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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.

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

Table 1
Effects of OSM treatment on CRP mRNA expression in primary human hepatocytes.

Exposure time to OSM	CRP mRNA expression (% of 18 S)	CRP mRNA fold-induction
0 h	0.0002 ± 0.0003	–
8 h	0.0141 ± 0.0102*	8453 ± 9934*
24 h	0.0567 ± 0.0373*	32,011 ± 39,266*
48 h	0.0260 ± 0.0171*	24,695 ± 43,352*

CRP mRNA fold-induction are defined as the ratio of CRP mRNA levels in OSM-treated primary hepatocytes versus those found in untreated counterparts.
Data are mean ± SD of values from six independent hepatocyte populations.
* $p < 0.05$ when compared to untreated hepatocytes (Student–Newman–Keuls test).

cultures (data not shown). As shown in Table 1, OSM markedly increased mRNA expression of CRP, whatever the exposure time, even if the level of induction varied according to hepatocyte populations; such a variety in the level of induction has already been reported for regulation of referent inflammatory markers, including CRP, by cytokines in human hepatocytes [16]. Since CRP is a well-established target of OSM [7], these data indicated that primary human hepatocytes were fully responsive to OSM in our hands and were thus suitable for investigating OSM effects towards drug transporter expression.

3.2. Effects of OSM treatment on drug transporter mRNA expression

We first analyzed the effects of OSM treatment on SLC transporter mRNA expression by RT-qPCR. As shown in Fig. 1A, human hepatocytes obtained from 6 individuals exhibited lower mRNA expression of OATP1B1, OATP1B3/SLCO1B3 and organic anion transporter 2 (OAT2)/SLC22A7, when exposed to OSM, comparatively to untreated counterparts, whatever the time of treatment (8 h, 24 h or 48 h). With respect to OATP1B3 and OAT2, the time of exposure did not affect the level of expression repression in a major way, i.e., reduced levels of transporter expression found in hepatocytes exposed to OSM for 8 h, 24 h or 48 h were not statistically different; by contrast, OATP1B1 expression was found to statistically decrease with the time of exposure (Fig. 1A). OSM was also found to repress organic cation transporter (OCT)1/SLC22A1, OATP2B1 and NTCP mRNA levels, but only in response to a 24-h or 48-h exposure (Fig. 1A).

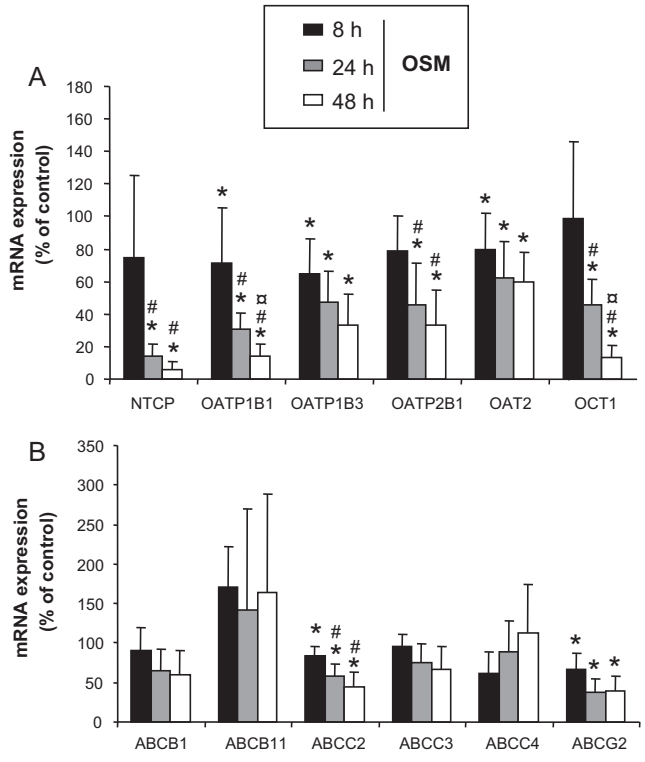

Fig. 1. Effects of OSM on drug transporter mRNA expression in human hepatocytes. Primary human hepatocytes were either untreated or exposed to 10 ng/ml OSM for 8 h, 24 h or 48 h. SLC (A) and ABC (B) transporter mRNA expression was then determined by RT-qPCR, as described under Section 2. Data are expressed for each transporter as percentage of expression found in control untreated hepatocytes, arbitrarily set at the value of 100%. They are the means ± SD of values from six independent hepatocyte populations. *$p < 0.05$ when compared to untreated cells; #$p < 0.05$ when compared to cells exposed to OSM for 8 h; ¤$p < 0.05$ when compared to cells exposed to OSM for 24 h (Student–Newman–Keuls test).

