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Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes

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Running title

Drug transporter regulation in primary human hepatocytes

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Abbreviations

ABC:	ATP binding cassette
AhR:	aryl hydrocarbon receptor
BCRP:	breast cancer resistance protein
BSEP:	bile salt export pump
CAR:	constitutive androstane receptor
CYP:	cytochrome P-450
MDR	multidrug resistance
MRP:	multidrug resistance protein
NQO1:	NAD(P)H quinone oxidoreductase 1
Nrf2:	nuclear factor E2-related factor 2
NTCP:	Na^+-taurocholate co-transporting 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
SLC:	solute carrier
TCDD:	2,3,7,8-tetrachlorodibenzo-p-dioxin

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ABSTRACT

Sinusoidal and canalicular hepatic drug transporters constitute key-factors involved in liver drug elimination. 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 (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 3 of 7 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. BSEP and NTCP, 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 inter-individual variability in responsiveness. This may deserve further attention with respect to drug-drug interactions and hepatic drug adverse effects.

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INTRODUCTION

Hepatic drug transporters constitute important players of 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/SLCO1B3) (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 (ABCB1) encoded by multidrug resistance 1 (MDR1) gene, multidrug resistance protein 2 (MRP2/ ABCC2), MRP6 (ABCC6) and breast cancer resistance protein (BCRP/ABCG2), are involved in secretion of drugs or their metabolites into the 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 co-transporting 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 Boyer, 2003); BSEP also transports xenobiotics like 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 CAR (constitutive androstane receptor), 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; Piton et al., 2005). PXR-activating drugs also enhance P-glycoprotein levels (Geick et al., 2001) and expression of the mouse Oatp1a4 (Cheng et al., 2005). These results suggest a coordinate regulation between some transporters like P-glycoprotein/MDR1 and MRP2 and some metabolizing enzymes like cytochrome P-450 (CYP) 3A4, 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 analyse 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 since they are a valuable cellular model for investigating liver detoxification pathways, especially in humans (Gomez-Lechon et al., 2004). The tested chemicals, i.e. 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 Nrf2. Our data demonstrate a complex pattern of hepatic transporter regulation in response to xenobiotics, in addition to inter-individual variability in responsiveness.

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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 from Cooper (Melun, France) whereas OPZ was kindly supplied by Dr F. Ballet (Sanofi-Aventis, Evry, France). RIF, TCDD, and OPZ were commonly used as stocked solution in dimethylsulfoxide, whose 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 Roche (Meylan, France) and Invitrogen (Cergy-Pontoise, France), respectively.

Cell isolation and culture

Human hepatocytes were obtained from adult donors undergoing hepatic resection, via the Biological Ressource Center (Rennes, France). Donor data, including sex, age and disease state, are provided in Table 1. Cells were prepared by perfusion of histologically-normal liver fragments using a collagenase solution as previously described (Jigorel et al., 2005). All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee. Hepatocytes were primary cultured on plastic dishes in Williams'E medium, as already reported (Courtois et al., 2002); such culture conditions were retained since they allowed the study of liver drug detoxifying proteins, including drug metabolizing enzymes and transporters (Morel et al., 1993; Courtois et al., 2002; Jigorel et al., 2005). Xenobiotic-treatments, initiated 48 h after cell seeding, were renewed every day for 3 days; a shorter exposure to PB (24 h) was also performed.

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RNA isolation and RT-qPCR analysis

Total RNA was isolated from cells using a SV total RNA isolation kit (Promega, Madison, WI). Total RNA was then subjected to RT-qPCR assays using the fluorescent dye SYBR Green methodology and an ABI Prism 7000 detector (Applied Biosystem, Foster City, CA) as previously described (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 informations were taken into account for each target gene to avoid detection of genomic DNA. The specificity of each gene amplification was moreover verified at the end of each qPCR reaction 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 previously described (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, Chemicon International, Temecula, CA), BCRP (clone BXP-21, Alexis Biochemicals, Lausen, Switzerland) or P-glycoprotein (clone C219, Centocor, Malvern, PA). A peroxydase-conjugated anti-mouse antibody was thereafter used as secondary antibody. After washing, immuno-labelled proteins were visualized by chemiluminescence. Crude membranes from P-glycoprotein-positive K562 R/7 cells, BCRP-

positive HEK-293 cells (kindly provided by Dr A. Di Pietro, Institut de Biologie et Chimie des Proteines, UMR 5086 CNRS/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

In order to take individual variations into account and to minimize 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) comparatively to those found in solvent-treated counterparts (for TCDD, RIF and OPZ treatment) or untreated counterparts (for PB treatment), in at least 3 of the 7 analyzed cultures, was applied for assessing alteration of transporter mRNA expression.

