Photochemotherapeutic Agent 8-Methoxypsoralen Induces Cytochrome P450 3A4 and Carboxylesterase HCE2: Evidence on an Involvement of the Pregnane X Receptor

Jian Yang*§ and Bingfang Yan**†

*Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island 02881; and
†Department of Pharmacology, Nanjing Medical University, Nanjing, Jiangsu 210029, China

Received August 1, 2006; accepted September 18, 2006

8-Methoxypsoralen (8-MOP) is a prototype photochemotherapeutic agent and used to treat various skin disorders such as psoriasis and cutaneous T-cell lymphoma. Animal studies demonstrate that repeated treatment with 8-MOP markedly increases the capacity of drug metabolism. In this study, we report that 8-MOP is a potent inducer of cytochrome P450 3A4 (CYP3A4) and carboxylesterase 2 (HCE2), two major human enzymes that catalyze oxidative and hydrolytic reactions, respectively. In human primary hepatocytes, 8-MOP markedly induced the expression of CYP3A4 (approximately sixfold) and HCE2 (approximately threefold) and the induction occurred in a concentration-dependent manner (0–50 μM). RNA interference of the expression of the pregnane X receptor (PXR) proportionally decreased the induction. In a reporter assay, 8-MOP stimulated both CYP3A4 and HCE2 promoters, and the stimulation was enhanced by cotransfection of PXR. Several natural variants of PXR differed markedly from the wild-type receptor in responding to 8-MOP. In addition to human PXR (hPXR), 8-MOP activated rat PXR, and the activation was comparable to that of hPXR (EC₅₀ = ~14 μM). PXR is recognized as a master regulator of the genes encoding drug-metabolizing enzymes and transporters. The involvement of PXR in 8-MOP induction suggests that this chemotherapeutic drug-metabolizing enzymes and transporters. The involvement of PXR in 8-MOP induction suggests that this chemotherapeutic agent causes a broader range of drug-drug interactions, and the differential activation of certain PXR variants suggests that the magnitude of the interactions varies from person to person.

Key Words: psoralen; 8-methoxypsoralen; photochemotherapy; cytochrome P450 3A4; carboxylesterase HCE2; PXR.
metabolism of 8-MOP itself is markedly increased in rats with repeated doses of 8-MOP. Likewise, rats pretreated with 8-MOP exhibit marked increases (approximately sixfold) in the clearance of subsequently administered theophylline. In rat primary hepatocytes treated with 8-MOP, the expression of CYP1A1 is increased by more than 10-fold depending on the concentrations (Bauergart et al., 2005). In addition to phase I enzymes, 8-MOP has been shown to induce the expression of UDP-glucuronosyltransferase-1A6, a major phase II enzyme involved in the metabolism of estrogen (Diawara and Kulkosky, 2003). Increased metabolism of estrogen likely contributes to the reproductive toxicity commonly associated with this chemotherapeutic agent (Diawara and Kulkosky, 2003). These findings suggest that 8-MOP increases the expression of a wide range of drug-metabolizing enzymes.

In this study, we report that 8-MOP is a potent inducer of cytochrome P450 3A4 (CYP3A4) and carboxylesterase 2 (HCE2), two human enzymes that play important roles in oxidative and hydrolytic biotransformation, respectively (Bu, 2006; Satoh and Hosokawa, 1998). In human primary hepatocytes and Huh7 hepatoma cells, both CYP3A4 and HCE2 were inductively expressed by 8-MOP, and the induction was proportionally abolished by RNA interference (RNAi) against pregnane X receptor (PXR). 8-MOP efficaciously stimulated both CYP3A4 and HCE2 promoters and the stimulation was enhanced by PXR cotransfection. Several natural variants of PXR differed markedly from the wild-type receptor in responding to 8-MOP. Interestingly, 8-MOP comparably activated both human PXR isoforms (hPXR)s and rat PXR isoforms (rPXR)s, although many chemicals exhibit species-dependent activation.

