Differential Regulation of Sinusoidal and Canaliculare Hepatic Drug Transporter Expression by Xenobiotics Activating Drug-Sensing Receptors in Primary Human Hepatocytes

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ABSTRACT:

Sinusoidal and canalicular hepatic drug transporters constitute key factors involved in drug elimination from liver. Regulation of their expression via activation of xenosensors, such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), and nuclear factor E2-related factor 2 (Nrf2), remains incompletely characterized. The present study was therefore designed to carefully analyze expression of major drug transporters in primary human hepatocytes exposed to dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) (an AhR activator), rifampicin (RIF) (a PXR activator), phenobarbital (PB) (a CAR activator), and oltipraz (OPZ) (a Nrf2 activator), using mainly reverse transcription-real time polymerase chain reaction assays. With a threshold corresponding to a 1.5-fold factor change in mRNA levels, observed in at least three of seven independent human hepatocyte cultures, efflux transporters such as MDR1, MRP2 and BCRP were up-regulated by PB, RIF, and OPZ, whereas MRP3 was induced by OPZ and RIF. MDR1 and BCRP expression was also increased by TCDD- and RIF-augmented mRNA levels of the influx transporter OATP-C. Bile acid transporters, i.e., bile salt export pump and Na+-taurocholate cotransporting polypeptide, and the sinusoidal transporter, OAT2, were down-regulated by all the tested chemicals. Influx transporters such as OCT1, OATP-B, and OATP8 were repressed by PB and TCDD. PB also decreased MRP6 expression, whereas mRNA levels of OCT1 and OATP8 were down-regulated by RIF and OPZ, respectively. Taken together, these data establish a complex pattern of transporter regulation by xenobiotics in human hepatocytes, in addition to interindividual variability in responsiveness. This may deserve further attention with respect to drug-drug interactions and adverse effects of hepatic drugs.

Hepatic drug transporters constitute important factors in the hepatobiliary elimination of xenobiotics (Chandra and Brouwer, 2004). They belong to the solute carrier (SLC) or the ATP-binding cassette (ABC) superfamilies of transporters (Schinkel and Jonker, 2003). SLC transporters, especially organic cation transporter 1 (OCT1/SLC22A1) (Jonker and Schinkel, 2004), organic anion transporting polypeptides (OATP-B/SLCO2B1, OATP-C/SLCO1B1, and OATP8/SLCOIB3) (Hagenbuch and Meier, 2003), and organic anion transporter 2 (OAT2/SLC22A7) (Kobayashi et al., 2005), located at the sinusoidal membrane of hepatocytes, mediate the uptake of endogenous and foreign compounds from blood. Canalicular ABC transporters, such as P-glycoprotein (ABC1) encoded by multidrug resistance 1 (MDR1) gene, multidrug resistance protein 2 (MRP2/ABC2), MRP6 (ABC6), and breast cancer resistance protein (BCRP/ABCG2), are involved in secretion of drugs or their metabolites into bile (Schinkel and Jonker, 2003; Fardel et al., 2005). The efflux pump MRP3 (ABCC3) is located at the sinusoidal pole, where it is thought to mediate secretion of drug metabolites into the bloodstream for subsequent urinary elimination (Zelcer et al., 2005). Hepatic transport of bile acids is mainly mediated by the SLC transporter Na+-taurocholate cotransporting polypeptide (NTCP/SLC10A1) and the ABC transporter bile salt export pump (BSEP/ABCB11), located at the sinusoidal and canalicular membranes of hepatocytes, respectively (Trauner and Bouyer, 2003). BSEP also transports xenobiotics such as pravastatin (Hirano et al., 2005).

Like drug-metabolizing enzymes, hepatic transporters have been shown to be regulated by xenobiotic receptors acting as drug sensors (Klaassen and Slitt, 2005). Rifampicin (RIF), activating pregnane X receptor (PXR), phenobarbital (PB), activating constitutive androstane receptor (CAR), and oltipraz (OPZ), activating nuclear factor E2-related factor 2 (Nrf2), have thus been found to induce expression of MRP2 in human liver cells (Kauffmann et al., 2002; Fardel et al., 2005). Their expression, whereas mRNA levels of OCT1 and OATP8 were down-regulated by RIF and OPZ, respectively. Taken together, these data establish a complex pattern of transporter regulation by xenobiotics in human hepatocytes, in addition to interindividual variability in responsiveness. This may deserve further attention with respect to drug-drug interactions and adverse effects of hepatic drugs.

