Differential Regulation of Drug Transporter Expression by Hepatocyte Growth Factor in Primary Human Hepatocytes

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ABSTRACT:

Hepatocyte growth factor (HGF) is known to down-regulate expression of drug-detoxifying proteins such as cytochromes P450 (P450s) in human hepatocytes. The present study was designed to determine whether HGF may also impair expression of uptake and efflux drug transporters, which constitute important determinants of the liver detoxification pathway, such as P450s. Exposure of primary human hepatocytes to 20 ng/ml HGF for 48 h was found to down-regulate mRNA levels of major sinusoidal uptake transporters, including sodium taurocholate-cotransporting polypeptide (NTCP), organic anion-transporting polypeptide (OATP) 2B1, OATP1B1, organic cation transporter (OCT) 1, and organic anion transporter 2. HGF concomitantly reduced NTCP, OATP2B1, and OATP1B1 protein expression and NTCP, OATP, and OCT1 transport activities. With respect to efflux pumps, HGF decreased mRNA expression of the canalicular bile salt export pump, whereas that of the multidrug resistance (MDR) 1 gene was transiently increased. Moreover, Western blot analysis indicated that HGF up-regulated expressions of MDR1/P-glycoprotein and breast cancer resistance protein in human hepatocytes, whereas those of multidrug resistance gene-associated protein (MRP) 2 and MRP3 were unchanged. However, HGF prevented constitutive androstane receptor-related up-regulation of MRP2 occurring in phenobarbital-treated hepatocytes. Taken together, these data demonstrate that HGF differentially regulates transporter expression in human hepatocytes, i.e., it represses most of the sinusoidal uptake transporters, whereas expression of most of the efflux transporters is unchanged or increased. Such changes probably contribute to alterations of pharmacokinetics in patients with diseases associated with increased plasma levels of HGF such as fulminant hepatitis.

Hepatocyte growth factor (HGF) is a heterodimeric molecule composed of a 69-kDa α-chain and a 34-kDa β-chain, known to be the ligand of the c-met membrane receptor tyrosine kinase and initially characterized as a potent stimulator of DNA synthesis in hepatocytes (Nakamura et al., 1989; Rubin et al., 1993). It is therefore thought to contribute in a major way to liver regeneration and, consistent with such a role, its plasma levels are increased in patients with fulminant hepatic failure or liver cirrhosis (Tsubouchi et al., 1991). In addition to this mitogenic role, HGF has been shown to regulate expression of various genes, such as the cytochrome P450 (P450) 7A1 gene, encoding the cholesterol 7α-hydroxylase involved in bile acid synthesis (Song et al., 2007). P450s involved in drug detoxification have also been demonstrated to be regulated by HGF (Kakizaki et al., 2007). HGF treatment thus resulted in decreased expression of CYP1A2 and CYP3A4 at both mRNA and protein levels in primary human hepatocytes (Donato et al., 1998); CYP1A2, CYP3A4, and also CYP2B6 activities were concomitantly decreased, suggesting reduced metabolism of drugs administered during the course of human liver diseases associated with up-regulation of HGF production (e.g., viral or toxic fulminant hepatitis). Down-regulation of detoxication capacity has been similarly observed in rats during liver regeneration after partial hepatectomy (Habib et al., 1994), a physiopathological situation well known to be associated with increased production of HGF (Lindroos et al., 1991).

Besides P450s, hepatic drug transporters, which constitute important determinants of the liver detoxification pathway (Funk, 2008), may be targets for HGF. Indeed, sinusoidal uptake solute-carrier (SLC) transporters such as sodium taurocholate-cotransporting polypeptide (Ntcp) (Slc10a1), organic anion-transporting polypeptide (Oatp) 1a1 (Slco1a1), and Oatp2 (Slco1a2) have been shown to be down-regulated in HGF-positive proliferating rat liver after partial hepatectomy (Gerloff et al., 1999), whereas, in contrast, the canalicular ATP-binding cassette (ABC) transporter multidrug resistance (Mdr) 1b gene (Abcb1b)/P-glycoprotein is up-regulated (Vos et al., 1999). Moreover, HGF overexpression in mice led to decreased expression of the sinusoidal uptake transporters organic anion trans-
porter (Oct) 1 (Slc22a1) (Kakizaki et al., 2007). This putative regulation of hepatic drug transporter expression by HGF remains poorly characterized, however, especially in human hepatocytes. Therefore, the present study was designed to gain insights about this point, using primary human hepatocytes, which are well recognized as a convenient model to study regulatory ways of drug detoxification proteins (Gomez-Lechon et al., 2004). Our data indicate that exposure to HGF differentially regulates drug transporters in human hepatocytes, i.e., most of the uptake SLC transporters, unlike the majority of efflux ABC transporters, were down-regulated. Such changes may contribute to alterations of pharmacokinetics in humans with diseases associated with high plasma levels of HGF.