**MATERIALS AND METHODS**

**Chemicals and supplies.** 5,6-Dichlororibosidylbenzimidazole (DRB), p-nitrophenylacetate, Hanks balanced salt solution, 8-MOP, rifampicin (RIF), and William’s E medium were purchased from Sigma (St Louis, MO). Dulbecco’s modified Eagle’s medium (DME), Insulin-Transferrin-Selenium G supplement, LipofectAMINE, and Plus Reagent were purchased from Invitrogen (Carlsbad, CA). Kits for luciferase detection and P450-Glo were from Promega (Madison, WI). Delipidated and normal fetal bovine sera were from HyClone (Carlsbad, CA). Kits for luciferase detection and P450-Glo were from Promega (Madison, WI). Delipidated and normal fetal bovine sera were from HyClone (Logan, UT). The goat anti-rabbit IgG conjugated with alkaline phosphatase was from Pierce (Rockford, IL). Nitrocellulose membranes were from Bio-Rad (Hercules, CA). Unless otherwise specified, all other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

**Culture and treatment of human primary hepatocyte and hepatoma cells.** Plated human primary hepatocytes (in six-well plate) were obtained from Liver Tissues Procurement and Distribution System (University of Minnesota, Twin Cities, MN) or commercial source CellzDirect (Pittsboro, NC). A total of four donors were included with two males (21 and 48 years old) and two females (35 and 72 years old). Among the donors, three were Caucasian and one Africa American (the 21-year male). None of them was smoker. Upon arrival, cell supernatants were replaced with rich William’s E medium containing penicillin (100 U/ml)/streptomycin (10 μg/ml) (Ma et al., 2005). After incubation at 37°C with 5% CO2 for 24 h, hepatocytes were treated with 8-MOP at various concentrations (0–50μM) or RIF (at 25μM). To determine an involvement of transactivation, cells were cotreated with DRB (50μM), an inhibitor of RNA synthesis (Clement and Wilkinson, 2000).

Typically, the treatment lasted for 30 h with a change of fresh medium at 24 h. 8-MOP reportedly undergoes extensive metabolism (Busch et al., 1978; Mays et al., 1987a; Schmid et al., 1980), and the schedule of treatment was designed to minimize potential complication from 8-MOP metabolites. Huh7 cells were maintained in DMEM containing 10% fetal bovine serum, penicillin/streptomycin, and 1% nonessential amino acids. Cells were usually seeded at a density of 2 × 10^3 cells/well (12-well plates) in medium containing 10% delipidated fetal bovine serum and treated similarly as primary hepatocytes.

**Reverse transcription-coupled polymerase chain reaction.** Total RNA was isolated with an RNA kit from Qiagen (Valencia, CA), and the integrity of the RNA was confirmed by formaldehyde gel electrophoresis. Total RNA (1 μg) was subjected to the synthesis of the first-strand cDNA in a total volume of 25 μl with random primers and Moloney murine leukemia viral reverse transcriptase. The reactions were conducted at 42°C for 50 min. The cDNAs were then diluted 10 times and subjected to PCR amplification (5 μl of the diluted cDNA). The typical cycling parameters were 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s for a total of 22–35 cycles depending on the mRNA level of a gene to be analyzed. The primers for CYP3A4 amplification were 5′-TGCCAGTATGGAGATGTGT-3′ (forward) and 5′-AATTGATTGGGC-CAAGACTC-3′ (reverse); the primers for HCE2 amplification were 5′-GCCATCGAACAATGTTACTC-3′ (forward) and 5′-CACGAGCT-3′ (reverse); and the primers for housekeeping gene GAPDH were 5′-CATCTGATGCTGTCACCC-3′ (forward) and 5′-CATGTGAAGCTGATGCCCT-3′ (reverse). The PCR-amplified products were resolved by agarose gel electrophoresis and detected by Typhoon 9410.

**Plasmid constructs and site-directed mutagenesis.** PXR expression constructs (human, rat, and cimeric) and CYP3A4 promoter reporter (−7836 to −6939 to −362/−35) were described elsewhere (Song et al., 2005). The HCE2 promoter reporter and siPXR construct were kindly provided by Drs Dolan (Wu et al., 2003) and Kemper (Bhalla et al., 2004), respectively. PXR natural variants were prepared by site-directed mutagenesis as described previously (Li et al., 2004). Complementary oligonucleotides were synthesized to introduce a substitution. The primers were annealed to the human expression construct and subjected to a thermocycler for a total of 15 cycles. The resultant PCR-amplified constructs were then digested with Dpn I to remove the non-mutated parent construct. The mutared PCR-amplified constructs were used to transform XL1-Blue bacteria. All mutated constructs were subjected to sequence analysis.