We next studied the effects of OSM treatment on ABC transporter mRNA expression. As shown in Fig. 1B, expression of ABCB1, bile salt export pump/ABCB11, ABCC3 and ABCC4 remained statistically unchanged in hepatocytes exposed to OSM for 8 h, 24 h or 48 h, when compared to untreated counterparts. By contrast, OSM down-regulated ABCC2 and ABCG2 expression, whatever the time of exposure; ABCC2 mRNA levels, unlike ABCG2 mRNA levels, were however lower in hepatocytes exposed to 24-h or 48-h to OSM when compared to those found in counterparts exposed for only 8-h, suggesting a time-dependent effect of the cytokine towards mRNA levels of this transporter.

When considering the repression factor after a 24-h exposure to OSM, i.e., the ratio of mRNA levels in untreated hepatocytes versus those found in treated counterparts, NTCP was the most repressed among the transporters affected by OSM, followed by OATP1B1, ABCG2, OATP2B1, OATP1B3, OCT1, ABCC2 and OAT2 (Table 2). Interestingly, these transporters, excepted OAT2 which has not been analyzed, were also found to be repressed by OSM in human highly differentiated hepatoma HepaRG cells (Table 2), known to represent a useful alternative to primary human hepatocytes for investigating hepatic detoxifying proteins, including transporters [22,28]. A 8-h exposure to 10 ng/ml OSM moreover markedly induced CRP mRNA expression by a 517.4 ± 264.0-fold factor in HepaRG cells, confirmating that these hepatoma cells were fully responsive to OSM.

The dose–response relationship for OSM effects towards some transporters was next characterized in primary human hepatocytes. As shown in Fig. 2, repressing effects of OSM towards NTCP, OATP1B1, OATP1B3, OCT1, ABCC2 and ABCG2 were dose-dependent, with EC50 ranging around 0.1–1 ng/ml. Treatment by 20 ng/ml OSM led to down-regulations of transporter expression similar to those observed with 10 ng/ml OSM. The use of OSM at 1 ng/ml also repressed transporters, but in a more moderate manner. The dose of 0.1 ng/ml was still partially active, except for ABCG2,

Table 2
Repression of drug transporter expression in primary human hepatocytes and hepatoma HepaRG cells exposed to OSM.

Transporter	Fold repression (mRNA levels)	
	Human hepatocytes	HepaRG cells
NTCP	9.8 ± 6.2	17.0 ± 2.9
OATP1B1	3.6 ± 1.2	4.6 ± 0.6
ABCG2	3.2 ± 1.6	3.1 ± 0.3
OATP2B1	2.6 ± 1.1	3.3 ± 0.6
OATP1B3	2.5 ± 1.1	2.3 ± 0.3
OCT1	2.4 ± 1.0	2.8 ± 1.0
ABCC2	1.8 ± 0.5	2.8 ± 0.4
OAT2	1.8 ± 0.7	Not done

Fold repression are defined as the ratio of drug transporter mRNA levels in control untreated cells versus those found in counterparts exposed to 10 ng/ml OSM for 24 h.
Data are the mean ± SD of values from six independent populations (human hepatocytes) or from three independent experiments (HepaRG cells).