Materials and Methods

Chemicals and Reagents. Recombinant human HGF was provided by R&D Systems (Minneapolis, MN). [3H(G)]Taurocholic acid (specific activity 2.4 mCi/mmol), and [1-14C]TEA (specific activity 2.4 mCi/mmol) were purchased from Connaught Life and Analytical Sciences (Waltham, MA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA). All other compounds and antibodies against MDR1/P-glycoprotein and breast cancer resistance protein (BCRP) (ABCG2) were from Alexis Biochemicals (Lausen, Switzerland), whereas those against MRP2 and MRP3 were provided by Millipore Bioscience Research Reagents (Temecula, CA).

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 (Jigorel et al., 2005). Hepatocytes were then seeded on plastic dishes at a density of 2 x 10^5 cells/cm^2 in Williams’ E medium (In Vitrogen, Cergy-Pontoise, France), supplemented with 10% fetal calf serum (Perbio Sciences, Pontoise, France), supplemented with 10% fetal calf serum (Perbio Sciences, Pontoise, France), 5 μg/ml bovine insulin (Sigma-Aldrich), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (In Vitrogen). After 24 h, this seeding medium was discarded, and primary hepatocytes were routinely cultured in the fetal calf serum-containing Williams’ E medium defined as above and supplemented with 5 x 10^-5 M hydrocortisone hemisuccinate (Upjohn, Paris La Défense, France) and 2% dimethyl sulfoxide, as reported previously (Chouteau et al., 2001; Le Vee et al., 2008). All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee.

RNA Isolation and Analysis. Total RNA was isolated from cells using TRIzol reagent (In Vitrogen). RNA was then subjected to real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) using fluorescent dye SYBR Green methodology and an ABI Prism 7300 detector (Applied Biosystems, Foster City, CA), as reported previously (Jigorel et al., 2005). Gene primers for drug transporters, CYP2B6 and 18S RNA were exactly as described previously (Jigorel et al., 2005). Hepatocytes were then seeded on plastic dishes at a density of 2 x 10^5 cells/cm^2 in Williams’ E medium (In Vitrogen, Cergy-Pontoise, France), supplemented with 10% fetal calf serum (Perbio Sciences, Pontoise, France), supplemented with 10% fetal calf serum (Perbio Sciences, Pontoise, France), 5 μg/ml bovine insulin (Sigma-Aldrich), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (In Vitrogen). After 24 h, this seeding medium was discarded, and primary hepatocytes were routinely cultured in the fetal calf serum-containing Williams’ E medium defined as above and supplemented with 5 x 10^-5 M hydrocortisone hemisuccinate (Upjohn, Paris La Défense, France) and 2% dimethyl sulfoxide, as reported previously (Chouteau et al., 2001; Le Vee et al., 2008). All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee.

FIG. 1. Effects of HGF on CYP7A1 and HIF-1α mRNA expression in human hepatocytes. Primary human hepatocytes were either untreated (UNT) or exposed to 20 ng/ml HGF for 8, 24, or 48 h. CYP7A1 (top panel) and HIF-1α (bottom panel) mRNA expression was then determined by RT-qPCR, as described under Materials and Methods. Data are expressed for each transporter as a percentage of expression found in untreated hepatocytes, arbitrarily set at the value of 100%, and are the means ± S.E.M. of values from six independent hepatocyte populations. * p < 0.05 compared with untreated cells.