ABBREVIATIONS: SLC, solute carrier; ABC, ATP binding cassette; AhR, aryl hydrocarbon receptor; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CAR, constitutive androstane receptor; MDR, multidrug resistance; MRP, multidrug resistance protein; NQO1, NAD(P)H quinine oxidoreductase 1; Nrf2, nuclear factor E2-related factor 2; NTCP, Na+-taurocholate cotransporting polypeptide; OCT, organic cation transporter; OATP, organic anion-transporting polypeptide; OAT, organic anion transporter; OPZ, oltipraz; PB, phenobarbital; PXR, pregnane X receptor; RIF, rifampicin; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.
2005; Piton et al., 2005). PXR-activating drugs also enhance P-glycoprotein levels (Geick et al., 2001) and expression of the mouse Oatpl4a1 (Cheng et al., 2005). These results suggest a coordinate regulation between some transporters, like P-glycoprotein/MDR1 and MRP2, and some metabolizing enzymes, like CYP3A4, whose expression is closely controlled by PXR (Eloranta et al., 2005).

Due to the increasing understanding of the important role played by transporters in pharmacokinetics, data about alteration of transporter levels in response to xenobiotic exposure may be important to consider, especially with respect to drug-drug interactions and drug adverse events (Shitara et al., 2005). Unfortunately, regulation of drug transporters remains incompletely characterized, especially in human hepatocytes. The present study was therefore designed to analyze expression of major sinusoidal and canalicular membrane transporters in xenobiotic-treated primary human hepatocytes, using mainly reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays. Primary hepatocyte cultures were used because they are a valuable cellular model for investigating liver detoxification pathways, especially in humans (Gomez-Lechon et al., 2004). The tested chemicals, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), PB, RIF, and OPZ, allowed us to investigate regulation by major hepatic drug sensors such as aryl hydrocarbon receptor (AhR), CAR, PXR, and Nr2f. Our data demonstrate a complex pattern of hepatic transporter regulation in response to xenobiotics, in addition to interindividual variability in responsiveness.

Materials and Methods

Chemicals and Reagents. RIF and TCDD were provided by Sigma-Aldrich (St. Louis, MO) and Cambridge Isotope Laboratories (Cambridge, MA), respectively. PB was obtained from Cooper (Melun, France), while OPZ was kindly supplied by Dr F. Ballet (Sanofi-Aventis, Evry, France). RIF, TCDD, and OPZ were commonly used as stock solution in dimethyl sulfoxide, for which the final concentration in culture media did not exceed 0.2% (v/v); control cultures received the same dose of solvent. PB was directly dissolved in culture medium. Collagenase and Williams' E medium were obtained from Biomedical Research, Cambridge, MA, and known intron-exon boundary (Table 2) were designed with the Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA), as described previously (Jigorel et al., 2005). Gene-specific primers (Table 2) were designed with the Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA), and known intron-exon boundary information was taken into account for each target gene to avoid detection of genomic DNA. Moreover, the specificity of each gene amplification was verified at the end of each qPCR by analysis of dissociation curves of the PCR products. The curves of amplification were read 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 tested cDNA to an 18S RNA endogenous reference and corresponds to the mean of a triplicate determination.