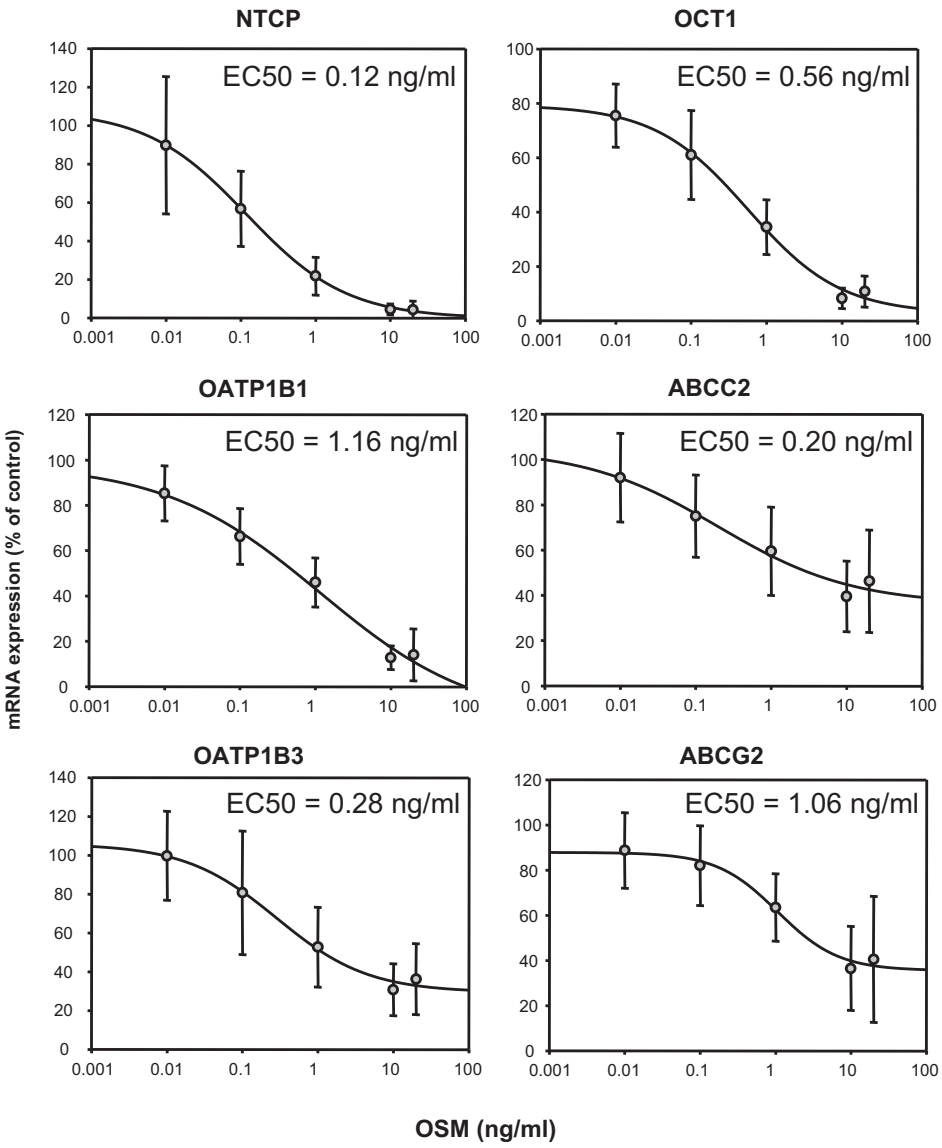

Fig. 2. Dose–response of OSM effects towards drug transporter expression. Primary human hepatocytes were either untreated or exposed to 0.01, 0.1, 1, 10 or 20 ng/ml OSM for 48 h. Transporter mRNA expression was then determined by RT-qPCR, as described under Section 2. Data are expressed for each transporter as percentage of expression found in control untreated hepatocytes, arbitrarily set at the value of 100%, and are the means ± SD of values from four independent hepatocyte populations. They were fitted with OSM concentrations, allowing to determine OSM EC50 for each transporter regulation.

whereas the lower concentration of 0.01 ng/ml has no obvious, or only very limited, effects on transporter mRNA expression (Fig. 2).

3.3. Effects of OSM treatment on drug transporter protein expression

To determine whether some of the changes in transporter mRNA levels induced by OSM also occur at the protein level, we next performed Western-blot analysis of crude membranes from OSM-treated primary human hepatocytes and untreated counterparts. As indicated in Fig. 3, exposure to OSM for 48 h reduced expression of NTCP, OATP1B1, OATP2B1 and ABCG2. By contrast, it failed to obviously alter P-glycoprotein/ABCB1, ABCC2 and ABCC3 expression (Fig. 3).

3.4. Effects of OSM treatment on transporter activities

Owing to the limited availability of human hepatocytes, we focussed on the effects of cytokine treatments on activities of NTCP and OATPs, whose mRNA expressions were among the most repressed by OSM (Table 2). As shown in Fig. 4, exposure to OSM for 48 h resulted in decreased NTCP and OATP transport activity in human primary hepatocytes, when compared to untreated counterparts.