FIG. 2. Effects of HGF on drug transporter mRNA expression in human hepatocytes. Primary human hepatocytes were either untreated or exposed to 20 ng/ml HGF for 8, 24, or 48 h. SLC (A) and ABC (B) transporter mRNA expression was then determined by RT-qPCR, as described under Materials and Methods. Data are expressed for each transporter as a percentage of expression found in untreated hepatocytes, arbitrarily set at the value of 100%, and are the means ± S.E.M. of values from six independent hepatocyte populations. * p < 0.05 compared with untreated cells.
Western Blot Analysis. Crude membrane extracts were prepared from primary human hepatocytes as described previously (Jigorel et al., 2006). 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 NTCP (SLC10A1) (Kullak-Ublick et al., 1997), OATP2B1 (SLCO2B1), OATP1B1 (SLCO1B1) (Zollner et al., 2003), MDR1 (ABCB1), MRP2 (ABCC2), MRP3 (ABCC3), or BCRP (ABCG2). Peroxidase-conjugated monoclonal antibodies were thereafter used as secondary antibodies. After washing, immunolabeled proteins were visualized by chemiluminescence. Gel loading and transfer were checked by staining membranes with Ponceau red. The intensities of stained bands were measured.

Fig. 3. Dose response of HGF effects toward transporter expression. Primary human hepatocytes were either untreated or exposed to various concentrations of HGF (from 0.005 to 50 ng/ml) for 48 h. Transporter and CYP7A1 mRNA expression was then determined by RT-qPCR, as described under Materials and Methods. Data are expressed for each transporter and for CYP7A1 as a percentage of expression found in untreated hepatocytes, arbitrarily set at the value of 100%, and are the means ± S.E.M. of values from four independent hepatocyte populations. *, p < 0.05 compared with untreated cells.

Fig. 4. Effects of HGF on drug transporter protein expression in human hepatocytes. Primary human hepatocytes were either untreated (UNT) or exposed to 20 ng/ml HGF for 48 h. Transporter protein content was then determined by Western blot analysis, followed, for each transporter, by densitometric analysis and expressed relative to transporter expression found in untreated cells, arbitrarily set at the value of 100% and indicated by a dotted line (top panel); data are the means ± S.E.M. of values from six independent hepatocyte populations. *, p < 0.05 compared with untreated cells. P-gp, MDR1/P-glycoprotein. Representative blots from one hepatocyte population are also shown for each transporter (bottom panel).
**Results**

**Effects of HGF Treatment on Expression of Referent HGF Target Genes.** Primary human hepatocytes from six liver donors were exposed to HGF for 8, 24, or 48 h. HGF was first used at a concentration (20 ng/ml) close to those used for treating primary human hepatocytes (Donato et al., 1998; Song et al., 2007; Barreiros et al., 2009); this HGF concentration did not exert toxicity as demonstrated by phase-contrast microscopic examination of the cultures and analysis of cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay (data not shown). As shown in Fig. 1, HGF markedly repressed CYP7A1 mRNA expression and concomitantly induced HIF-1α mRNA levels at all three time intervals. Because CYP7A1 and HIF-1α can be considered as prototypical markers known to be down-regulated or up-regulated by HGF, respectively (Tacchini et al., 2004; Song et al., 2007), primary human hepatocytes were responsive to HGF and thus suitable for investigating HGF effects toward drug transporter expression.

**Effects of HGF Treatment on Drug Transporter mRNA Expression.** The effects of HGF treatment on SLC transporter mRNA expression were first analyzed by RT-qPCR. As shown in Fig. 2A, HGF-treated hepatocytes from six individuals exhibited lower mRNA expression of NTCP, OATP2B1, OATP1B1, OCT1, and OAT 2 (SLC22A7) compared with that for untreated counterparts at all times of treatment (8, 24, or 48 h). In contrast, mRNA levels of OATP1B3 (SLCO1B3) remained unchanged. With respect to OATP2B1 and OCT2, the time of exposure did not significantly affect the level of repression in a major way; i.e., similar reduced levels of transporter expression were found in hepatocytes exposed to HGF for 48 or 48 h (p > 0.05). In contrast, expressions of NTCP, OATP1B1, and OCT1 in hepatocytes treated with HGF for 48 h were lower than those found in counterparts exposed to the cytokine for only 8 h (p < 0.05), probably indicating that repression increased with exposure time for these transporters. When the repression factor after a 48-h exposure to HGF was considered, i.e., the ratio of mRNA levels in untreated hepatocytes versus those found in treated counterparts, OCT1 was the most repressed (by a 10.6-fold factor) among the transporters affected by HGF, followed by OATP1B1 (repression by a 3.8-fold-factor), NTCP (repression by a 3.7-fold factor), OAT2 (repression by a 2.9-fold factor), and OATP2B1 (repression by a 2.8-fold factor).