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RESULTS

Effects of chemical treatment on drug metabolizing enzyme expression.

Hepatocytes prepared from 7 donors were primary cultured and 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). In order 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

In order to verify that primary human hepatocytes exhibited basal expression of liver transporters like their *in vivo* counterparts, we analysed mRNA levels of these detoxifying proteins in xenobiotic-untreated 3-day-old cultured hepatocytes from 7 donors using RT-qPCR. As shown in Fig. 1A and 1B, mRNAs of both ABC and SLC liver transporters were detected in primary human hepatocytes, with levels ranging approximately from 20 % to 320

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% 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 inter-individual variations are commonly observed when using isolated human hepatocytes for detoxifying studies (Shibata et al., 2002), results from the 7 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 level values 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.

In order to classify our data with taking individual variations into account and to minimize 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) comparatively to xenobiotic-untreated counterparts, in at least 3 of the 7 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 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 2.4-fold factor). MRP3 was up-regulated by RIF and OPZ with, however, only 3 and 4 responsive hepatocyte populations, respectively (Table 4). BCRP mRNA levels were enhanced by all the chemicals; the number of responsive cultures differed however according to the inducers: 3 (for TCDD), 6 (for PB), 4 (for RIF) and 5 (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 similar manner to that used for ABC transporters. Data obtained from the 7 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 than 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 expressions were 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 which 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 analysed 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 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.

We finally 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 when compared to untreated counterparts.

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DISCUSSION

The present work was performed in order to analyze regulation of human hepatic drug transporters by prototypical chemicals activating drug-sensing receptors. These chemicals, i.e. TCDD, PB, RIF and OPZ, markedly induced expression of their known targets, i.e. CYP1B1, CYP2B6, CYP3A4 and NQO1 (Moore et al., 2003; Nebert et al., 2004; Piton et al., 2005), demonstrating 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 expressions were markedly induced by RIF, as previously described (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 previously described (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 favour 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 in addition 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 regulations 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-regulations of BSEP, NTCP and OAT2 by PB, RIF and OPZ, also constitute 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.

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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 inter-species 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 of PB-mediated regulations, i.e. those concerning MDR1, BCRP and MRP2, were documented through Western blot experiments. In the same way, RIF-mediated up-regulations of MRP2 and P-glycoprotein have been previously observed at protein levels (Greiner et al., 1999; Kauffmann 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. On the other hand, the fold inductions of mRNA transporters in response to xenobiotics, which range from 1.6- to 4.4-fold factor, are rather low when compared to those observed for drug metabolizing enzymes. Such induction levels however may be sufficient to have clinical implications. Indeed, PXR-mediated up-regulation of Oatp1a4 in rats leads to

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increased hepatic extraction of ouabain handled by Oatp1a4 (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 hepatocytes 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 yet 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 inter-individual variability in transporter regulation.

A great number of transporters was found to be affected by PB; some of these regulations corresponded to inductions (MDR1, MRP2, BCRP) whereas others were repressions (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 since 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

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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 CITCO 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 mrp2 (Burk et al, 2005; Kast et al., 2002). CAR-independent effects of PB on transporters can however not be discarded. Indeed, mrp3 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). PB however 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 constitute therefore 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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LEGENDS TO FIGURE

Fig. 1. Basal expression of transporters in untreated primary human hepatocytes

Levels of ABC (A) and SLC (B) transporter mRNAs were determined in 3-day-old cultured hepatocytes by RT-qPCR as described in Materials and Methods. Data are expressed as % of transporter mRNA levels found in freshly isolated hepatocytes, arbitrarily set at 100%; they are the means ± SD of values from 7 independent hepatocyte populations.

Fig. 2. Expression of ABC transporter mRNAs in xenobiotic-treated human hepatocytes.

