Induction Profile of Rat Organic Anion Transporting Polypeptide 2 (oatp2) by Prototypical Drug-Metabolizing Enzyme Inducers That Activate Gene Expression through Ligand-Activated Transcription Factor Pathways

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ABSTRACT

Knowledge of regulation of transporters would aid in predicting pharmacokinetics and drug-drug interactions. Treatment of rats with pregnenolone-16α-carbonitrile (PCN) and phenobarbital increases hepatic uptake of cardiac glycosides. Rat organic anion transport polypeptide 2 protein (oatp2; Slc21a5) transports cardiac glycosides with high affinity. Levels of rat hepatic oatp2 protein and mRNA are regulated by PCN and phenobarbital treatment; however, the effects of other microsomal enzyme inducers on oatp2 have not been investigated. Therefore, the purpose of this study was to further determine whether oatp2 is regulated by a broader scale of drug-metabolizing enzyme inducers that are ligands or activators for the aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor (PPAR), and antioxidant/electrophile response element (ARE/EpRE). Oatp2 protein levels determined by Western blot were decreased 56 to 72% by the AhR ligands, increased 84 to 132% by the CAR ligands, and increased 230 to 360% by PXR ligands. The PPAR ligands and ARE/EpRE activators generally had minimal effects on oatp2 protein levels. Oatp2 mRNA levels, determined by the bDNA technique, generally did not show a correlation with the altered oatp2 protein levels, e.g., among PXR ligands, only PCN increased oatp2 mRNA levels, but spironolactone and dexamethasone did not. Furthermore, only PCN, but not spironolactone and dexamethasone, increased the transcription of the oatp2 gene as the amount of hnRNA was increased when determined by reverse transcription-polymerase chain reaction. In conclusion, some drug-metabolizing enzyme inducers regulate oatp2 protein levels, especially the CYP3A inducers. However, there is no correlation between their ability to increase levels of oatp2 protein and mRNA, suggesting that regulation of oatp2 by drug-metabolizing enzyme inducers occurs at both the transcriptional and post-translational levels.

For some drugs and xenobiotics to be biotransformed by the phase I and II drug-metabolizing enzymes, they have to be transported into the liver cell. A family of transporter proteins, known as the organic anion transporting polypeptide (oatp), participates in the transport of drugs and xenobiotics into liver. However, whether the drug-metabolizing enzyme inducers regulate these hepatic uptake transporters has not been studied thoroughly. Whereas activation of drug transporters is beneficial in cases when higher clearance rate is desired, it will be detrimental when effective therapeutic concentrations need to be maintained.

This laboratory has shown that phenobarbital and PCN, which induce the drug-metabolizing enzymes via the CAR and PXR receptor, respectively, enhance the plasma disappearance and biliary excretion of cardiac glycosides (Klaassen and Plaa, 1968; Klaassen, 1970a, 1970b, 1974a). In contrast, 3,4-benzo[a]pyrene, which induce drug-metabolizing enzymes via the Ah receptor, did not increase the biliary excretion of the cardiac glycosides (Klaassen, 1970b, 1974a, 1974b). It was later shown that phenobarbital and PCN, but not 3,4-benzo[a]pyrene, increased the uptake of cardiac glycosides into isolated hepatocytes (Eaton and Klaassen, 1979), indicating a specificity in the ability of drug-metabolizing enzyme inducers to enhance the hepatic uptake of xenobiotics.