Preparation of Crude Membranes and Western Blotting. Crude membranes were prepared from cultured human hepatocytes by differential centrifugation as described previously (Courtois et al., 2002). Proteins were then separated on a 7% polyacrylamide gel and electrophoretically transferred to nitrocellulose membranes. After blocking in Tris-buffered saline containing 4% bovine serum albumin, membranes were hybridized overnight at 4°C with mouse primary monoclonal antibodies directed against MRP2 (clone M2III-6; Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Human Liver Identification</th>
<th>Sex</th>
<th>Age</th>
<th>Disease State</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL 1</td>
<td>Male</td>
<td>61</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HL 2</td>
<td>Male</td>
<td>63</td>
<td>Metastasis of colon cancer</td>
</tr>
<tr>
<td>HL 3</td>
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<td>75</td>
<td>Metastasis of colon cancer</td>
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<td>HL 5</td>
<td>Female</td>
<td>60</td>
<td>Metastasis of colon cancer</td>
</tr>
<tr>
<td>HL 6</td>
<td>Male</td>
<td>55</td>
<td>Metastasis of colon cancer</td>
</tr>
<tr>
<td>HL 7</td>
<td>Female</td>
<td>39</td>
<td>Hepatocellular adenoma</td>
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</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5' - 3')</th>
<th>Reverse Primer (5' - 3')</th>
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</thead>
<tbody>
<tr>
<td>CYP2B6</td>
<td>TTCTCACCGCTCTCCCTCTATCCAA</td>
<td>GTCGCAATCTCCACACGCTCA</td>
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<tr>
<td>CYP1B1</td>
<td>GTCGCAATCTCCCTCTCTCTCTCT</td>
<td>CCCACCACTCTGATCAATCTCTCT</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>CCTCTCCTCTAGGCAGTAAAT</td>
<td>TCCCCACCTGATCAACGCAA</td>
</tr>
<tr>
<td>NQ01</td>
<td>GCCCCGACCTTTGATATT</td>
<td>TGAACCTCTCCCTAAACCCAG</td>
</tr>
<tr>
<td>ABC transporters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDR1/ABCB1</td>
<td>GCCAAACGCTTTAGACGAGC</td>
<td>TCCCAATGCTCTGCGACATTA</td>
</tr>
<tr>
<td>MRP2/ABCC2</td>
<td>TGACAAGTGTTGACCCACAT</td>
<td>AGCTCTTCTCTGCGCTTCTCTCT</td>
</tr>
<tr>
<td>MRP3/ABCC3</td>
<td>GTCCTACCAAGTTCGAGTGA</td>
<td>TACCATATTGGCTATATT</td>
</tr>
<tr>
<td>MRP6/ABCB6</td>
<td>TOTGCCCTTTTGAAATCCTC</td>
<td>AGACGACGCTGGAAATCTAT</td>
</tr>
<tr>
<td>BCRP/ABCB2</td>
<td>TGACACGCTTACTGGCGAAGA</td>
<td>TCTCCAGACACCAAGAG</td>
</tr>
<tr>
<td>BSEP/ABCB11</td>
<td>TGACTCGTATCCAAGGAGA</td>
<td>TGTTCTTGAGAAAATCTTC</td>
</tr>
<tr>
<td>SLC transporters</td>
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<td></td>
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<tr>
<td>OAT4/SLC1O1A</td>
<td>GGGACATTTGGGATGTG</td>
<td>AGGGGCTGGAGAAATCTTG</td>
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<td>OAT5/SLC1C1B1</td>
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<td>OATP6/SLC1B3</td>
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<tr>
<td>NTCP/SLC1O1A</td>
<td>GCCGACGGGTCGATGATGATGAT</td>
<td>ATGGAGTTCAAGGACATCTTC</td>
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<tr>
<td>OAT2/SLC22A2</td>
<td>GCCGACCGCTAGAGGTTGAAATTC</td>
<td>AGCCGCTAGAGGTTGAAATTC</td>
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This text provides a detailed description of the methods used for the study of drug transporter regulation in primary human hepatocytes. It includes information about the chemicals and reagents used, the materials and methods section, and specific tables and figures illustrating the results. The text also discusses the importance of transporters in pharmacokinetics and the role of xenobiotics in regulating these transporters.
TABLE 3
Up-regulation of referent drug-metabolizing enzymes by xenobiotics

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Xenobiotic</th>
<th>-Fold Inductiona</th>
<th>Induction Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1B1</td>
<td>TCDD</td>
<td>445 ± 234.2</td>
<td>14.8–720.7</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>PB</td>
<td>23 ± 13.6</td>
<td>6.7–49.1</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>RIF</td>
<td>36.9 ± 35.5</td>
<td>7.5–110.1</td>
</tr>
<tr>
<td>NQO1</td>
<td>OPZ</td>
<td>8.6 ± 5.4</td>
<td>2.5–15.3</td>
</tr>
</tbody>
</table>

aData defined as the ratio of mRNA levels in treated hepatocytes versus those found in xenobiotic-untreated counterparts and expressed as mean ± S.D. of values from seven independent hepatocyte populations.