The effects of HGF treatment on ABC transporter mRNA expression were next studied. As shown in Fig. 2B, expression of BCRP, MRP2, MRP3, and MRP4 (ABCC4) remained unchanged in hepatocytes exposed to HGF for 8, 24, or 48 h, compared with that for untreated counterparts. In contrast, HGF down-regulated BSEP (ABCB11), whatever the time of exposure; BSEP mRNA levels in hepatocytes exposed to HGF for 48 h were thus reduced by a 4.5-fold factor compared with those for untreated cells. HGF treatment was also found to induce MDR1 mRNA levels by a 2-fold factor after an 8-h exposure to HGF (Fig. 2B); however, this up-regulation of MDR1 expression was transient because longer exposure to HGF (24 and 48 h) failed to alter MDR1 mRNA levels.

The dose-response relationship for HGF effects toward transporter expression was then characterized. For this purpose, we only analyzed transporters whose mRNA expression was decreased by at least 50% in human hepatocytes exposed to 20 ng/ml HGF for 48 h, i.e., NTCP, OATP2B1, OATP1B1, OCT1, OAT2, and BSEP (Fig. 2). These transporters were similarly down-regulated by 50 ng/ml HGF (Fig. 3). HGF used at 5 ng/ml also significantly repressed transporter expression, although in a more moderate manner for NTCP, OATP2B1, OATP1B1, and OAT2 (Fig. 3). Lower concentrations of HGF such as 0.005, 0.05, or 0.5 ng/ml failed to obviously alter expression of...
transporters; they also did not affect CYP7A1 mRNA levels, which were, in contrast, markedly reduced by higher concentrations of HGF (Fig. 3).

Effects of HGF Treatment on Drug Transporter Protein Expression. To determine whether some of the changes in transporter mRNA levels induced by HGF treatment also occur at the protein level, Western blot analysis of crude membranes from HGF-treated primary human hepatocytes and untreated counterparts was performed. As indicated in Fig. 4, HGF treatment for 48 h reduced NTCP, OATP2B1, and OATP1B1 levels. In contrast, HGF induced P-glycoprotein and BCRP expression but failed to obviously alter MRP2 and MRP3 expression (Fig. 4).

Effects of HGF Treatment on Transporter Activities. Owing to the limited availability of human hepatocytes, we focused on the effects of HGF treatment on activities of NTCP, OATPs, and OCT1, whose mRNA expressions were among the most repressed by HGF (Fig. 2A). As shown in Fig. 5, exposure to HGF for 48 h resulted in decreased NTCP, OATP, and OCT1 transport activity in human primary hepatocytes, compared with that for untreated counterparts.

Correlation Analysis of the Repressing Effects of HGF, TNF-α, and IL-6 on Drug Transporter mRNA Expression in Human Hepatocytes. TNF-α and IL-6 are proinflammatory cytokines whose plasma levels are increased and correlated with up-regulated HGF levels during fulminant hepatic failure (Sekiyama et al., 1994) and have been previously demonstrated to repress various hepatic drug transporters (Le Vee et al., 2009). To search for a putative correlation between these alterations of drug transporter expression occurring in primary human hepatocytes exposed to HGF, TNF-α, or IL-6, drug transporters were ranked according to the down-regulation of their mRNA expression in response to a 48-h treatment by these soluble factors; data used for HGF effects were from the present study, whereas those for TNF-α and IL-6 effects were from Le Vee et al. (2009) and were obtained by using primary human hepatocytes cultured in the same conditions as those used in the present study. For each treatment, transporters were ranked from the most repressed transporter to the least repressed, according to mRNA expression levels. Correlations were analyzed by using Spearman’s rank correlation method. Results indicated that the effects of HGF were significantly correlated with those of TNF-α (Fig. 6). In contrast, HGF effects on drug transporter expression were not correlated with those of IL-6 (Fig. 6).