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ABBREVIATIONS: oatp, organic anion transporting polypeptide; AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; PXR, pregnane-X-receptor; PPAR, peroxisome proliferator-activated receptor; ARE/EpRE, antioxidant/electrophile response element; bDNA, branched DNA signal amplification technique; hnRNA, heterogeneous nuclear RNA; CYP, cytochrome P450; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; PCB, polychlorinated biphenyl; SD, Sprague-Dawley; BNF, β-naphthoflavone; DAS, diallyl sulfide; SPIRO, spironolactone; DEX, dexamethasone; CLOF, clofibric acid; DEHP, diethylhexylphthalate; PFDA, perfluorodecanoic acid; BHA, butylated hydroxyanisole; EQ, ethoxyquin; OLTI, oltipraz; TBST, Tris-buffered saline/Tween 20; RT-PCR, reverse transcription-polymerase chain reaction; I-3-C, indole-3-carbinol.
Recent advances in cloning transporters during the last decade make the in-depth investigation of the hepatic uptake of xenobiotics possible. Rat oatp2, a recently cloned organic anion transporting polypeptide, is mainly localized to the sinusoidal membranes of hepatocytes (Noe et al., 1997; Abe et al., 1998; Gao et al., 1999; Reichel et al., 1999). Oatp2 has been shown to mediate the uptake of many structurally unrelated compounds, with a very high affinity to cardiac glycosides (Noe et al., 1997; Abe et al., 1998; Kakyo et al., 1998; Reichel et al., 1999). Rats treated with phenobarbital showed increased levels of oatp2 protein, and rats treated with PCN exhibited increased levels of both protein and mRNA (Rausch-Derra et al., 2001).

Marked progress on the mechanisms by which the induction of drug-metabolizing enzymes occurs has been made during the last few years. Several ligand-activated transcription factor pathways involved in the induction of some phase I and II enzymes have been discovered (Waxman, 1999; Fuhr, 2000). For example, the AhR mediates the induction of cytochrome P450 1A1/2 (CYP1A1/2; Safe and Krishnan, 1995), 2000). For example, the AhR mediates the induction of cyto-

Rats treated with phenobarbital showed increased levels of oatp2 protein, and rats treated with PCN (Reichel et al., 1999). Rats treated with phenobarbital (PB, 80 mg/kg, 4 days, i.p. in saline), diethyl ether, 5-thiouracil (5-Thu, 75 mg/kg, 4 days, i.p. in corn oil), and perfluorodecanoic acid (PFDA, 40 mg/kg, 1 day, killed on day 5, i.p. in corn oil); and ARE/EpRE activators: butylated hydroxyanisole (BHA, 75 mg/kg, 4 days, gavage in corn oil), ethoxyquin (EQ, 150 mg/kg, 4 days, gavage in corn oil), and oltipraz (OLTI, 150 mg/kg, 4 days, gavage in corn oil). All injections were at a volume of 5 ml/kg. Livers were removed on day 5, snap-frozen in liquid nitrogen, and stored at −80°C.

Preparation of Membranes. Liver membrane samples were prepared by homogenizing liver samples from each individual animal (0.1–0.5 g) in 10 ml of buffer (0.25 M sucrose, 10 mM Tris-HCl [pH 7.5], 25 μg/ml leupeptin, 50 μg/ml aprotinin and antipain, 0.5 μg/ml pepstatin, and 40 μg/ml phenylmethylsulfonyl fluoride), using a Teflon pestle and a 15-ml glass homogenizing vessel (Wheaton, Millville, NJ). The homogenate was centrifuged at 4°C for 100,000×g for 1 h. The pellet was then resuspended in 0.3 mM sucrose and 20 mM HEPES (pH 7.5). The protein concentration of each sample was determined by the bichinonic acid procedure using a BCA kit by Pierce (Rockford, IL).