Primary human hepatocytes were either untreated or exposed to 10 nM TCDD, 3.2 mM PB, 50 µM RIF or 50 µM OPZ for 72 h. Levels of ABC transporter mRNAs were then determined by RT-qPCR as described in Materials and Methods. Data shown correspond to transporter mRNA levels found in xenobiotic-treated hepatocytes and are expressed as % of those found in xenobotic-untreated counterparts, arbitrarily set at 100 %. Values from each of the 7 analyzed hepatocyte populations are indicated; each value corresponds to a triplicate determination. Bar, mean of the mRNA values from the 7 hepatocyte populations.

Fig. 3. Expression of SLC transporter mRNAs in xenobiotic-treated human hepatocytes.

Primary human hepatocytes were either untreated or exposed to 10 nM TCDD, 3.2 mM PB, 50 µM RIF or 50 µM OPZ for 72 h. Levels of SLC transporter mRNAs were then determined by RT-qPCR as described in Materials and Methods. Data shown correspond to transporter mRNA levels found in xenobiotic-treated hepatocytes and are expressed as % of those found in xenobiotic-untreated counterparts, arbitrarily set at 100 %. Values from each of the 7 analyzed hepatocyte populations are indicated; each value corresponds to a triplicate determination. Bar, mean of the mRNA values from the 7 hepatocyte populations.

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Fig. 4. Features of PB-mediated regulation of transporter mRNAs in primary human hepatocytes.

Primary human hepatocytes were either untreated or exposed to 3.2 mM PB for 24 h or to various PB concentrations (from 0.1 mM to 3.2 mM) for 72 h. mRNA levels of CYP2B6, MDR1, MRP2, BCRP, MRP6, BSEP, OAT2 and NTCP were then determined by RT-qPCR as described in Materials and Methods. Data shown correspond to transporter mRNA levels found in PB-treated hepatocytes and are expressed as % of those found in untreated counterparts, arbitrarily set at 100 %; they are the means $\pm$ SD of independent values from at least 3 independent responsive hepatocyte populations.

Fig. 5. Expression of MDR1/P-glycoprotein, MRP2 and BCRP in PB-treated hepatocytes.

Primary human hepatocytes were either untreated (UNT) or exposed to 3.2 mM PB for 72 h. Crude membrane proteins were then prepared as described in Materials and Methods. Expression of MDR1/P-glycoprotein (P-gp), MRP2 and BCRP was next investigated by Western blotting. Data shown correspond to the analysis of three independent hepatocyte populations. Crude membranes from P-glycoprotein-positive K562 R/7 cells, freshly isolated human hepatocytes (FIH) and BCRP-positive HEK-293 cells were used as positive controls for P-glycoprotein, MRP2 and BCRP expression. Molecular weights of transporters are indicated on the right.

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TABLE 1

Clinical characteristics of liver donors

Human liver identification	Sex	Age (Years)	Disease state
HL 1	M	61	Hepatocellular carcinoma
HL 2	M	63	Metastasis of colon cancer
HL 3	F	75	Metastasis of colon cancer
HL 4	F	61	Secondary sclerosing cholangitis
HL 5	F	60	Metastasis of colon cancer
HL 6	M	55	Metastasis of colon cancer
HL 7	F	39	Hepatocellular adenoma

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TABLE 2

Primer sequences for RT-qPCR assays

Gene	Forward Primer (5'- 3')	Reverse Primer (5'- 3')
Drug metabolizing enzymes		
CYP2B6	TTCCTACTGCTTCCGTCTATCAAA	GTGCAGAATCCCACAGCTCA
CYP1B1	GCTGCAGTGGCTGCTCCT	CCCACGACCTGATCCAATTCT
CYP3A4	CTTCATCCAATGGACTGCATAAAT	TCCCAAGTATAACACTCTACACAGACAA
NQO1	GCCGCAGACCTTGTGATATT	TGAACACTCGCTCAAACCAG
ABC transporters		
MDR1/ABCB1	GCCAAAGCCAAAATATCAGC	TTCCAATGTGTTCGGCATTA
MRP2/ABCC2	TGAGCAAGTTTGAAACGCACAT	AGCTCTTCTCCTGCCGTCTCT
MRP3/ABCC3	GTCCGCAGAATGGACTTGAT	TCACCACTTGGGGATCATTT
MRP6/ABCC6	TGTCGCTCTTTGGAAAATCC	AGGAACACTGCGAAGCTCAT
BCRP/ABCG2	TGCAACATGTACTGGCGAAGA	TCTTCCACAAGCCCCAGG
BSEP/ABCB11	TGATCCTGATCAAGGGAAGG	TGGTTCCTGGGAAACAATTC
SLC transporters		
OATP-A/SLCO1A2	TGGGGAACTTTGAAATGTGG	AAGGCTGGAACAAAGCTTGA
OATP-B/SLCO2B1	TGATTGGCTATGGGGCTATC	CATATCCTCAGGGCTGGTGT
OATP-C/SLCO1B1	GCCCAAGAGATGATGCTTGT	ATTGAGTGGAAACCCAGTGC
OATP8/SLCO1B3	GGGTGAATGCCCAAGAGATA	ATTGACTGGAAACCCATTGC
NTCP/SLC10A1	GGGACATGAACCTCAGCATT	CGTTTGGATTTGAGGACGAT
OAT2/SLC22A7	GAGGATGAACCTGCCACAGT	TCTGCTCACACACCAGATCC
OCT1/SLC22A1	TAATGGACCACATCGCTCAA	AGCCCCTGATAGAGCACAGA
18S	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTCGCTC

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TABLE 3

Up-regulation of referent drug metabolizing enzymes by xenobiotics

Human hepatocytes were treated by prototypical xenobiotics for 72 h. mRNA levels were then determined by RT-qPCR

Enzyme	Xenobiotic	Fold induction [a]	Induction range
CYP1B1	TCDD	445 ± 243.2	14.8 - 720.7
CYP2B6	PB	23 ± 13.6	6.7 - 49.1
CYP3A4	RIF	36.9 ± 35.5	7.5 - 110.1
NQO1	OPZ	8.6 ± 5.4	2.5 - 15.3

[a] defined as the ratio of mRNA levels in treated hepatocytes versus those found in xenobiotic-untreated counterparts and expressed as mean ± SD of values from 7 independent hepatocyte populations.

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TABLE 4

Summary of drug transporters induced by xenobiotics

Human hepatocytes were treated by prototypical xenobiotics for 72 h. mRNA levels were then determined by RT-qPCR

Transporter	Xenobiotic	Number of responsive cases	Fold induction [a]
MDR1	TCDD	3/7	2.3 ± 0.5
	PB	7/7	4.4 ± 1.9
	RIF	7/7	2.9 ± 1.0
	OPZ	6/7	2.2 ± 0.8
MRP2	PB	7/7	3.8 ± 0.8
	RIF	7/7	2.5 ± 0.9
	OPZ	5/7	2.4 ± 0.6
MRP3	RIF	3/7	1.7 ± 0.1
	OPZ	4/7	1.8 ± 0.5
BCRP	TCDD	3/7	1.6 ± 0.2
	PB	6/7	3.7 ± 1.0
	RIF	4/7	2.7 ± 1.2
	OPZ	5/7	2.6 ± 0.4
OATP-C	RIF	5/7	2.4 ± 0.8

[a] defined for responsive hepatocyte populations as the ratio of mRNA levels in treated hepatocytes versus xenobiotic-untreated hepatocytes and expressed as mean ± SD of values from 3 to 7 responsive independent hepatocyte populations.

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TABLE 5

Summary of drug transporters repressed by xenobiotics

Human hepatocytes were treated by prototypical xenobiotics for 72 h. mRNA levels were then determined by RT-qPCR

Transporter	Xenobiotic	Number of responsive cases	Fold repression [a]
MRP6	PB	6/7	2.1 ± 0.6
BSEP	TCDD	6/7	2.5 ± 0.9
	PB	5/7	9.5 ± 4
	RIF	6/7	5.1 ± 1.6
	OPZ	6/7	2.7 ± 1
OCT1	TCDD	3/7	3 ± 1.2
	PB	3/7	3.6 ± 1.3
	RIF	5/7	2.6± 1
OATP-B	TCDD	4/7	1.8 ± 0.3
	PB	6/7	3.3 ± 2.6
OATP8	TCDD	7/7	2.3 ± 0.5
	PB	3/7	2.4 ± 0.8
	OPZ	5/7	2.4 ± 1.2
OAT2	TCDD	4/7	2.2 ± 0.4
	PB	7/7	16.6 ± 9.3
	RIF	3/7	2.1 ± 0.2
	OPZ	7/7	6.1 ± 8.4
NTCP	TCDD	4/7	2.8 ± 0.7
	PB	7/7	15.3 ± 9.3
	RIF	6/7	2.9 ± 1.3
	OPZ	4/7	2.7 ± 0.5

[a] defined for responsive hepatocyte populations as the ratio of mRNA levels in xenobiotic-untreated hepatocytes versus treated hepatocytes and expressed as mean ± SD of values from 3 to 7 responsive independent hepatocyte populations.