Chemicon International, Temecula, CA). BCRP (clone BXP-21; Alexis Biocins, Lausen, Switzerland), or P-glycoprotein (clone C219; Centocor, Malvern, PA). A peroxidase-conjugated anti-mouse antibody was thereafter used as secondary antibody. After washing, immunolabeled proteins were visualized by chemiluminescence. Crude membranes from P-glycoprotein-positive K562 R7 cells, BCRP-positive HEK-293 cells (kindly provided by Dr A. Di Pietro, Institut de Biologie et Chimie des Proteines, UMR 5086 Centre National de la Recherche Scientifique/Université de Lyon), and freshly isolated human hepatocytes were used as positive controls for expression of P-glycoprotein, BCRP, and MRP2, respectively.

Analysis of Data. To take individual variations into account and to minimize the impact of weak xenobiotic-induced changes in transporter levels, a threshold of a 1.5-fold change in mRNA levels [i.e., mRNA levels in xenobiotic-treated hepatocytes greater than 150% (for induction) or lower than 67% (for repression)] compared with those found in solvent-treated counterparts (for TCDD, RIF, and OPZ treatment) or untreated counterparts (for PB treatment), in at least three of the seven analyzed cultures, was applied for assessing alteration of transporter mRNA expression.

Results
Effects of Chemical Treatment on Drug-Metabolizing Enzyme Expression. Primary culture of hepatocytes prepared from seven donors was performed, and the cultured hepatocytes were exposed to TCDD, PB, RIF, and OPZ for 72 h. None of these chemicals exert toxicity at the used concentrations, as demonstrated by phase-contrast microscopic examination of the cultures and analysis of viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay (data not shown). To determine whether these compounds were fully active in our hepatocyte cultures with respect to regulation of detoxifying pathways, mRNA levels of referent drug-metabolizing enzymes were determined using RT-qPCR; data were expressed as the -fold variation of mRNA levels in treated hepatocytes versus xenobiotic-untreated counterparts (Table 3). In each hepatocyte population, CYP1B1, CYP2B6, CYP3A4, and NAD(P)H quinone oxidoreductase 1 (NQO1) mRNA levels were markedly induced in TCDD-, PB-, RIF-, and OPZ-treated hepatocytes, respectively. The means of -fold induction range from 8.6 (for NQO1 in OPZ-exposed hepatocytes) to 445 (for CYP1B1 in TCDD-treated hepatocytes) (Table 3). Overall, these data indicate that the primary human hepatocytes were responding appropriately to the inducers (Moore et al., 2003; Nebert et al., 2004; Piton et al., 2005).

Basal Levels of Transporter mRNA Expression in Untreated Primary Human Hepatocytes. To verify that primary human hepatocytes exhibited basal expression of liver transporters like their in vivo counterparts, we analyzed mRNA levels of these detoxifying proteins in xenobiotic-untreated 3-day-old cultured hepatocytes from seven donors using RT-qPCR. As shown in Fig. 1, A and B, mRNAs of both ABC and SLC liver transporters were detected in primary human hepatocytes, with levels ranging from approximately 20% to 320% of those found in freshly isolated hepatocytes; similar ranges of transporter expression have also been observed in cultured hepatocytes maintained in the presence of extracellular matrix components (Luttringer et al., 2002). Primary human hepatocytes thus retain notable expression of influx and efflux liver transporters in the used culture conditions, and they can therefore be considered as a convenient cellular model for studying the regulation of transporters, as already claimed (Courtois et al., 2002; Jigorel et al., 2005).

Effects of Chemical Treatment on ABC Transporter mRNA Expression. Levels of ABC transporter mRNAs in xenobiotic-treated hepatocytes were determined using RT-qPCR and were expressed comparatively to those found in xenobiotic-untreated counterparts, arbitrarily set at the value of 100%. Due to the fact that interindividual variations are commonly observed when using isolated human hepatocytes for detoxifying studies (Shibata et al., 2002), results from the seven studied hepatocyte populations were provided (Fig. 2). Such data confirmed that differences in response to xenobiotics occurred among hepatocyte populations: for example, although PB induced MDR1 and MRP2 mRNA expression in all the hepatocyte cultures, mRNA levels in PB-exposed hepatocytes ranged from 180 to 630% and from 260 to 500% for MDR1 and MRP2, respectively (Fig. 2). Another example was provided by MDR1 mRNA levels in TCDD-treated hepatocytes, which ranged from 60 to 270% of the values found in untreated counterparts.