Effect of HGF Treatment on Phenobarbital-Mediated Regulation of Drug Transporters. Because HGF has been previously shown to alter regulation of some P450s in response to chemical inducers (Donato et al., 1998), whether HGF may also act on xenobiotic-mediated regulation of transporters was investigated. For this purpose, we focused on phenobarbital-related induction of ABC transporters because 1) phenobarbital is one of the chemical inducers whose effects toward P450s, especially Cyp2b10, is impaired by HGF (Koike et al., 2007), 2) the xenobiotic receptor CAR, which is thought to mediate many of the effects of phenobarbital, has been shown to be down-regulated by HGF in mouse hepatocytes and human hepatoma
cells (Kakizaki et al., 2007; Osabe et al., 2008), and 3) phenobarbital appears to be one of the most robust inducers of drug transporters such as MDR1, MRP2, and BCRP in primary human hepatocytes (Jigorel et al., 2006). HGF was first demonstrated to repress mRNA expression of CAR in primary human hepatocytes by a 3.8-fold factor (Fig. 7A), which agrees with previous data from human hepatoma HepG2 cells (Osabe et al., 2008). HGF was next shown to markedly inhibit induction of the CAR prototypical target CYP2B6 occurring in phenobarbital-exposed human hepatocytes (Fig. 7B), therefore demonstrating that HGF impairs CAR-related regulatory ways of detoxifying proteins in primary human hepatocytes. HGF also decreased basal levels of CYP2B6 mRNAs (Fig. 7B), which probably has to become integrated into the overall repressing action of HGF toward P450 expression (Donato et al., 1998). Last, the effects of HGF on phenobarbital-mediated induction of MDR1, MRP2, and BCRP mRNA expression were investigated. As shown in Fig. 8, HGF failed to alter phenobarbital-related up-regulation of MDR1 and BCRP but inhibited concomitant induction of MRP2 mRNA levels.

Discussion

The data reported in the present study demonstrate that HGF can markedly alter drug transporter expression in human hepatocytes, especially after a 48-h treatment, as summarized in Table 1. In particular, mRNA levels of uptake transporters NTCP, OATP2B1, OATP1B1, OCT1, and OCT2 are decreased in response to HGF treatment in a dose-dependent manner; moreover, some of these transporters, i.e., NTCP, OATP2B1, and OATP1B1, are also down-regulated at protein levels and NTCP, OATP, and OCT1 transport activities are concomitantly reduced. Taken together, these data indicate that these sinusoidal uptake transporters probably constitute major targets of HGF in human hepatocytes; they may also similarly represent putative targets of HGF in rodent hepatocytes because some of them have been reported to be down-modulated in regenerating rat liver, a physiopathological situation associated with up-regulation of HGF (Gerloff et al., 1999) or in liver of HGF-overexpressing mice (Kakizaki et al., 2007). However, it should be kept in mind that OATP1B3 mRNA levels were not altered in HGF-treated human hepatocytes; likewise, HGF did not change Oatp1 mRNA expression in primary rat hepatocytes (Iwakiri et al., 2008). Taken together, these data thus demonstrate that HGF impairs expression of a majority, but not the totality, of uptake SLC transporters in hepatocytes.

Besides its action on uptake transporters, HGF also acts on expression of efflux ABC transporters in human hepatocytes but in a different and more limited manner (Table 1). BSEP and MDR1 were thus the only ABC efflux transporters whose mRNA expressions were altered by HGF, i.e., down-regulated (for BSEP) or transiently up-regulated (for MDR1). In addition, BCRP protein expression was induced in HGF-exposed human hepatocytes, as it was for P-glycoprotein expression. In contrast, basal expression of MRP2, a major

![Fig. 7. Effects of HGF on CAR mRNA expression (A) and phenobarbital-related induction of the CAR target CYP2B6 (B).](image-url)