Western Blot Analysis. Polyacrylamide-gel electrophoresis (runn-

Materials and Methods

Chemicals. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was a gift from Dr. Karl Rozman (University of Kansas Medical Center, KS). Oltipraz was a gift from Dr. L. Lubet (National Cancer Institute, Bethesda, MD). Polychlorinated biphenyl 99 (PCB 99) and polychlorinated biphenyl 126 (PCB 126) were obtained from AccuStandard (New Haven, CT). All other chemicals, unless otherwise indicated, were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Animals and Treatment. Male Sprague-Dawley (SD) rats (Harlan Sprague-Dawley Inc., Indianapolis, IN), weighing approximately 225 g, were used throughout the study except for the rats administered PCB 126 and PCB 99, which were from Sasco Laboratories, Inc. (Wilmington, MA). Five animals as a group were administered one of the following chemicals: AhR ligands: TCDD (3.9 μg/kg, 1 day, i.p. in corn oil), indole-3-carbolin (1-3-C, 56 mg/kg, 4 days, gavage in corn oil), β-naphthoflavone (BNF, 100 mg/kg, 4 days, i.p. in corn oil), and PCB 126 (40 μg/kg, 7 days, gavage in corn oil); CAR ligands: phenobarbital (PB, 80 mg/kg, 4 days, i.p. in saline), diallyl sulfide (DAS, 500 mg/kg, 4 days, i.p. in corn oil), and PCB 99 (16 mg/kg, 7 days, gavage in corn oil); PXR ligands: pregnenolone-16α-carbonitride (PCN, 50 mg/kg, 4 days, i.p. in corn oil), spironolactone (SPIRO, 75 mg/kg, 4 days, i.p. in corn oil), and dexamethasone (DEX, 50 mg/kg, 4 days, i.p. in corn oil); PPAR ligands: clofibrate (CLOF, 200 mg/kg, 4 days, i.p. in saline), diethylhexylphthalate (DEHP, 1200 mg/kg, 4 days, gavage in corn oil), and perflo-
Branched DNA (bDNA) Technique. Rat oatp2 mRNA levels were assessed using the Quantigene expression kit (bDNA technique; Bayer, Walpole, MA) as described in the manufacturer's protocol and validated in this laboratory (Hartley and Klaassen, 2000). Briefly, the oligonucleotide probes (capture extenders, label extenders, and blockers) were combined and diluted to 50 fmol/ml in wash buffer (100 mM HEPES buffer, pH 7.6, 0.65 mg/ml proteinase K, 1% lithium lauryl sulfate, 800 mM lithium chloride, 8 mM EDTA, and 0.5% Micro-O-protect). Total RNA (1 µg/µl, 10 µl) was added to each well containing 50 µl of capture hybridization buffer (100 mM HEPES buffer, pH 7.6, 3 mg/ml Boehringer-Mannheim blocking reagent, 1% lithium lauryl sulfate, 8 mM EDTA, and 0.5% Micro-O-protect) and 50 µl of each probe set in wash buffer. RNA was allowed to hybridize to each probe set overnight at 53°C. The plate was allowed to cool to 46°C and washed with washing buffer [twice with 0.1× SSC (1× SSC contains 0.15 M sodium chloride and 0.015 M sodium citrate), 1% sodium dodecyl sulfate, and 0.5 mg/ml sodium azide]. Samples were hybridized with the bDNA (branched DNA) amplification molecule (100 µM/vell at 0.2 fmol/µl bDNA) in the amplifier/label probe buffer (100 mM HEPES buffer, pH 7.6, 1.5 mg/ml Boehringer-Mannheim blocking reagent, 1% lithium lauryl sulfate, 10 µM ZnCl2, 1 mM MgCl2, 1% brij 35, and 0.5 Micro-O-protect) for 1 h at 46°C. The plate was allowed to cool to room temperature, and the wells were washed with high volume wash buffer (twice). Label probe diluted in amplifier/label probe buffer was added to each well (100 µl/well at 0.4 fmol/µl of alkaline phosphatase), and the alkaline phosphatase-conjugated label probe was allowed to hybridize to the bDNA-RNA complex for 1 h at 46°C. Each plate was cooled to room temperature and washed with high volume wash buffer (twice). The reaction was triggered by the addition of a dioxetane substrate solution [100 µl/Well of Lumiphos Plus (Lumigen, Inc., Southfield, MI) containing 0.3% sodium dodecyl sulfate], and the alkaline phosphatase-conjugated probe was allowed to hybridize to the bDNA-RNA complex for 1 h at 46°C. Each plate was cooled to room temperature and washed with high volume wash buffer (twice). The enzyme reaction was allowed to proceed for 1 h at 37°C, and the luminescence was measured with the QuantiLum 320 Luminometer equipped to read 96-well plates (Chiron Corp., Emeryville, CA).