To classify our data, taking individual variations into account, and to minimize the impact of weak xenobiotic-induced changes in transporter levels, a threshold of a 1.5-fold change in mRNA levels, i.e., mRNA levels in xenobiotic-treated hepatocytes greater than 150% (for induction) or lower than 67% (for repression) compared with xenobiotic-untreated counterparts, in at least three of the seven analyzed cultures, was applied for assessing alteration of transporter expression. With these criteria, MDR1 mRNA expression was found to be induced by all the chemicals (Table 4); the mean of -fold induction range from xenobiotic-untreated counterparts and expressed as mean ± S.D. of values from seven independent hepatocyte populations.
induction varied from 2.2 (for OPZ) to 4.4 (for PB). MRP2 expression was also induced by PB, RIF, and OPZ; the most efficient induction (by a 3.8-fold factor) was obtained with PB, whereas RIF and OPZ triggered a more moderate increase (by a 2.5- and a 2.4-fold factor).

MRP3 was up-regulated by RIF and OPZ with, however, only three and four responsive hepatocyte populations, respectively (Table 4).

BCRP mRNA levels were enhanced by all the chemicals; however, the number of responsive cultures differed according to the inducers: three (for TCDD), six (for PB), four (for RIF), and five (for OPZ) (Table 4). PB decreased MRP6 mRNA levels by a 2.1-fold factor (Table 5). BSEP expression was reduced by all the chemicals, PB and RIF being the most efficient, with 9.5- and 5.1-fold factors of repression, respectively (Table 5).

Effects of Chemical Treatment on SLC Transporter Expression. SLC transporter mRNA levels were analyzed in xenobiotic-treated human hepatocytes in a manner similar to that used for ABC transporters. The results are summarized in Tables 4 and 5.
transports. Data obtained from the seven studied hepatocyte populations are shown in Fig. 3. As for ABC transporters, individual variations were observed for some SLC transporter responses to xenobiotics. Thus, PB decreased OATP-C expression in two cases, whereas it induced it in a third case and had no obvious effects in the remaining four cases (Fig. 3). Therefore, we applied the same criteria as those described above for ABC transporters for assessing induction or repression of SLC transporter expression.

With such criteria, OATP-C was the only inducible SLC transporter (Table 4); this occurred in response to RIF with a 2.4-fold factor of induction. OCT1, OATP-B, and OATP8 mRNA levels were decreased by TCDD and RIF (Table 5). RIF and OPZ also down-regulated OCT1 and OATP8, respectively. OAT2 and NTCP expression was diminished by all the chemicals, especially by PB (Table 5); OAT2 and NTCP mRNA levels in PB-treated hepatocytes thus corresponded to less than 10% of those found in untreated counterparts (Fig. 3).

PB-Mediated Regulation of Transporters in Human Hepatocytes. In the last part of our study, we focused on PB effects that appeared to be among the most striking when considering the number of affected transporters and the levels of induction or repression. We first determined whether a short exposure (1 day) to 3.2 mM PB was sufficient to alter transporter expression. As shown in Fig. 4, up-regulation of CYP2B6, MDR1, MRP2, and BCRP, and down-regulation of MRP6, OAT2, and NTCP were observed from a 24-h treatment. By contrast, BSEP expression was not affected by this short PB exposure.

We next analyzed the dose-response of PB effects on transporter levels. As shown in Fig. 4, the use of an elevated dose of PB (2 mM or 3.2 mM) for 3 days allowed it to reach a maximal effect, for both inducing CYP2B6, MDR1, MRP2, and BCRP and repressing MRP6, BSEP, OAT2, and NTCP expression; by contrast, the use of lower PB concentrations (0.1 or 0.5 mM) had no or only reduced effect.

Finally, we determined whether PB-mediated up-regulation of MDR1, MRP2, and BCRP mRNA levels was associated with concomitant increase of corresponding transporter proteins by Western blotting. As shown in Fig. 5, PB-exposed human hepatocytes from three donors exhibited enhanced levels of P-glycoprotein/MDR1, MRP2, and BCRP compared with untreated counterparts.