![Fig. 8. Effects of HGF on phenobarbital-related induction of the ABC transporters MDR1, BCRP, and MRP2. Primary human hepatocytes were either untreated (UNT) or exposed to 20 ng/ml HGF for 48 h and then were treated or not with 3.2 mM phenobarbital (PB) in the presence or absence of HGF for an additional 24 h. CYP2B6 mRNA expression was then determined by RT-qPCR, as described under Materials and Methods. Data are expressed as a percentage of expression found in untreated hepatocytes, arbitrarily set at the value of 100%, and are the means ± S.E.M. of values from four independent hepatocyte populations. *p < 0.05. NS, not significant.)](image-url)
canalicular efflux transporter handling organic anions (Keppler, 1999), was not altered by HGF at either mRNA and protein levels. MRP2 levels have also been shown not to be altered during rat liver regeneration after partial hepatectomy, whereas Mrd1b/P-glycoprotein expression was induced (Gerloff et al., 1999; Vos et al., 1999), thus fully supporting the conclusion that these hepatic ABC transporters are differentially sensitive to growth factors. MRP3, located at the basolateral pole of hepatocytes and thought to be involved in back transport of drugs from hepatocytes to blood (Borst et al., 2006), was also not targetted by basal HGF.

Although basal MRP2 expression was not impaired by HGF, phenobarbital-mediated induction of MRP2 was prevented by exposure of human hepatocytes to HGF. These data most likely illustrate the fact that HGF down-regulates the CAR-related signaling pathway, which has been shown to be involved in phenobarbital-mediated MRP2 induction in hepatocytes (Kast et al., 2002). This down-regulation of the CAR-related regulatory pathway in primary human hepatocytes, clearly demonstrated by the inhibition of phenobarbital-mediated up-regulation of the CAR prototypical target CYP2B6, is probably due to the repression of CAR expression by HGF, initially reported in human hepatoma HepG2 cells (Osabe et al., 2008) and now extended to primary normal human hepatocytes in the present study. In addition, HGF may also impair the CAR-related pathway through inhibiting ligand-induced nuclear accumulation of this drug-sensing receptor, as recently described in mouse primary hepatocytes (Koike et al., 2007).

In contrast with that of MRP2, phenobarbital-mediated inductions of MDR1 and BCRP were not altered by HGF in primary human hepatocytes. These data therefore produce an indirect argument for discarding a role for CAR in induction of these ABC transporters by phenobarbital. Such CAR-independent effects of phenobarbital have also been reported for the up-regulation of Mrp3 by phenobarbital in mice (Xiong et al., 2002; Cherrington et al., 2003). However, it is noteworthy that MRP3 is not induced by phenobarbital in primary human hepatocytes (Jigorel et al., 2006; Richert et al., 2009), thus suggesting species-dependent regulation of this transporter.

Cellular and molecular mechanisms involved in HGF-related regulation of transporters in human primary hepatocytes remain to be determined. Transcriptional mechanisms, including suppression in transcription and changes in binding activities of transporter gene transactivators or coactivators in response to HGF, may be involved, as already suggested for cytokine-mediated regulation of hepatic organic anion transporter in mouse liver (Geier et al., 2005). In addition, post-transcriptional mechanisms have to be considered, especially for the up-regulation of BCRP expression. Such post-transcriptional mechanisms have already been hypothesized to play a major role in some transporter regulation, especially that of MRP2 and BSEP, down-regulated in response to lipopolysaccharide in human liver slices (Elferink et al., 2004) and that of MRP3 and BCRP, up-regulated in response to TNF-α in primary human hepatocytes (Le Vee et al., 2009). It is interesting to note that an HGF effect on the transporter expression profile was found to be significantly correlated to that of TNF-α, using rank correlation analysis. This finding may suggest some inter-relationships or cross-talk between HGF-signaling pathways and those linked to TNF-α in human hepatocytes. The fact that HGF and TNF-α similarly repress CYP7A1, CYP1A2, and CYP3A4 expressions (Abdel-Razzak et al., 1993; Donato et al., 1998; De Fabiani et al., 2001; Song et al., 2007) may argue in favor of this hypothesis. In contrast, HGF and IL-6 effects toward transporters were demonstrated not to be correlated, thus favoring the idea that they mobilized independent signaling pathways.