3.5. Correlation analysis of cytokine repressing effects on drug transporter mRNA expression in human hepatocytes

Besides OSM, various cytokines such as IL-1β, TNF-α, IL-6 and IFN-γ, have been previously shown to down-regulate mRNA expression of various hepatic transporters [15,16,29]. In order to search for a putative correlation between these repressive effects of cytokines, drug transporters were ranked according to the down-regulation of their mRNA expression in response to a 24-h treatment by cytokines. Data used for OSM effects were from Fig. 1, whereas those related to IL-1β, TNF-α, IL-6 and IFN-γ effects have been previously obtained using primary human hepatocytes cultured in the same conditions than those used in the present study [15,19,29]. For each treatment, transporters were ranked

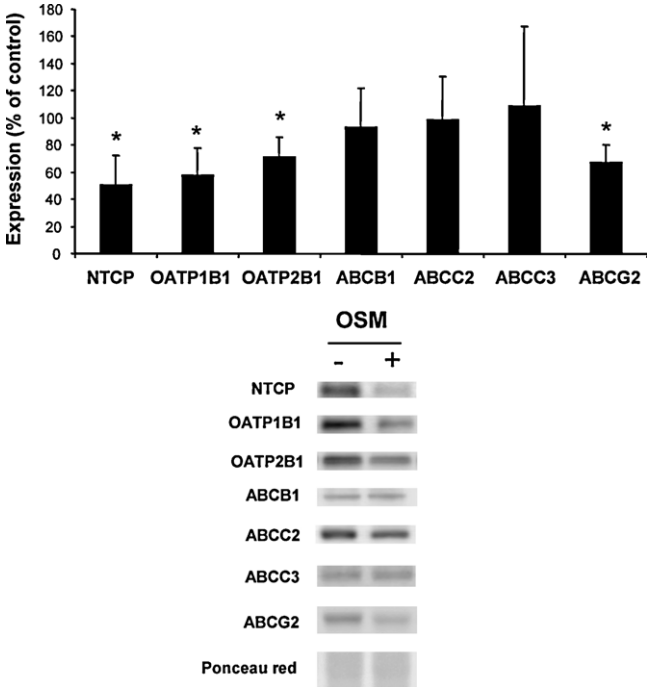

Fig. 3. Effects of OSM on drug transporter protein expression in human hepatocytes. Primary human hepatocytes were either untreated or exposed to 10 ng/ml OSM for 48 h. Transporter protein content was then determined by Western-blot analysis. For each transporter, data were quantified by densitometric analysis and expressed relatively to transporter expression found in control untreated cells, arbitrarily set at the value of 100%; they are the means ± SD of values from three (NTCP) or six (other transporters) independent hepatocyte populations (upper panel). A representative blot is also shown for each transporter (lower panel). $^{*}p < 0.05$ when compared to untreated cells (Student's *t* test).

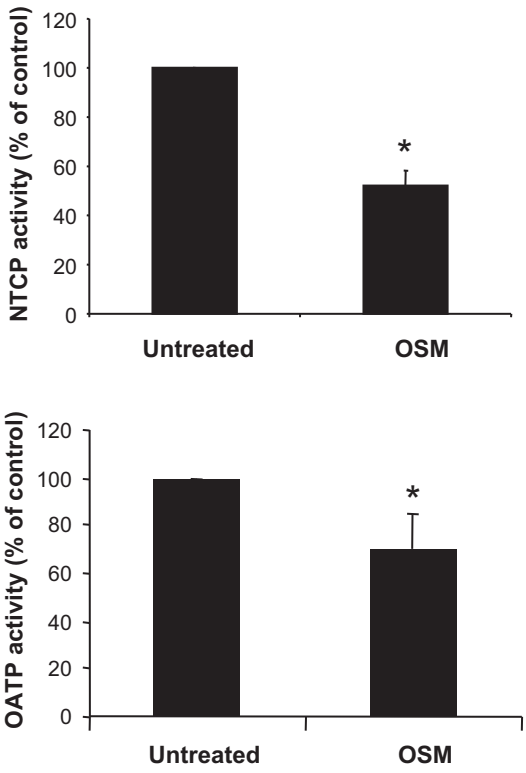

Fig. 4. Effects of OSM on drug transporter activities in human hepatocytes. Primary human hepatocytes were either untreated or exposed to 10 ng/ml OSM for 48 h. NTCP and OATP activities were then determined using radiolabeled substrates as described under Section 2. Data are expressed relatively to transporter activity found in control untreated cells, arbitrarily set at the value of 100%; they are the means ± SD of values from five (NTCP activity) or six (OATP activity) independent hepatocyte populations. $^{*}p < 0.05$ when compared to untreated cells (Student's *t* test).

from the most repressed transporter to the less repressed according to mRNA expression levels. Correlations were analyzed using the Spearman's rank correlation method. Results indicated that the effects of OSM towards drug transporters were highly correlated with those of IL-6 and, in a more moderate manner, with those of IL-1β (Fig. 5). By contrast, they were not correlated with those of TNF-α and IFN-γ (Fig. 5).