**Transient cotransfection experiment.** Cells (Huh7) were plated in 24-well plates in DMEM supplemented with 10% delipidated fetal bovine serum at a density of 8 × 10^3 cells per well. Transfection was conducted by lipofection with LipofectAMINE and Plus Reagent (Song et al., 2005). Transfection mixtures contained 100 ng of a PXR plasmid, 100 ng of a reporter plasmid, and 10 ng of null Resilla luciferase plasmid. Cells were transfected for 4 h, and the medium was replaced with fresh medium. After 12 h, the medium was changed again with the same medium containing a chemical or solvent DMSO (final concentration of 0.1%). The transfected cells were incubated for an additional 24 h, washed once with PBS, and collected by scraping. The collected cells were subjected to two cycles of freeze/thaw. The reporter enzyme activities were assayed with a Dual-Luciferase Reporter Assay System. This system contained two substrates, which were used to determine the activities of two luciferases sequentially. The firefly luciferase activity, which represented the reporter activity, was initiated by mixing an aliquot of lysates (10 μl) with Luciferase Assay Reagent II. Then, the firefly luminescence was quenched and the Renilla luminescence was simultaneously activated by adding Stop & Glo Reagent to the sample tubes. The firefly luminescence signal was normalized based on the Renilla luminescence signal, and the ratio of normalized luciferase activity from chemical over DMSO treatment served as fold of induction.

**Modulation of PXR expression by RNAi and overexpression.** To define the role of PXR in the induction by 8-MOP, the expression of PXR was regulated by RNAi and overexpression. For the RNAi experiment, cells (Huh7) were plated in six-well plates and transfected with the siPXR construct.
(800 ng/well). After a 72-h incubation with a change of fresh medium at 36 h, the cells were treated with 8-MOP (25 μM), RIF (25 μM), or DMSO (0.1%) for 30 h. The expression of CYP3A4, HCE2, and PXR was monitored by reverse transcriptase–polymerase chain reaction (RT-PCR). For the overexpression, cells were transfected with a PXR construct or the corresponding vector. After a 12-h incubation, cells were treated similarly but the treatment lasted for 72 h. RT-PCR and Western blots were both used to determine the expression of CYP3A4, HCE2, and PXR.

**Western analysis.** Cell lysates (30 μg) were resolved by 7.5% SDS-PAGE in a mini-gel apparatus and transferred electrophoretically to nitrocellulose membranes. After nonspecific binding sites were blocked with 5% nonfat milk, the blots were incubated with an antibody against CYP3A4, HCE2, or PXR. The preparation of the antibodies was described elsewhere (Sachdeva et al., 2003; Zhu et al., 2000). The primary antibodies were subsequently localized with goat anti-rabbit IgG conjugated with horseradish peroxidase. Horseradish peroxidase activity was detected with a chemiluminescent kit (SuperSignal West Pico). The chemiluminescent signal was captured by KODAK Image Station 2000, and the relative intensities were quantified by KODAK 1D Image Analysis Software.

**Enzymatic assay.** Huh7 cells were treated with 8-MOP (50 μM) or the same volume of DMSO for 48 h, and microsomes were prepared by differential centrifugation as described previously (Zhu et al., 2000). The activity of CYP3A4 was determined with a P450-Glo kit, essentially according to the manufacturer’s manual. Briefly, microsomes (10 μg in 12.5 μl) were mixed with 12.5 μl of CYP3A4 substrate Luciferin-BE (4 ×). After a 10-min preincubation at 37°C, the NADPH regeneration mixture (25 μl containing 400 mM KPO4) was added to initiate the enzymatic reaction. The reaction lasted for 30 min at 37°C and was terminated by adding 50 μl of Luciferin Detection Reagent. After an additional 10-min incubation at room temperature, the luminescent signal was determined. The activity of HCE2 was determined spectrophotometrically with standard substrate p-nitrophenylacetate. Sample cuvette (1 ml) contained 10 μg microsomes in 100 mM potassium phosphate buffer, pH 7.4, and 1 mM substrate at room temperature. Reactions were initiated by adding p-nitrophenylacetate (10 μl of 100 mM stock in acetonitrile), and hydrolytic rate was recorded from an increase in absorbance at 400. The extinction coefficient (E400) was determined to be 13 mM−1 cm−1. Several controls were performed including incubation without microsomes or the regeneration system.