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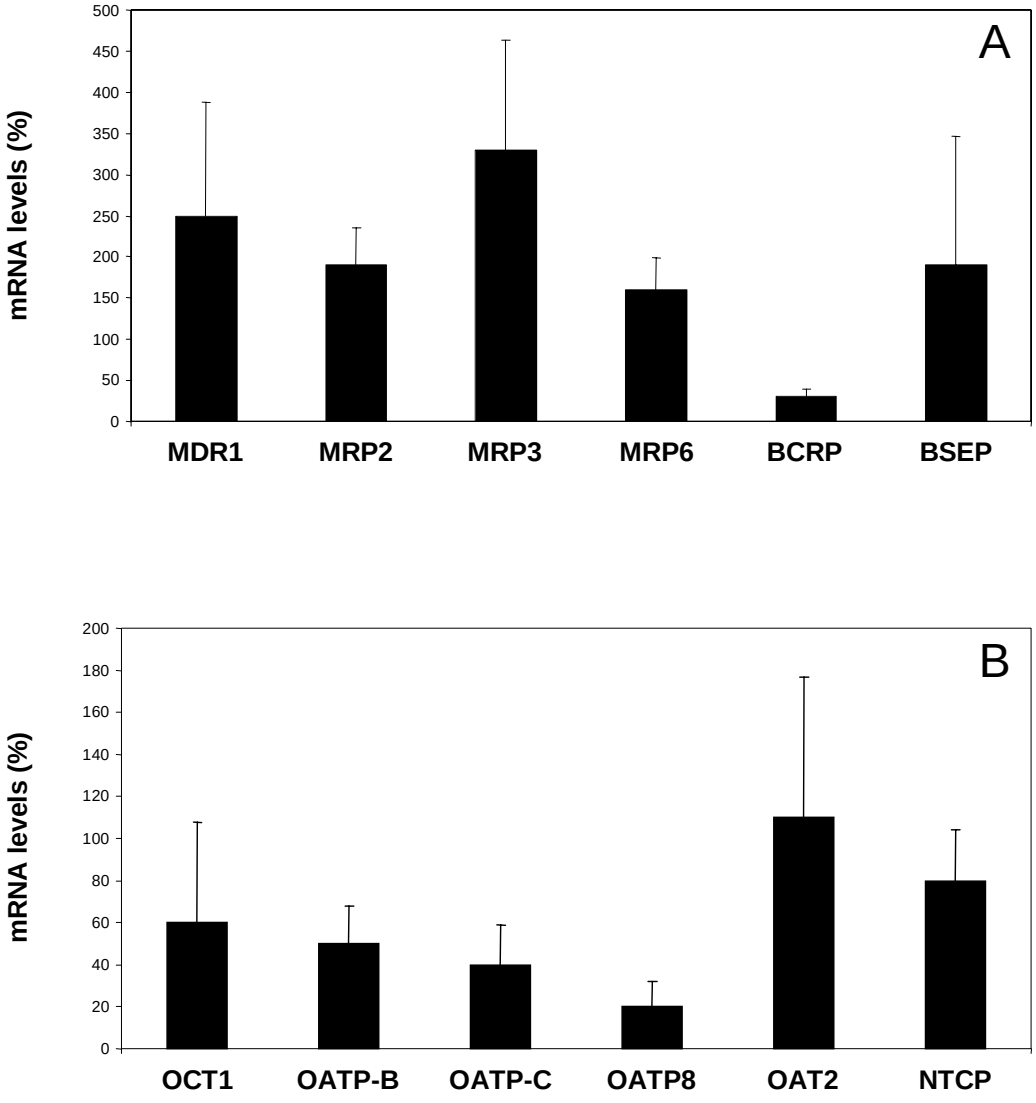