3.6. Involvement of type II OSM receptor in OSM effects towards drug transporters

To investigate the implication of the type II OSM receptor in the repressing effects of OSM towards drug transporters, we performed knock-down of its subunits OSMRβ and gp130 in HepaRG cells using siRNA transfection. As shown in Fig. 6A, HepaRG cells transfected with siRNAs targeting OSMRβ (si-OSMRβ) or gp130 (si-gp130) exhibited reduced expressions of OSMRβ and gp130 mRNAs, which represent less than 25% of mRNA levels found in control counterparts transfected with non-targeting siRNAs (si-NT). In order to determine whether these type II OSM receptor subunit down-regulations have functional consequences, we analyzed their implication towards OSM-mediated CRP mRNA induction. As indicated in Fig. 6B, si-OSMRβ- and si-gp130-transfected cells exposed to OSM exhibited reduced up-regulation of CRP when compared to that occurring in control si-NT-transfected counterparts. Moreover, they also displayed reduced levels of phospho-ERK, whereas total ERK expression remained unchanged (Fig. 6C), knowing that the ERK pathway constitutes one of the major signaling events activated by OSM, i.e., ERK is activated through phosphorylation in response to OSM [30]. Taken together, these data strongly suggest that siRNA-mediated repression of type II OSM receptor subunits was functionally relevant in HepaRG cells, i.e., it resulted in decreased activity of OSM.

We next analyzed the consequences of siRNA-mediated OSMRβ and gp130 subunit knockdown on OSM-mediated down-regulation of NTCP, OATP1B1 and OATP2B1, which are the three most repressed drug transporters in OSM-exposed HepaRG cells (Table 2). OSM-mediated repression of OATP1B1 and OATP2B1 was found to be counteracted in a major way by siRNA-mediated silencing of either OSMRβ (Fig. 7A) or gp130 (Fig. 7B) subunits of type II OSM receptor. OSM-related repression of NTCP was also significantly attenuated (Fig. 7A and B).

4. Discussion

The data reported in the present study demonstrate that treatment by OSM can repress expression of major drug transporters in human hepatocytes. SLC transporters are notably affected in a major way, because OSM down-regulates mRNA levels of NTCP, involved in sinusoidal uptake of bile acids, of OATP1B1, OATP1B3, OATP2B1 and OAT2, involved in sinusoidal uptake of organic anions, and of OCT1, mediating sinusoidal uptake of organic cations, in a dose-dependent manner. Moreover, some of these transporters such as NTCP, OATP1B1 and OATP2B1 are also repressed at protein levels, and NTCP and OATP transport activities are concomitantly reduced by OSM.

OSM also targets ABC efflux transporters, but in a more limited manner. Indeed, if ABCG2 expression was repressed at both mRNA and protein level by OSM treatment in primary human hepatocytes, other major ABC transporters such as P-glycoprotein/ABCB1, ABCB11, ABCC3 and ABCC4 were not affected. With respect to the