**Other analyses.** Protein concentrations were determined with BCA assay (Pierce) based on albumin standard. Data are presented as mean ± SD of at least three separate experiments, except where results of blots are shown, in which case, a representative experiment is depicted in the figures. Comparisons between two values were made with t-test at p < 0.05.

**RESULTS**

**Induction of CYP3A4 and HCE2 as a Function of 8-MOP**

In animal, 8-MOP has been shown to increase the overall capacity of drug metabolism. In this study, we made an effort to examine whether 8-MOP causes induction, initially, in human primary hepatocytes. The focus was on the expression of CYP3A4 and carboxylesterase HCE2. CYP3A4 is the most abundant CYP enzyme and metabolizes more than half of therapeutic agents (Bu, 2006). HCE2, abundantly expressed in the gastrointestinal tract, liver, and kidney, is a major pharmacokinetic determinant of many drugs containing ester linkages (Satoh and Hosokawa, 1998). Hepatocytes were treated with 8-MOP at various concentrations, and the expression of both genes was monitored by semiquantitative PCR. As shown in Figure 1, the levels of CYP3A4 and HCE2 mRNA were markedly increased by 8-MOP and the increases occurred in a concentration-dependent manner. The maximum induction of CYP3A4 was approximately sixfold, whereas the maximal induction of HCE2 was approximately threefold. In both cases, the maximum induction by 8-MOP (50 μM) was comparable to that by RIF at 25 μM (Fig. 1).

**Transcriptional Involvement in CYP3A4 and HCE2 Induction by 8-MOP**

The increases of CYP3A4 and HCE2 mRNA suggested two possibilities: (1) 8-MOP stimulates the transcription and/or (2) decreases the degradation of mRNA. In order to test the first possibility, a transcriptional inhibition assay was performed with
The abolished induction by DRB suggested that 8-MOP increases the expression of CYP3A4 and HCE2 through transactivation. This possibility was tested with CYP3A4 and HCE2 promoter reporters. The CYP3A4 reporter, containing the proximal promoter and a distal region, has been shown to respond to many CYP3A4 inducers (Song et al., 2005). The HCE2 reporter contained a 1783-bp upstream sequence, which represents the entire promoter (Wu et al., 2003). Cells (Huh7) were transfected with a promoter reporter and the Renilla plasmid, and the transfected cells were treated with 8-MOP or DMSO. After a 30-h incubation, cells were lysed and the luciferase activities were determined. As shown in Figure 2B, treatment with 8-MOP significantly increased the activity of both reporters, although the magnitude of the increased transactivation was less than that detected by RT-PCR (Fig. 2A). It is likely that the endogenous genes are more sensitive targets than the heterologous promoters, and supplement of limited factors (e.g., PXR) is required to fully activate the reporters (discussed below).

**Involvement of PXR in the Induction of CYP3A4 and HCE2 by 8-MOP**

The increased activity of the reporters by 8-MOP suggested that this chemotherapeutic agent stimulates the promoters of CYP3A4 and HCE2. PXR is recognized as a master regulator of many genes encoding drug-metabolizing enzymes (Carnahan and Redinbo, 2006; Kliewer, 2003). In order to test whether PXR plays a role in the increased transactivation by 8-MOP, we performed knockdown and overexpression experiments to selectively modulate the expression of PXR. For the knockdown experiment, Huh7 cells were transfected with siPXR construct or the corresponding vector, treated with 8-MOP or DMSO, and analyzed for the levels of CYP3A4 and HCE2 mRNA. As shown in Figure 3A, the levels of both CYP3A4 and HCE2 mRNA were significantly decreased in the cells transfected with the siPXR construct compared with those in the vector-transfected cells. The level of PXR was significantly decreased in the cells transfected with the siPXR construct (RT-PCR). Similar decreases on RIF-mediated induction were detected as well (Fig. 3A).