Northern Blot Analysis of Rat oatp2 mRNA. Total RNA was isolated from livers of the aforementioned SD male rats treated with corn oil, PCN, spironolactone, and dexamethasone (five rats for each treatment group), using RNAzol B reagent following the manufacturer's protocol and validated in this laboratory (Rausch-Derra et al., 2001). Shown in Fig. 1A is a representative Western blot of liver membrane fractions probed with anti-oatp2 antibody after treatment with the aforementioned drug-metabolizing enzyme inducers. The data are also quantitatively expressed as the optical density after scanning each Western blot from each rat (n = 5) (Fig. 1B). All four AhR ligands (TCDD, 388-fold; I-3-C, 4.7-fold; BNF, 294-fold; and PCB 126, 279-fold). The mRNA levels of CYP2B1/2 were increased by the CAR ligand PB (53-fold) and phenobarbital-like compounds DAS (65-fold) and PCB 99 (55-fold). The mRNA levels of CYP3A1 were increased by the PXR ligands (PCN, 20-fold; SPIRO, 12-fold; and DEX, 33-fold). The mRNA levels of CYP4A2/3 were increased by the PPAR ligands (CLOF, 6.8-fold; DEHP, 10.3-fold; and PFDA, 10.8-fold). The mRNA levels of quinone reductase were increased by the ARE/EpRE activators (BHA, 2.1-fold; EQ, 4.6-fold; and OLTI, 6.3-fold).

Results

Male SD rats were dosed with five groups of prototypical drug-metabolizing enzyme inducers. The dosing paradigm was designed according to what is commonly cited in the literature. Furthermore, the mRNA levels of CYP1A1, CYP2B1/2, CYP3A1, CYP4A2/3, and quinone reductase were determined by the bDNA technique after treatment with these microsomal enzyme inducers to confirm the induction (data not shown). For example, the mRNA levels of CYP1A1 were increased by the treatment of AhR ligands (TCDD, 388-fold; I-3-C, 4.7-fold; BNF, 294-fold; and PCB 126, 279-fold). The mRNA levels of CYP2B1/2 were increased by the CAR ligand PB (53-fold) and phenobarbital-like compounds DAS (65-fold) and PCB 99 (55-fold). The mRNA levels of CYP3A1 were increased by the PXR ligands (PCN, 20-fold; SPIRO, 12-fold; and DEX, 33-fold). The mRNA levels of CYP4A2/3 were increased by the PPAR ligands (CLOF, 6.8-fold; DEHP, 10.3-fold; and PFDA, 10.8-fold). The mRNA levels of quinone reductase were increased by the ARE/EpRE activators (BHA, 2.1-fold; EQ, 4.6-fold; and OLTI, 6.3-fold).

Effects of the Prototypical Drug-Metabolizing Enzyme Inducers on the Protein Levels of Rat Hepatic oatp2. To determine whether oatp2 is regulated by the prototypical drug-metabolizing enzyme inducers that induce phase I and II enzymes through ligand-activated transcription factor pathways, oatp2 protein levels were determined by Western blotting using the anti-oatp2 antiserum developed in this laboratory (Rausch-Derra et al., 2001). Shown in Fig. 1A is a representative Western blot of liver membrane fractions probed with anti-oatp2 antibody after treatment with the aforementioned drug-metabolizing enzyme inducers. The data are also quantitatively expressed as the optical density after scanning each Western blot from each rat (n = 5) (Fig. 1B). All four AhR ligands (TCDD, I-3-C, BNF, and PCB 126) reduced oatp2 protein levels by 56, 56, 60, and 72%, respectively. All three CAR ligands (PB, DAS, and PCB 99) increased oatp2 protein levels moderately, by 80, 100, and 130%, respectively. All three PXR ligands (PCN, SPIRO, and DEX) dramatically increased oatp2 protein levels by 360, 270, and 230%, respectively. The PPAR ligands (CLOF, DEHP, and PFDA) and the ARE/EpRE activators (BHA, EQ, and OLTI) had minimal effects on oatp2 protein levels, except for the PPAR ligand, DEHP, which increased oatp2 protein levels significantly (120%).