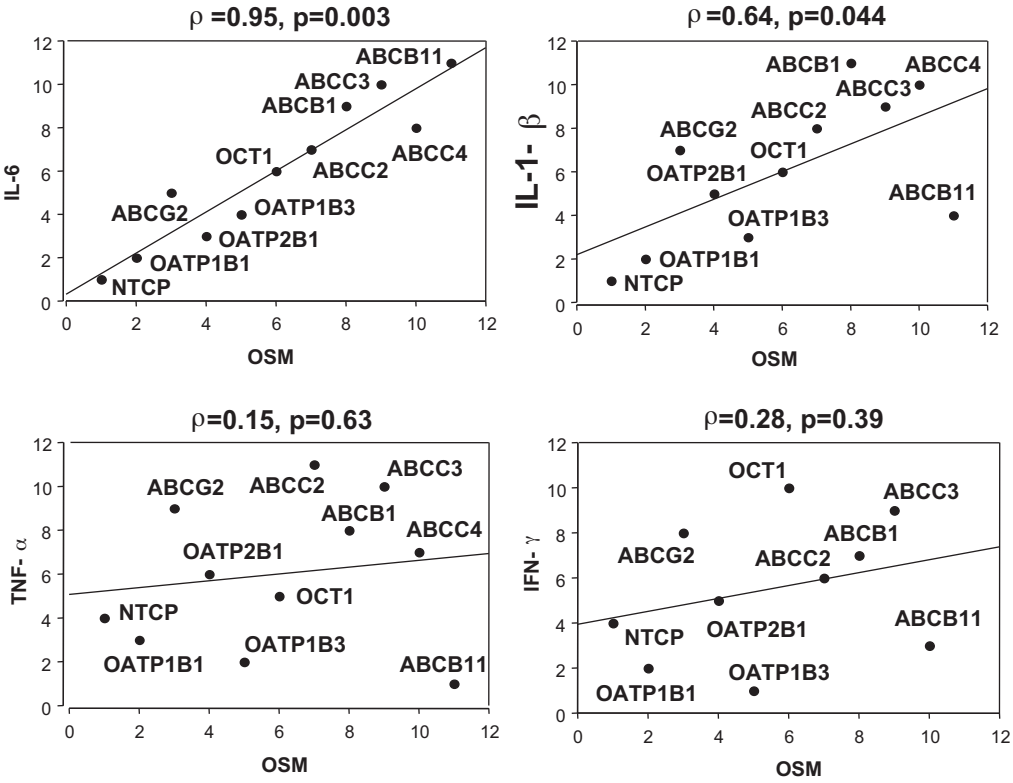

Fig. 5. Rank correlation analysis of repressing effects of OSM, IL-6, TNF-α, IL-1β and IFN-γ towards drug transporter mRNA expression in human hepatocytes. Drug transporters were ranked according to the down-regulation of their mRNA expression in response to a 24-h treatment by OSM, IL-6, TNF-α, IL-1β or IFN-γ, from data from Fig. 1 for OSM effects and from previous published data for the effects of the other cytokines [15,19,29]. For this purpose, transporters were ranked for each treatment from the most repressed transporter to the less repressed, through considering the repression factor for each transporter, i.e., the ratio of mRNA levels in untreated hepatocytes versus those found in treated counterparts. Correlations were analyzed using the Spearman's rank correlation method. Spearman's rank coefficients (ρ) and p values are provided on the top of each correlation graph.

organic anion efflux transporter ABCC2, its mRNA expression was down-regulated by OSM, whereas its protein expression remained unaltered; the reason for such a discrepancy remains unclear, but it could be linked to the relative low level of repression of ABCC2 mRNA expression by OSM in primary human hepatocytes (by only a 1.8-fold-factor after a 24-h exposure to OSM; Table 2) or could reflect divergent transcriptional and post-transcriptional effects of OSM towards ABCC2 expression. Such differential effects of cytokines towards mRNA and protein expressions of drug transporters have already been reported for the effects of IL-6 and TNF-α towards some hepatic transporters. Indeed, IL-6 decreased ABCB1 mRNA levels in primary human hepatocytes, without however affecting P-glycoprotein content, whereas TNF-α increased protein expression of ABCG2 and ABCC3, without concomitant changes in mRNA levels of these transporters [19].

The mechanism by which OSM altered expression of transporters remains to be characterized. Type II OSM receptor, which has already been demonstrated to be implicated in hepatic effects of OSM [6], is most likely involved. Indeed, knock-down of gp130 and OSMRβ subunits of this receptor counteracted down-regulation of NTCP, OATP2B1 and OATP1B1 mRNA expression by OSM in HepaRG cells. It can therefore be hypothesized that signaling pathways that are activated by this receptor, especially by its signal-transducing component gp130 subunit, may play a major role in OSM-related changes in transporter expression. The fact that these transporter expression alterations due to OSM treatment were highly correlated with those triggered by exposure to IL-6 fully supports this hypothesis, because IL-6 receptor also uses gp130 subunit for transduction pathway [31]. Through gp130 subunit, OSM and also IL-6, are known to activate several distinct signaling ways, including Janus kinase/signal transducer and activator of transcription cascade, MAPK pathway and phosphatidylinositol 3-kinase pathway [32]; in agreement with these data, the MAPK ERK was found to be activated by OSM in HepaRG cells (Fig. 6C). Analysis of the role that may play these different signaling pathways in OSM-related transporter repression would likely deserve further studies. In this context, it is however noteworthy that the effects of OSM towards transporter were not correlated with those of TNF-α or IFN-γ, suggesting that these cytokines mobilize independent signaling pathways for acting on transporters. With respect to the effects of IL-1β on transporters, a correlation with those of OSM was observed; this likely indicate some partial interrelationships or crosstalks between IL-1β-signaling pathways and those linked to OSM with respect to hepatic transporter regulation. The fact that at least MAPKs and STAT3 can be activated by both OSM and IL-1β may support this hypothesis [15,31,33].