Next we examined the effect of PXR overexpression on the induction of CYP3A4 and HCE2 by 8-MOP. Similarly, transfection was conducted to modulate the expression of PXR; however, a construct encoding hPXR was used to replace the siPXR construct. The transfected cells were treated with 8-MOP, RIF, or DMSO. In addition to RT-PCR, Western blotting was used to monitor the expression as well. Both methods could detect the expression of HCE2 regardless of the cells transfected with the PXR construct or the vector (Fig. 3B). However, the expression of CYP3A4 in vector-transfected cells could be detected by RT-PCR only, even under induced conditions (Fig. 3B). As expected, treatment with 8-MOP markedly increased the levels of both CYP3A4 and HCE2 (Fig. 3B).
More importantly, significantly higher levels were detected in the cells overexpressing PXR. Interestingly, PXR transfection alone (DMSO treatment) increased the basal expression of CYP3A4 and HCE2 (Fig. 3B), suggesting that the expression of PXR in the cell line is a limited factor. We also examined whether the induction determined by RT-PCR or Western analysis reflects the induction on the enzymatic activity. Huh7 cells were treated with DMSO or 8-MOP, and microsomes were assayed for the activities of CYP3A4 and HCE2 as described in the Materials and Methods. *Significantly different from vector-transfected cells according to t-test (p < 0.05).

FIG. 3. Induction of CYP3A4 and HCE2 as a function of PXR. (A) Effect of siPXR on the induction of CYP3A4 and HCE2. Huh7 cells in 6-well plates were transiently transfected with a mixture containing 800 ng of siPXR or the corresponding vector (per well). After 72 h incubation, the transfected cells were treated with 8-MOP (25 μM), RIF (25 μM), or the same volume of DMSO (0.1%) for 30 h. Total RNA was isolated and analyzed for the expression of CYP3A4, HCE2, PXR, and GAPDH by RT-PCR. The PCR cycles were 35 for CYP3A4, 30 for HCE2, 32 for PXR, and 22 for GAPDH. Three independent experiments were performed, and the data are expressed as mean ± SD. *Significantly different from vector-transfected cells according to t-test (p < 0.05). (B) Effect of PXR overexpression on the induction of CYP3A4 and HCE2. Huh7 cells in 6-well plates were transiently transfected with a mixture containing 800 ng (per well) of PXR construct or the corresponding vector. After 12 h incubation, the transfected cells were treated with 8-MOP (25–50 μM) or the same volume of DMSO (0.1%) for 72 h. Total RNA or cell lysates were isolated and analyzed for the expression of CYP3A4, HCE2, PXR, and GAPDH by RT-PCR. The PCR cycles were 29 for CYP3A4, and Western analysis was performed with 30 μg protein. (C) Enzymatic activity of CYP3A4 and HCE2. Huh7 cells were treated with DMSO or 8-MOP (50 μM) for 48 h. Microsomes were prepared by differential centrifugation and assayed for the activities of CYP3A4 and HCE2 as described in the Materials and Methods. *Significantly different from vector-transfected cells according to t-test (p < 0.05).
8-MOP Comparably Activates hPXR and rPXR

The knockdown and overexpression studies demonstrated that PXR supports the induction by 8-MOP. In rodents, 8-MOP was shown to markedly increase the overall capacity of drug metabolism (Baumgart et al., 2005; Diawara and Kulkosky, 2003; Mays et al., 1987). We next examined whether 8-MOP activates both hPXR and rPXRs (Song et al., 2005). In addition to wild-type PXRs, several rat-human chimeric PXRs were included as well. These chimeric receptors were prepared to gradually replace the ligand-binding domain of hPXR with the corresponding rat sequence at an increasing length of 20 residues (Song et al., 2005). Cotransfection was performed with a transfection mixture containing a PXR construct, the CYP3A4 reporter and the Renilla luciferase plasmid. The transfected cells were treated with 8-MOP or RIF, and the increased activity of the reporter was expressed as fold of activation.

Several important observations were made with the cotransfection study (Fig. 4A). First, RIF activated hPXR and a few of chimeric PXRs containing limited length of rPXR sequence (up to rh226) (Fig. 4A). In contrast, 8-MOP activated all receptors (both wild-type and all chimeric PXRs). Second, the maximum activation by RIF was detected with hPXR (wild type), whereas the maximum activation by 8-MOP was detected with chimeric receptor rh226 (Fig. 4A). Third, the chimeric PXRs containing the replacement from residues 286 to 366 (rh286 to rh366) were less responsive to 8-MOP. And more interestingly, 8-MOP comparably activated both hPXR and rPXR (~15-fold) with similar EC₅₀ values (14.3 vs. 13.9 µM). It should be noted that all receptors were expressed to a similar extent based on Western analysis (not shown).

