (Minneapolis, MN). [^{3}H(G)]taurocholic acid (sp. act. 1.19 Ci/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). Probenecid was from Sigma–Aldrich (Saint-Quentin Fallavier, France). Antibodies against the ABC transporters P-glycoprotein/multidrug resistance gene 1/ABCB1 and breast cancer resistance protein/ABCG2 were provided by Alexis Biochemicals (Lausen, Switzerland), whereas those against multidrug resistance-associated protein (MRP)2/ABCC2 and MRP3/ABCC3 were from Chemicon International (Temecula, CA), and those directed against the mitogen-activated protein kinase (MAPK) phospho-extracellular signal regulated kinase (ERK) and total ERK from Cell Signaling Technology (Beverly, MA). All other compounds and reagents were commercial products of the highest purity available. Vehicle for OSM was phosphate-buffered saline; control cultures received the same dose of vehicle as treated counterparts.

2.2. Cell isolation and culture

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 [18]. They were primary cultured on plastic dishes in Williams'E medium, as already reported [19,20]. 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 [21,22].

2.3. SiRNA transfection

SiRNA transfection was performed in HepaRG cells as previously described [23]. Briefly, HepaRG cells were trypsinized and replated in 24-multiwells with 100 nM chemically synthesized, double-stranded, siRNAs targeting mRNAs of gp130 or OSMRβ subunits, provided by Sigma–Aldrich, or control non-targeting siRNAs (si-NT), provided by Dharmacon (Lafayette, CO), 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 72 h in Williams'E medium described above, before being treated with OSM.

2.4. RNA isolation and analysis

Total RNA was isolated from cells using the TRIzol^R 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 [24]. Gene primers for drug transporters and CRP were exactly as previously described [19]. Other primers were MRP4/ABCC4 sense, GCTCAGGTTGCCTATGTGCT, ABCC4 antisense, CGGTTACATTTCCTCCTCCA, OSMRβ sense, ATGCCATCATGACCTGGAA, OSMRβ antisense, CTCGCGCCATGTACTCTGT, gp130 sense, ATGAAGGTGGGAAGGATGG, gp130 antisense, TGCCTTGGAGGAGTGAG. 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. Western-blot analysis

Total cellular or crude membrane extracts were prepared from primary human hepatocytes as previously described [15]. 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 sodium-taurocholate cotransporting polypeptide (NTCP)/SLC10A1, organic anion transporting polypeptide (OATP)2B1/SLCO2B1, OATP1B1/SLCO1B1 [25], P-glycoprotein/ABCB1, ABCC2, ABCC3, ABCG2, phospho-ERK or total ERK. 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).

2.6. Transport assays

Transport activities due to NTCP or OATPs were analyzed through measuring sodium-dependent-intracellular accumulation of the NTCP substrate taurocholate and probenecid-sensitive uptake of the OATP substrate estrone-3-sulfate, as previously described [18]. Briefly, cells were incubated at 37 °C for 10 min with 0.17 μM [^{3}H]taurocholate in the presence or absence of sodium or with 1.7 nM [^{3}H]estrone-3-sulfate in the presence or absence of the OATP inhibitor probenecid used at 2 mM. After washing in phosphate-buffered saline, cells were lyzed and accumulation of radiolabeled substrates was determined through scintillation counting. Taurocholate accumulation values in the presence of sodium minus accumulation values in the absence of sodium and estrone-3 sulfate uptake values in the absence of probenecid minus uptake values in the presence of probenecid, are thought to represent NTCP- and OATP-related transport activities [18].

2.7. Statistical analysis

Quantitative data were usually expressed as means ± SD. They were statistically analyzed using the Student's t test, Kruskal–Wallis one-way analysis of variance followed by the Student–Newman–Keuls test, or the nonparametric Spearman's rank correlation method. The criterion of significance was $p < 0.05$. Data from dose–response studies were fitted using the SigmaPlot software (Systat software, San Jose, CA).

3. Results

3.1. Effects of OSM treatment on CRP expression

Primary human hepatocytes from 6 liver donors were exposed to 10 ng/ml OSM for 8 h, 24 h or 48 h. This concentration of OSM was retained since it has been previously used for treating cultured hepatic cells in various studies [7,26,27]; it did not exert toxicity as demonstrated by phase-contrast microscopic examination of the

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