Fig. 1

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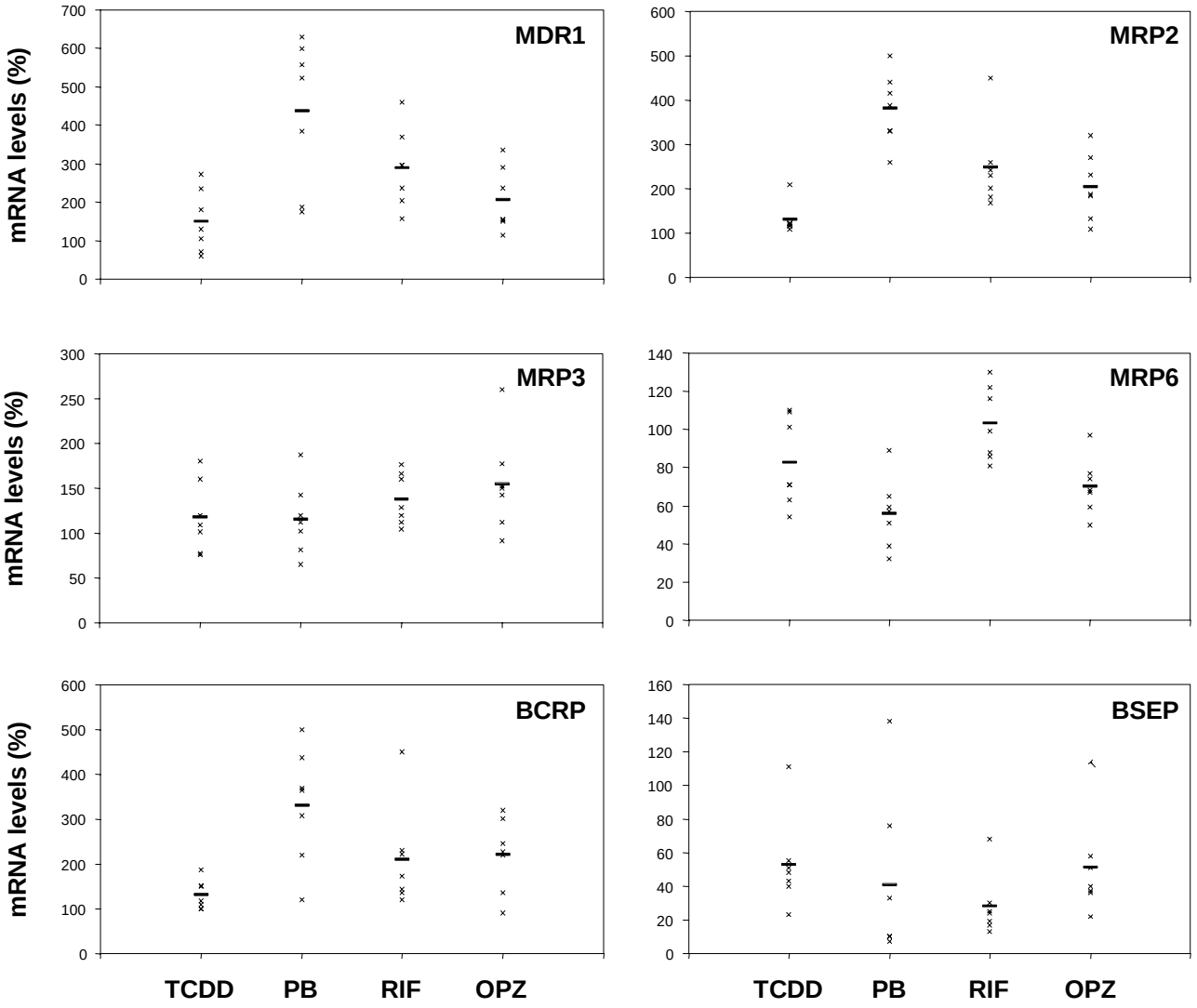