Activation of PXR Variants by 8-MOP

hPXR and rPXRs are almost identical in the DNA-binding domain (95%) but much more diverse (75%) in the ligand-binding domain (Zhang et al., 1999). The comparable activation of hPXR and rPXRs suggests that 8-MOP tolerates more structural restraint compared with chemicals (e.g., RIF) that activate PXR in a species-dependent manner (Carnahan and Redinbo, 2006; Kliewer, 2003). Consistent with this species-dependent induction, RIF is an efficacious activator of hPXR and PCN is a potent inducer of rodent PXR (Carnahan and Redinbo, 2006; Kliewer, 2003). The ligand-binding domain of PXR is ~293 residues long. Interestingly, replacement of the first 100 residues from the N-terminus (switch from human to rat) completely abolishes the ability to respond to RIF (up to chimeric rh246) (Fig. 4A). In contrast, replacement of additional 100 residues gains the full responsiveness to PCN (Song et al., 2005). These findings suggest that the residues from 246 to 346 functions as a transient region from human to rat specificity. 8-MOP is a naturally occurring photoactive compound that distinguishes 8-MOP from many other PXR activators. Compounds such as RIF and pregnenolone 16α-carbonitrile (PCN) exert profound species-dependent activation. RIF is a potent inducer in humans, whereas PCN is a potent inducer in rodents (Carnahan and Redinbo, 2006; Kliewer, 2003). Consistent with the species-dependent induction, RIF is an efficacious activator of hPXR and PCN is a potent activator of rodent PXR (Carnahan and Redinbo, 2006; Kliewer, 2003). The ligand-binding domain of PXR is ~293 residues long. Interestingly, replacement of the first 100 residues from the N-terminus functions as a transient region from human to rat specificity. 8-MOP is an activator of both hPXR and rPXR with less apparent transient region (Fig. 4A).

The nonselectivity of 8-MOP toward hPXR and rPXR suggests that this photoactive compound tolerates structural restraint more than RIF does. In support of this notion, all chimeric PXRs are activated by 8-MOP, whereas RIF activates only those containing limited length of the replacement (from rh147 to rh226) (Fig. 4A). In contrast, among the variants containing substitutions in the ligand-binding domain, only two of them (hPXR₅₂₀₈F and hPXR₃₈₁₉W) differ by 50% from RIF (Fig. 5A). It should be noted that PXR and variants were expressed to a comparable extent (top of Fig. 5A). We further tested variants (hPXR₃₈₁₁W and hPXR₉₉₈C) with higher concentrations of 8-MOP and RIF and confirmed that their responsiveness was either significantly decreased or completely eliminated (Fig. 5B).