Discussion

The present work was performed to analyze regulation of human hepatic drug transporters by prototypical chemicals activating drug-sensing receptors. These chemicals, TCDD, PB, RIF, and OPZ, markedly induced expression of their known targets, CYP1B1, CYP2B6, CYP3A4, and NQO1 (Moore et al., 2003; Nebert et al., 2004; Piton et al., 2005), demonstrating that they have efficiently activated the drug sensors AhR, CAR, PXR, and Nrf2. Moreover, they also regulated transporters that have already been described to be affected by these compounds in human hepatocytes. Thus, MDR1 and MRP2 expression was markedly induced by RIF, as described previously (Fardel et al., 2005; Klaassen and Slitt, 2005), confirming that these transporters are at least partly under PXR control. RIF also increased OATP-C expression, as very recently reported (Sahi et al., 2006), suggesting a PXR implication in OATP-C regulation as demonstrated for the rodent Oatp1a4 (Cheng et al., 2005). OPZ-mediated up-regulation of MRP2 and MRP3 and PB-triggered induction of MDR1 and MRP2 were observed, in agreement with previous reports (Courtois et al., 2002; Piton et al., 2005; Sahi et al., 2006). In addition, TCDD was shown to induce MDR1 expression in only some hepatocyte cultures, as described previously (Schuetz et al., 1995). Taken together, these data indicate that the primary hepatocytes were responding appropriately to the inducers and that our primary human hepatocyte system is...
thus convenient for analyzing transporter regulation. The fact that notable basal expression of both ABC and SLC liver transporters was retained in our human hepatocyte culture system also illustrates the interest of this in vitro model for transporter studies, although it was not based on the use of extracellular matrix components, well known to favor the in vitro maintenance of detoxifying pathways (Luttringer et al., 2002).

In addition to confirming some previous published results on transporter regulation in human hepatocytes, several novel observations emerge from our study. Some of these findings correspond to an extension to human hepatocytes of data previously obtained with human tumoral cell lines. Thus, RIF-mediated induction of MRP3 has already been reported in human hepatoma cells (Teng et al., 2003). In the same way, induction of BCRP by TCDD has been recently described in intestinal cancerous Caco-2 cells, which is fully consistent with the conclusion that this transporter may be an AhR target (Ebert et al., 2005). Interestingly, OPZ-mediated induction of BCRP may also be related to AhR activation, since OPZ has been hypothesized to interact with AhR as well as to activate Nrf2 (Le Ferrec et al., 2002). Our study also extends certain transporter regulations, previously observed in rodent cells or tissues, to human hepatocytes. Thus, NTCP down-regulation in response to TCDD has been recently described in rat liver (Fletcher et al., 2005). RIF-mediated BCRP up-regulation in human hepatocytes may be related to a PXR-dependent regulation of this transporter, reported in mice (Anapolsky et al., 2006). PXR may also account for RIF-triggered induction of MRP3.
because expression of this transporter is increased by known PXR agonists such as pregnenalone-16α-carbonitrile and spironolactone in mouse liver (Maher et al., 2005).

The remaining data from our study correspond to the report of new original regulation of hepatic transporters by xenobiotics. Induction of BCRP by PB and repression of BSEP, OCT1, OATP-B, OATP8, and OAT2 by TCDD have thus not been reported. Down-regulation of BSEP, NTCP, and OAT2 by PB, RIF, and OPZ, also constitutes new findings. In the same way, reduced expression of MRP6, OCT1, and OATP-B in PB-treated human hepatocytes was previously not known; it is the same thing for the down-regulation of OCT1 and OATP8 in response to RIF and OPZ, respectively. Taken together, our data indicate a complex pattern of human hepatic transporter regulation in response to chemicals that activate drug-sensing receptors, with opposite global effects on SLC and ABC transporters; indeed, chemicals rather decrease expression of SLC transporters, whereas they induce that of ABC transporters.

Several transporters, known to be regulated by xenobiotics in rodent liver cells, were not affected in human counterparts. Indeed, MRP6 expression, although induced in response to TCDD and OPZ in mouse liver (Maher et al., 2005), was not altered by these chemicals in primary human hepatocytes. In the same way, MRP2 and MRP3 were up-regulated by TCDD in mouse liver (Maher et al., 2005), but not in human hepatocytes. In addition, transporters such as BSEP and NTCP, repressed in response to PB and RIF in human hepatocytes, were not affected in mice exposed to CAR or PXR agonists (Wagner et al., 2005): similarly, MDR1, up-regulated by PB in human hepatocytes, was not affected by this chemical in rat counterparts (Courtois et al., 2002). The reasons for these discrepancies are unclear, but they may be linked to interspecies differences occurring for liver-detoxifying proteins (Le Bigot et al., 1987).