The putative physiological and pathological relevance of OSM effects towards human hepatic transporter expression remains to be precisely described. OSM exposure is however likely to result in reduced sinusoidal uptake of drugs, owing to its global repressing effect towards influx SLC transporters. In addition, OSM-mediated down-regulation of ABCG2 expression may contribute to decreased biliary secretion of drugs such as the antibiotic nitrofurantoin handled by this canalicular ABC pump [34]. Associated with the known repression of drug metabolizing CYP activity triggered by OSM, these putative alterations of hepatic drug transport may contribute to alteration of pharmacokinetics. Such impairment of drug disposition may have notably to be considered in subjects exhibiting elevated circulating levels of OSM, such as preeclamptic

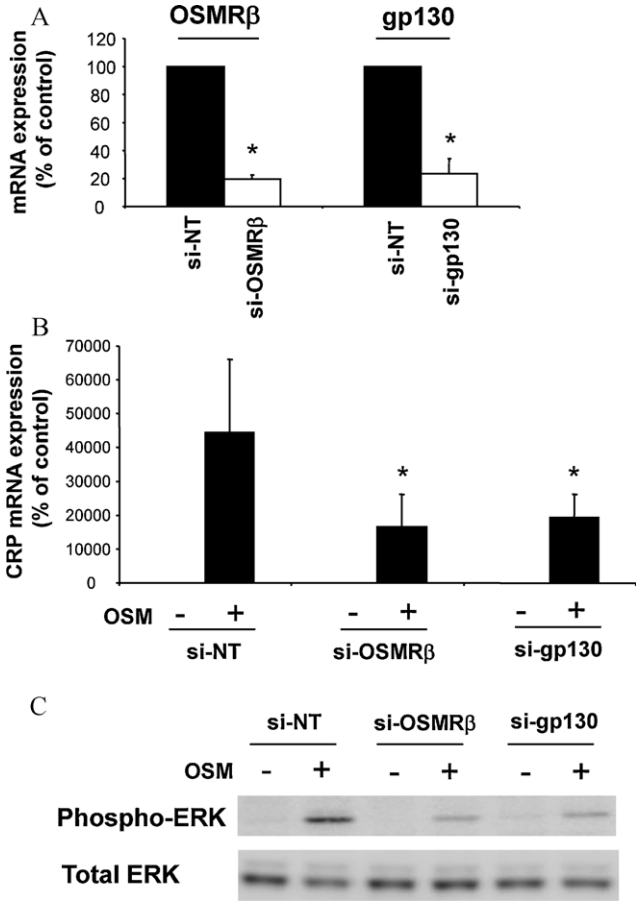

Fig. 6. Functional repression of OSMRβ and gp130 subunits of the type II OSM receptor by siRNA mediated transfection. HepaRG cells were transfected with non-targeting siRNAs (si-NT) or with siRNAs targeting OSMRβ (si-OSMRβ) or gp130 (si-gp130) as described under Section 2. (A) Expression of OSMRβ and gp-130 mRNAs was then determined by RT-qPCR. Data are expressed as percentage of expression found in control si-NT-transfected cells, arbitrarily set at the value of 100%, and are the means ± SD of values from four independent experiments. $^{*}p < 0.05$ when compared to si-NT-transfected cells (Student's *t* test). (B) Cells were exposed to 10 ng/ml OSM for 24 h. CRP mRNA expression was then determined by RT-qPCR; data are expressed as percentage of expression found in control OSM-untreated si-NT-transfected cells, arbitrarily set at the value of 100%, and are the means ± SD of values from four independent experiments. $^{*}p < 0.05$ when compared to OSM-treated si-NT-transfected cells (Student–Newman–Keuls test). (C) Cells were exposed to 10 ng/ml OSM for 30 min. Phospho-ERK and total ERK contents were next analyzed by Western-blot. Data shown are representative of two independent experiments.