DISCUSSION

8-MOP is a naturally occurring photoactive furocoumarin and widely used for treating skin disorders such as psoriasis, vitiligo, and cutaneous T-cell lymphoma (Arroyo and Tift, 2003; Wackernagel et al., 2006). This photoactive compound is found to prolong the presence of nicotine and has been studied for cigarette smoking cessation in recent years (Sellers et al., 2000). In rats, 8-MOP induces the expression of drug-metabolizing enzymes and causes pharmacokinetic interactions (Apseloff et al., 1990; Mays et al., 1987a). In this study, we report that 8-MOP is an efficacious inducer of CYP3A4 and HCE2, two major enzymes involved in the metabolism of a wide range of drugs and other xenobiotics. In human primary hepatocytes and hepatoma cells, 8-MOP markedly increases the expression of CYP3A4 and HCE2. The increases are proportionally decreased when the expression of PXR is knocked down. Certain natural variants of hPXR differ markedly from the wild-type PXR in responding to 8-MOP. Interestingly, 8-MOP activates both hPXR and rPXR to a similar extent. The nonselectivity of 8-MOP toward hPXR and rPXR distinguishes 8-MOP from many other PXR activators. Compounds such as RIF and pregnenolone 16α-carbonitrile (PCN) exert profound species-dependent activation. RIF is a potent inducer in humans, whereas PCN is a potent inducer in rodents (Carnahan and Redinbo, 2006; Kliewer, 2003). Consistent with the species-dependent induction, RIF is an efficacious activator of hPXR and PCN is a potent activator of rodent PXR (Carnahan and Redinbo, 2006; Kliewer, 2003). The ligand-binding domain of PXR is ~293 residues long. Interestingly, replacement of the first 100 residues from the N-terminus functions as a transient region from human to rat completely abolishes the ability to respond to RIF (up to chimeric rh246) (Fig. 4A). In contrast, replacement of additional 100 residues gains the full responsiveness to PCN (Song et al., 2005). These findings suggest that the residues from 246 to 346 functions as a transient region from human to rat specificity. 8-MOP is an activator of both hPXR and rPXR with less apparent transient region (Fig. 4A).
the wild type in responding to RIF whereas there are five toward 8-MOP (Fig. 5A). The precise mechanism for this phenomenon remains to be determined. It is likely that 8-MOP, much smaller than RIF (MW: 220 vs. 823), has multiple binding modes in the binding pocket. These binding modes differ from each other on the overall transactivation activity. As a result, substitution of a residue, with an ability to switch the binding mode, may cause a greater change on the transactivation. In support of this possibility, small ligand SR12813 has three distinct binding orientations based on X-ray crystallography, although interactions with a coregulator likely affect ligand-binding mode as well (Watkins et al., 2001, 2003). It should be noted that all residues substituted in the ligand-binding domain are conserved between hPXR and rPXRs.

It is interesting that certain substitutions outside of the ligand-binding domain also cause changes on the ligand-dependent activation. For example, variant hPXRR98C, containing a substitution in the DNA-binding domain, no longer responds to

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**FIG. 4.** Activation of hPXR and rPXRs and chimeric PXRs. (A) Activation of wild-type and chimeric PXRs Huh7 cells in 24-well plates were transiently transfected by with a mixture containing 100 ng of receptor plasmid, 100 ng of reporter plasmid (CYP3A4-DP-luc), and 10 ng of null-luciferase plasmid (reference). After 12 h incubation, the transfected cells were treated with 8-MOP (25 μM), RIF (25 μM), or the same volume of DMSO for 24 h. Cell lysates were assayed for the luciferase activities, and the data are expressed as fold of activation (n = 3). (B) Activation of hPXR and rPXRs as a function of 8-MOP. Huh7 cells were transfected and treated with various concentrations of 8-MOP (0–50 μM). The reporter activities were determined as described above.
either 8-MOP or RIF (Fig. 5). The precise mechanism for the complete loss of the transactivation activity remains to be determined. This residue is next to cysteine-97, which is likely involved in coordinating a zinc ion (PXR is a zinc finger transcription factor). Correct coordination of zinc ion is essential for these factors to bind DNA (Webster et al., 2001). Therefore, substitution of arginine-98 by a cysteine likely alters such coordination and leads to the loss of DNA-binding activity. hPXRG36R, on the other hand, shows differential changes. The activation of this variant is increased in response to 8-MOP, but decreased to RIF (Fig. 5A). Glycine-36 is located in the N-terminal region and appears not to involve DNA or ligand binding (Carnahan and Redinbo, 2006; Kliewer, 2003). Based on glutathione S-transferase pull-down assay (Johnson et al., 2006), this residue is not required for PXR-corepressor interaction either. Apparently, a comprehensive approach is needed to elucidate how substitution of glycine-36 with an arginine alters the ligand-dependent activation. Nevertheless, these findings suggest that substitutions, even not in the functionally important domains (e.g., DNA binding), may alter the ability to respond to a ligand.