Effects of the Prototypical Drug-Metabolizing Enzyme Inducers on Rat Hepatic oatp2 mRNA Levels. In general, the major mechanism by which chemicals increase drug-metabolizing enzyme mRNA levels is via increase of...
gene transcription. Although all AhR ligands (TCDD, I-3-C, BNF, and PCB 126) decreased oatp2 protein levels, their effects on oatp2 mRNA levels were not uniform; only PCB 126 decreased oatp2 mRNA levels significantly, TCDD and BNF had no effect, and I-3-C significantly increased oatp2 mRNA levels (Fig. 2). The CAR ligands (PB, DAS, and PCB 99) all increased oatp2 protein levels moderately, but only DAS significantly increased oatp2 mRNA levels; PB and PCB 99 had no effect. All three PXR ligands (PCN, SPIRO, and DEX) increased oatp2 protein levels markedly, but only PCN significantly increased oatp2 mRNA levels to a degree comparable with that observed at the protein levels. The two other PXR ligands, SPIRO and DEX, did not increase oatp2 mRNA levels. Treatment with PPAR ligands (CLOF, DEHP, and PFDA), or the ARE/EpRE activators (BHA, EQ, and OLTI) did not affect hepatic oatp2 mRNA levels.

**Determinations of Rat Hepatic oatp2 mRNA Levels after PXR Ligands Treatment by Northern Blot Analysis.** Because changes in oatp2 mRNA levels did not correspond to the changes in oatp2 protein levels, liver oatp2 mRNA content after PXR ligand treatments was also determined by Northern blot analysis. The amount of oatp2 mRNA after treatment with three PXR ligands was examined, because all three PXR ligands robustly increased oatp2 protein levels, but only PCN increased oatp2 mRNA levels, when quantified by the bDNA technique. Northern blotting agreed with the bDNA results and confirmed that PCN was the only PXR ligand that markedly increased oatp2 mRNA content in liver (Fig. 3). Therefore, the results of the Northern blots and bDNA method confirmed that among the PXR ligands, only PCN markedly increased oatp2 mRNA levels, whereas SPIRO and DEX did not significantly alter oatp2 mRNA levels.

**Determinations of oatp2 Gene Transcription by hnRNA RT-PCR after PXR Ligand Treatment.** Increased mRNA levels can be due to either increased transcription or increased stabilization of the mRNA. The amount of hnRNA, the primary transcripts that reside in the nucleus and give rise to mRNA after post-transcriptional modification, such as splicing and editing, are good indicators of gene transcription (Sharp, 1994). Figure 4 illustrates the relative amount of oatp2 hnRNA in livers from rats treated with either vehicle or the three PXR ligands (PCN, SPIRO, and DEX) by RT-PCR. The primers were designed from the intron regions of rat oatp2, which are present in the primary transcripts but not in mRNA. The results clearly showed that PCN, but not SPIRO and DEX, increased oatp2 hnRNA levels compared with that in corn-oil controls, indicating that PCN induction of oatp2 in liver is, at least partially, due to activation of oatp2 gene expression.
Discussion

Transporters are involved in xenobiotic absorption, distribution, and excretion. For some chemicals, uptake into liver is required before they are biotransformed by phase I and II drug-metabolizing enzymes. Subsequently, after the metabolites are formed, they need to be transported out of the cell. Therefore, regulation of transport into cells would alter the kinetics of some xenobiotics. Some export transporters are altered by chemical pretreatment in mice. For example, the phosphatidylcholine transporter, mdr2, was induced by the PPARγ ligand, clofibrate (Chianale et al., 1996; Miranda et al., 1997). In isolated rat hepatocytes, the organic anion exporter Mrp2 was induced by vincristin, tamoxifen, and rifampicin (Kauffmann et al., 1997), and by dexamethasone and PCN (Courtois et al., 1999). Another exporter Mrp3 was induced by treatment with phenobarbital (Ogawa et al., 2000).