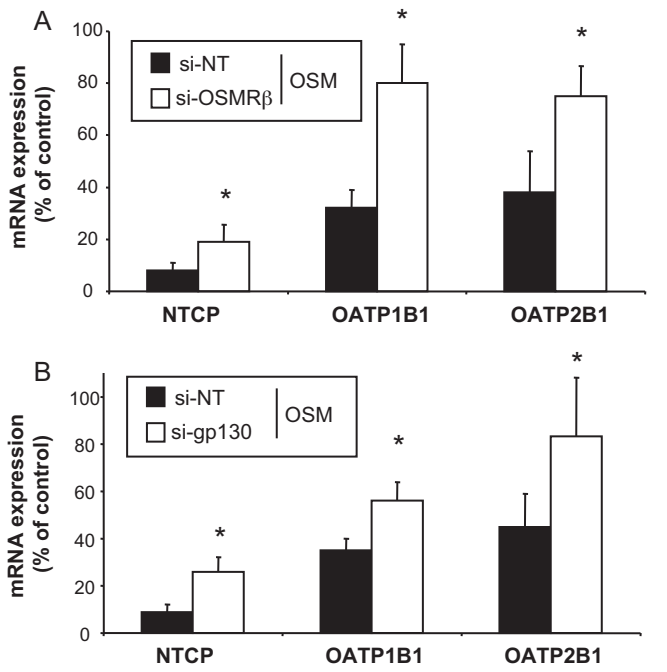

Fig. 7. Effects of OSMRβ and gp130 subunit knock-down on OSM-mediated repression of drug transporters. HepaRG cells, transfected with non-targeting siRNAs (si-NT) or with siRNAs targeting OSMRβ (si-OSMRβ) or gp130 (si-gp130) subunits of the type II OSM receptor, were exposed to 10 ng/ml OSM for 24 h. Expression of the transporters NTCP, OATP1B1 and OATP2B1 was then determined by RT-qPCR in si-OSMRβ- (A) and si-gp130- (B) transfected cells, as described under Section 2; data are expressed as percentage of expression found in control OSM-untreated si-NT-transfected cells, arbitrarily set at the value of 100%, and are the means ± SD of values from five independent experiments. $^{*}p < 0.05$ when compared to OSM-treated si-NT transfected cells (Student's *t* test).

pregnant women [35] or patients suffering from rheumatoid arthritis [36], systemic lupus erythematosus [37], periodontal diseases [38] or malignant hemopathies [39]. In this context, it is noteworthy that patients with these diseases may display serum OSM levels up to 1 ng/ml [35], which are therefore in the range of OSM concentrations exhibiting repressing activity on hepatic transporters *in vitro* (Fig. 2); this supports the idea that OSM serum levels up-regulated in some diseases may be sufficient to affect *in vivo* expression of drug transporters. Moreover, elevated hepatic expression of OSM has been reported in liver-injury conditions and cirrhosis [5,40] and this *in situ* hepatic production of OSM, in association with the up-regulation of other hepatic inflammatory cytokines such as IL-1β, TNF-α and IL-6, may therefore directly contribute to the decreased hepatobiliary secretion of drugs observed in these pathological situations [41]. In addition, the repressing effects of OSM towards NTCP expression and activity may lead to alteration of enterohepatic cycle of bile acids through impairment of their NTCP-mediated reuptake from blood at the sinusoidal pole of hepatocytes and, by this way, may contribute to cholestasis. Impairment of the biliary elimination of other endogeneous substrates for hepatic transporters, such as bilirubin handled by OATPs [42], may have also to be considered. Finally, it is noteworthy that OSM has been shown to impact brain endothelial cells and kidney tubular cells, which are well known to play a major role in drug disposition and elimination [30,43,44]; whether OSM may alter expression of drug transporters in these cells would likely deserve further studies.

In conclusion, the current data demonstrate that OSM down-regulates expression of drug transporters in human hepatocytes, especially that of sinusoidal uptake SLC transporters. Such changes may likely contribute to alterations of pharmacokinetics in patients suffering from diseases associated with increased production of OSM.

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