PXR is recognized as a master regulator of so-called chemical elimination genes, and efficacious activation of PXR by 8-MOP suggests that this chemotherapeutic agent induces many drug-metabolizing enzymes and transporters. Clearly, clinical trials are needed to precisely estimate the extent of pharmacokinetic interaction resulting from PXR activation by 8-MOP. The lowest concentration used in this study is 10 μM (a typical concentration for in vitro induction study), which causes a threefold induction of CYP3A4 (Fig. 1A). In humans, a single oral dose of 8-MOP leads to the plasma Cmax of ~330 ng/ml, and in some individuals, the Cmax could be as high as 712 ng/ml.
metabolism in both humans and rodents (Busch et al., 1999). These plasma levels are equivalent to concentrations of 1.5–3.5 μM. It is likely that the plasma concentration would be higher in patients who receive repeated doses, and the liver C_{max} would be higher than the plasma C_{max} immediately after oral administration. Therefore, it is expected that therapeutic doses of 8-MOP can cause enzyme induction (probably less than threefold). In further support of this possibility, 8-MOP is only slightly less potent than RIF with respect to the induction of CYP3A4 in human primary hepatocytes (Fig. 1A), and RIF has been clinically documented to increase the metabolism of many drugs through enzyme induction (Chen and Raymond, 2006). It should be noted that topical administration of 8-MOP is increasingly used as an alternative in treating skin disorders, although oral administration effectively controls the dose. More importantly, topical application achieves higher concentrations in the skin but significantly lower plasma concentrations (Tegeder et al., 2002). Therefore, topical application (skin disorders only) likely minimizes the induction of drug-metabolizing enzymes.

In addition to PXR targets, 8-MOP has been shown to increase the expression of CYP1A1, a target of the aryl hydrocarbon receptor (AhR) (Baumgart et al., 2005; Xu et al., 2006). Although PXR and AhR are structurally similar, they share little similarity on the ligand-binding specificity (Xu et al., 2006). It remains to be determined whether 8-MOP binds AhR directly and whether such binding leads to the induction of CYP1A1. Given the fact that 8-MOP undergoes extensive metabolism in both humans and rodents (Busch et al., 1978; Mays et al., 1987a; Schmid et al., 1980), certain metabolites rather than the parent compound are likely responsible for the activation of AhR. Alternatively, 8-MOP increases the expression of AhR, which in turn transactivates the CYP1A1 gene (e.g., through endogenous ligand). In support of this possibility, RIF (an hPXR ligand) increases the expression of AhR and also induces CYP1A1 in human hepatocytes (Maglich et al., 2002). Interestingly, PCN (a potent activator of rodent PXR) causes no changes on the expression of AhR in mice.

Induction of drug-metabolizing enzymes is not the only mechanism by which 8-MOP causes drug-drug interactions. It is well established that 8-MOP acts as a mechanism-based inhibitor of CYP2A6, a primary enzyme that metabolizes nicotine (Sellers et al., 2000). Prolonged presence of nicotine by 8-MOP has been proposed to serve as a basis for treating tobacco dependency. 8-MOP has also been shown to inhibit CYP3A4-mediated metabolism, although the nature of the inhibition remains to be determined (Guo et al., 2000; Rheeders et al., 2006). Nevertheless, the inhibition likely plays a dominant role immediately after dosing (particularly coadministration), whereas the induction, on the other hand, likely plays a more dominant role after repeated dosing of 8-MOP, or 8-MOP is given before a drug that is metabolized by an enzyme induced by 8-MOP (e.g., a CYP3A4 substrate).

In summary, our work points to several important conclusions. First, 8-MOP markedly increases the expression of CYP3A4 and HCE2; therefore, 8-MOP, particularly repeated dosing, likely increases the elimination of drugs metabolized by these enzymes. Second, the induction by 8-MOP is achieved through the activation of PXR; thus, 8-MOP likely induces the expression of all PXR target genes. Third, the activation by 8-MOP varies among PXR variants; thus, the overall induction likely exhibits individual variation. Finally, 8-MOP is a naturally occurring phototoxic compound and comparably activates both hPXR and rPXR, further establishing that activation of PXR serves as a general protection mechanism cross-species (so-called evolutionarily conserved). Pharmacologically, the involvement of PXR in 8-MOP–mediated induction suggests that this chemotherapeutic agent causes a broader range of drug-drug interactions, and the differential activation of certain PXR variants suggests that the magnitude of such interactions varies from person to person.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants F05AT003019, R01ES07965, and R01GM61988. The authors thank Drs. Dolan of the University of Chicago and Kemper of the University of Illinois for providing plasmid constructs and Dr. Rodgers of the University of Rhode Island for critical reading of the manuscript.

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