Because uptake by transporters is necessary for some xenobiotics for the subsequent biotransformation, a thorough study was conducted on the rat hepatic uptake transporter, oatp2, to determine its induction profile by prototypical drug-metabolizing enzyme inducers. Rat oatp2 is a member of the multispecific organic anion transporting polypeptide family (Noe et al., 1997; Abe et al., 1998). It transports many structurally unrelated compounds, including anions, cations, and neutral compounds (Abe et al., 1998; Eckhardt et al., 1999; Reichel et al., 1999). Among them, cardiac glycosides such as ouabain and digoxin are transported by oatp2 with very high affinity compared with other oatp family members (Noe et al., 1997). Research from this laboratory has shown that the hepatic uptake of cardiac glycosides is increased after treatment of the rats with PCN and phenobarbital (Klaassen, 1974a, 1974b). A study from this laboratory demonstrated that oatp2 was induced by PCN and phenobarbital (Rausch-Derra et al., 2001). Research from this laboratory has also shown that mouse oatp2 induction was abolished in PXR knockout mice after PCN treatment (Staudinger et al., 2001). It was discovered recently that phenobarbital and PCN are the classical ligands for orphan nuclear receptors, CAR and PXR, respectively. However, there is no information of the effects on rat oatp2 of other drug-metabolizing enzyme inducers that also activate gene expression through ligand-activated transcription factor pathways. Elucidation of the regulation of rat oatp2 will certainly help to understand the pharmacokinetics of xenobiotics and to understand and prevent drug-drug interactions. Therefore, the current study was designed to determine the induction profile of rat oatp2 by five classes of prototypical drug-metabolizing enzyme inducers that increase gene expression through ligand-activated transcription factor pathways.

Ligand-activated transcription factor pathways are responsible for the induction of most of the drug-metabolizing enzymes (Waxman, 1999; Fuhr, 2000). AhR, CAR, PXR, PPAR, and ARE/EpRE have been shown to be the major ligand-activated nuclear receptors for induction of CYP1A1/2, CYP2B1/2, CYP3A, CYP4A, and quinone reductase. Recently, liver X receptor (Janowski et al., 1996, 1999; Peet et al., 1998) and farnesoid X receptor (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999) have been discovered and seem to play important roles in maintaining cholesterol and bile acid homeostasis. However, the regulatory roles of ligand-activated transcription factor pathways on transporters have not been thoroughly examined.

The current study showed that oatp2 protein levels were regulated by several classes of drug-metabolizing enzyme inducers. Oatp2 protein levels were decreased 56 to 72% by AhR ligands, moderately increased by CAR ligands (84–132%), and dramatically increased by PXR ligands (233–363%).

The mechanism by which these drug-metabolizing enzyme inducers alter oatp2 protein levels is not apparent. For example, all three PXR ligands significantly increased oatp2 protein levels, but only PCN increased oatp2 mRNA content.
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in liver. This finding was confirmed by three independent experimental methods (bDNA, Northern blot, and hnRNA RT-PCR). Therefore, measurement of mRNA levels alone may not be an accurate marker for induction of oatp2. In addition, rat hepatic oatp2 mRNA levels do not correlate with the increased levels of rat hepatic oatp2 protein after treatment with prototypical CYP3A4 inducers, indicating that these chemicals may regulate oatp2 post-transcriptionally. A similar phenomenon has been reported for the differential regulation of CYP3A4 and P-glycoprotein by CYP 3A4 inducers. P-glycoprotein is induced by dexamethasone, but not by PCN and triacetyloleandomycin in male rat liver, whereas CYP3A4 is induced by all three chemicals, indicating the possibility of independent regulation of CYP3A4 and P-glycoprotein by the CYP3A4 inducers (Schuetz et al., 1996; Sulphati and Benet, 1998).