Fig. 2

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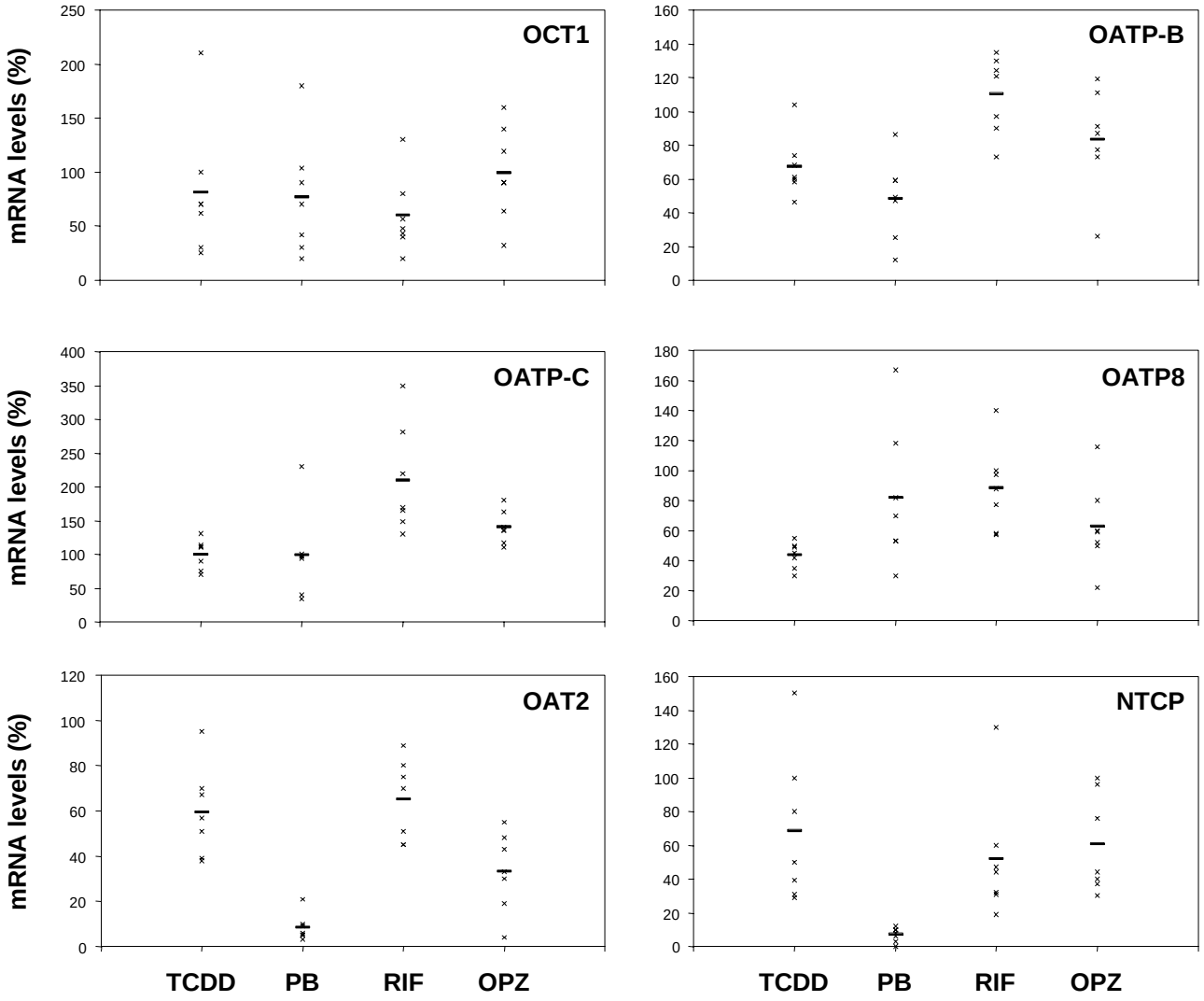