Whether the regulation of human hepatic drug transporters described in the present study may have functional and clinical implications remains to be determined. It is noteworthy that, although our study particularly addressed mRNA levels of transporters, some PB-mediated regulation, i.e., that concerning MDR1, BCRP, and MRP2, was documented through Western blot experiments. In the same way, RIF-mediated up-regulation of MRP2 and G-pglycoprotein has been previously observed at the protein level (Greiner et al., 1999; Kauffman et al., 2002). This suggests that variations in transporter mRNA levels are likely to be associated with changes in corresponding proteins. In this context, the marked down-regulation of both NTCP and BSEP by xenobiotics may deserve particular attention, since such alterations could markedly impair liver functions like bile acid secretion.

In contrast, the fold inductions of mRNA transporters in response to xenobiotics, which range from a 1.6- to a 4.4-fold factor, are rather low when compared with those observed for drug-metabolizing enzymes. Such induction levels, however, may be sufficient to have clinical implications. Indeed, PXR-mediated up-regulation of Oatplα4 in rats leads to increased hepatic extraction of ouabain handled by Oatplα4 (Klaassen and Slitt, 2005). Moreover, a 3.5-fold induction of intestinal P-glycoprotein content in response to RIF has been shown to cause a decrease in area under the plasma concentration time curve after oral administration of the P-glycoprotein substrate digoxin in humans (Greiner et al., 1999).

A highly variable response to inducers has been previously reported for some liver-detoxifying proteins, including glutathione S-transferases, among human hepatocyte cultures (Morel et al., 1993). For several transporter regulations, this phenotypic variability also occurred; i.e., primary hepatocytes from only some individuals were responsive. This was notably the case for up-regulation of MDR1 by TCDD, suggesting that the AhR pathway is not used for MDR1 regulation by TCDD (Schuetz et al., 1995). The cause for the polymorphism of response to xenobiotics with respect to transporter expression remains undetermined. It was not related to the age, sex, or disease state of the donors, and basal mRNA levels of transporters in untreated hepatocytes from responsive or unresponsive populations were not significantly different (data not shown), ruling out an implication of a putative difference of basal transporter expression for explaining the interindividual variability in transporter regulation.

A great number of transporters were found to be affected by PB; some of this regulation corresponded to induction (MDR1, MRP2, BCRP), whereas some was repression (MRP6, BSEP, OCT1, OATP-B, OATP8, OAT2, NTCP). PB-mediated regulation of MDR1, MRP2, MRp6, BCRP, OAT2, and NTCP can be considered as an early response because it was observed from a 24-h treatment; by contrast, a longer exposure to PB (72 h) was required to alter BSEP expression. PB effects on transporter expression were dose-dependent; interestingly, the use of a high PB concentration (2 or 3.2 mM) was the most efficient for both inducing CY2B6 and some transporters (MDR1, MRP2 and BCRP) and repressing others (NTCP, OAT2, BSEP, and MRP6). This may be consistent with the hypothesis that PB acts through a common mechanism on at least some of these transporters. In this context, a central role for CAR may be suspected, especially for MDR1 and MRP2. Indeed, the CAR agonist 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxide induced MDR1 expression in primary human hepatocytes (Maglich et al., 2003), and CAR has been recently demonstrated to be involved in the regulation of human intestinal P-glycoprotein and rat mrrp2 (Kast et al., 2002; Burk et al., 2005). CAR-independent effects of PB on transporters cannot, however, be discarded. Indeed, mrrp3 has been shown to constitute a CAR-independent target for PB in mice (Cherrington et al., 2003). MRP3 is also up-regulated in a CAR-independent manner in human hepatoma HepG2 cells (Xiong et al., 2002). However, PB failed to induce MRP3 expression in primary human hepatocytes, suggesting differences of responsiveness between normal and tumoral human hepatocytes with respect to transporter regulation.

In conclusion, xenobiotics activating drug-sensing receptors like AhR, CAR, PXR, and Nrf2 were shown to differentially regulate expression of sinusoidal and canalicular transporters in primary human hepatocytes. In addition to drug-metabolizing enzymes, drug transporters therefore constitute key targets of regulatory ways controlling liver detoxification. This point may be important to consider owing to the growing understanding of the role played by transporters in pharmacokinetics.

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