The physiological and pathological relevance of HGF effects to human hepatic transporter expression remains to be precisely described. However, HGF exposure is likely to decrease sinusoidal uptake of drugs, due to its global repressing effect on uptake transporters. In contrast, biliary efflux of drugs may be preserved (for drugs handled by MRP2) or even enhanced (for drugs handled by P-glycoprotein or BCRP). Taken together, these data indicate that HGF may reduce xenobiotic accumulation in hepatocytes, through inhibiting influx, whereas efflux remains constant or is increased. This transport regulation may be considered as a protective mechanism by which HGF-stimulated hepatocytes, which are presumed to replicate, decrease uptake and accumulation of foreign chemicals during their proliferation, a physiological stage known to be very sensitive to xenobiotics, especially genotoxic compounds. Such HGF-induced alterations of the drug transport process may therefore contribute to the resistance to hepatotoxins acquired by proliferating hepatocytes (Roberts et al., 1983), as already suggested for mdr gene up-regulation occurring during rat liver regeneration (Thorgeirsson et al., 1987). In the same way, the HGF-mediated down-regulation of NTCP and BSEP, involved in sinusoidal uptake and canalicular secretion of bile acids, respectively (Trauner and Boyer, 2003), and associated with the repression of bile acid synthesis due to concomitant CYP7A1 inhibition (Song et al., 2007), may have the aim of protecting the regenerating liver from accumulating toxic bile acids. Finally, it is noteworthy that HGF effects on hepatic transporter expression could have major pharmacokinetic consequences for patients exhibiting pathological states, especially fulminant hepatitis but also chronic hepatitis. Indeed, HGF plasma levels in these patients can reach values (5–10 ng/ml) (Tsubouchi et al., 1991) that are in the range of HGF concentrations affecting drug transporter expression (Fig. 3). This finding suggests that hepatocytes from such patients exhibit HGF-mediated repression of uptake transporters associated with concomitant down-regulation of P450 expression, which is likely to result in an overall decrease in the drug detoxification capacity of the liver. Such a down-regulation of the hepatic drug elimination pathway may moreover be reinforced by the up-regulation of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, which occur during fulminant hepatitis (Muto et al., 1988; Sekiyama et al., 1994) and which are presumed to also contribute to repression of both drug uptake transporters and P450s in human hepatocytes (Abdel-Razzak et al., 1993; Le Vee et al., 2008, 2009).

In conclusion, the current data demonstrate that HGF differentially regulates transporter expression in human hepatocytes, i.e., it markedly represses most of the uptake of SLC transporters, whereas expression of most of the efflux transporters is unchanged or increased. Such changes probably contribute to alterations of pharma-

### Table 1
Summary of the effects of HGF on human hepatic transporter expression

<table>
<thead>
<tr>
<th>Transporter</th>
<th>mRNA</th>
<th>Protein</th>
<th>Activity</th>
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<tbody>
<tr>
<td>MDR1</td>
<td>↔</td>
<td>↑</td>
<td>N.D.</td>
</tr>
<tr>
<td>BSEP</td>
<td>↓</td>
<td>N.D.</td>
<td>N.D.</td>
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<tr>
<td>BCRP</td>
<td>↔</td>
<td>↑</td>
<td>N.D.</td>
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<tr>
<td>MRP2</td>
<td>↔</td>
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<td>N.D.</td>
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<tr>
<td>MRP3</td>
<td>↔</td>
<td>↔</td>
<td>N.D.</td>
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<tr>
<td>MRP4</td>
<td>↓</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>NTCP</td>
<td>↓</td>
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<td>↓</td>
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<tr>
<td>OATP2B1</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>↑</td>
<td>N.D.</td>
<td>↑</td>
</tr>
<tr>
<td>OCT1</td>
<td>↓</td>
<td>N.D.</td>
<td>↓</td>
</tr>
<tr>
<td>OCT2</td>
<td>↓</td>
<td>N.D.</td>
<td>↓</td>
</tr>
</tbody>
</table>

* ↔, no change; ↓, significant induction; ↑, significant repression; N.D., not done.
* Activity was measured using the pan-OATP substrate estrone-3-sulfate and may therefore not reflect specific OATP2B1, OATP1B1, or OATP1B3-mediated transport.

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References


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