Fig. 3

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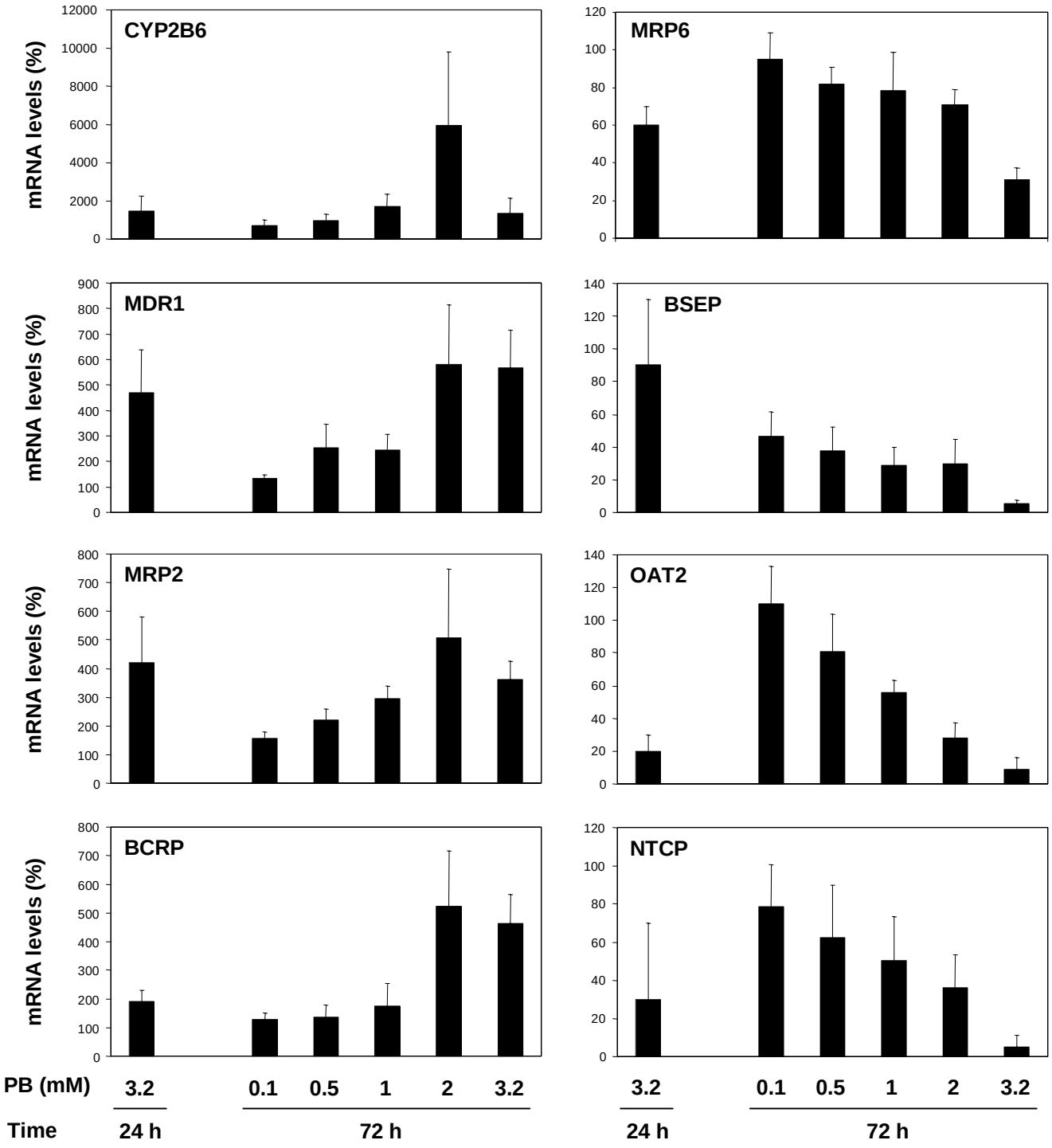

Fig. 4

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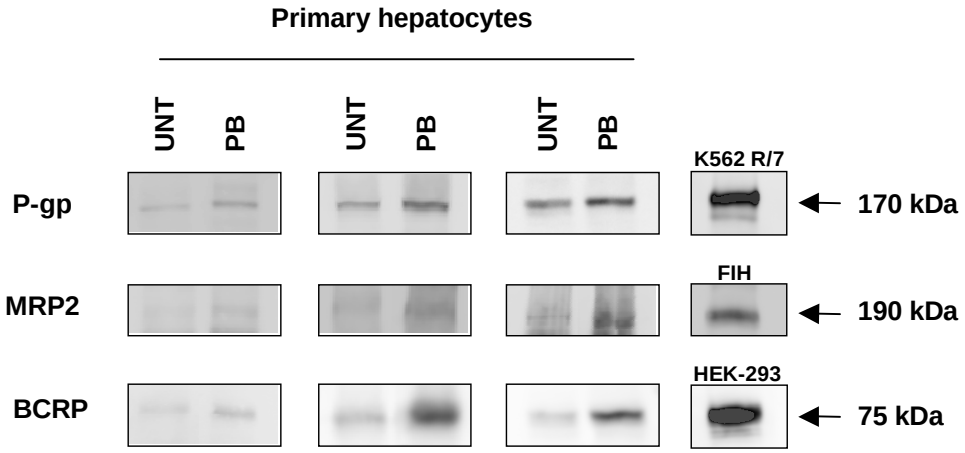

Fig. 5