PCN produced the largest increase in oatp2 protein and mRNA levels. Because levels of hnRNA were increased after PCN treatment, the increase in oatp2 mRNA levels appears to be due to an increase in oatp2 gene transcription. PCN is a potent ligand for rodent PXR and activation of the PXR activates CYP3A4 gene expression in several species, including mouse (Kliwer et al., 1998), rat (Zhang et al., 1999), rabbit (Savas et al., 2000), and human (Lehmann et al., 1998; Jones et al., 2000). Recently, PXR was shown to play a pivotal role in rifampin-mediated induction of P-glycoprotein, the gene product of MDR1 (Geick et al., 2001). Oatp2 is not only increased by PCN in adult rats but also during postnatal development, which strongly suggests that oatp2 is a potential PXR targeted gene. The mechanism by which PCN increases oatp2 expression can only be understood after thorough studies of the oatp2 promoter. The increase of oatp2 protein levels by two other PXR ligands, spironolactone and dexamethasone, does not appear to be due to increased transcription of the rat oatp2 gene. It is speculated that some post-translational effects such as a decrease in oatp2 protein turnover may be mediated through an (some) unknown PXR targeted gene(s), or may be mediated through unknown PXR-independent pathway(s) that is (are) activated by PCN, but not by spironolactone and dexamethasone.

The CAR ligands phenobarbital, diallyl sulfide, and PCB 99 all moderately increased oatp2 protein levels (84–132%). However, only diallyl sulfide significantly increased hepatic rat oatp2 mRNA levels. Phenobarbital has been shown to induce CYP2B1/2 via activation of the CAR (Honakoski et al., 1998; Kawamoto et al., 1999; Sueyoshi et al., 1999; Wei et al., 2000). Although CAR and PXR are both ligand-activated orphan nuclear receptors, the mechanism by which CAR and PXR ligands induce CYP2B1/2 and CYP3A4, respectively, is different (Sueyoshi and Negishi, 2001). CAR resides in the cytosol, and migrates into the nucleus upon binding of its ligands (Sueyoshi and Negishi, 2001). The receptor-ligand complex then dimerizes with RXRα and binds to response element(s) of the gene(s) that it regulates, which results in gene activation (Kawamoto et al., 1999; Sueyoshi and Negishi, 2001). Cross-talk between CAR and PXR has been reported, in that CAR ligands have been shown to be weak PXR ligands and induce CYP3A4 (Xie et al., 2000). In the present study, all CAR ligands increased oatp2 protein levels but not mRNA levels, indicating that post-translational effects, mediated by activation of either CAR or PXR by CAR ligands, possibly resulted in a decreased turnover of oatp2 protein. Also, CAR- or PXR-independent pathways cannot be excluded.

In the present study, oatp2 protein levels but not the mRNA levels were decreased by AhR ligands (TCDD, I-3-C, BNF, and PCB 126). Although AhR ligands are known for their induction of CYP1A1/2 and CYP1B1, they down-regulate a number of genes, such as the human estrogen receptor (White and Gasiewicz, 1993). AhR ligand down-regulation of oatp2 protein could have significant effects, which may result in increased plasma concentrations of oatp2 substrates, many of them important endogenous chemicals or pharmaceuticals. The PPAR ligands and the ARE/EpRE activators had minimal effects on oatp2 protein levels, except for the PPAR ligand, DEHP, which increased oatp2 protein levels significantly. DEHP also induces biological effects that occur independent of the PPARα (Melnick, 2001), therefore, it is possible that DEHP increases oatp2 protein levels through pathways that are independent of PPAR.

In conclusion, the present study provides evidence that some prototypical drug-metabolizing enzyme inducers that act through ligand-activated transcription factor pathways regulates rat oatp2 protein levels. The induction profile of oatp2 protein by these prototypical drug-metabolizing enzyme inducers mimics that of CYP3A4. However, rat hepatic oatp2 mRNA levels after treatment with microsomal enzyme inducers generally do not parallel the alteration of oatp2 protein levels. The highest induction of oatp2 was seen after PCN treatment, indicating that oatp2 is a potential PXR-targeted gene. The mechanism by which other drug-metabolizing enzyme inducers regulate oatp2 appears to occur at the post-translational level. In addition, altered protein levels of rat oatp2 after some microsomal enzyme inducer treatment indicates that drug-drug interactions may occur at the hepatic uptake level.

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References


