Peroxisome Proliferator-activated Receptor α Induces Hepatic Expression of the Human Bile Acid Glucuronidating UDP-glucuronosyltransferase 2B4 Enzyme*

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Glucuronidation, a major metabolic pathway for a large variety of endobiotics and xenobiotics, is catalyzed by enzymes belonging to the UDP-glucuronosyltransferase (UGT) family. Among UGT enzymes, UGT2B4 conjugates a large variety of endogenous and exogenous molecules and is considered to be the major bile acid conjugating UGT enzyme in human liver. In the present study, we identify UGT2B4 as a novel target gene of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), which mediates the hypolipidemic action of fibrates. Incubation of human hepatocytes or hepatoblastoma HepG2 and Huh7 cells with synthetic PPARα agonists, fenofibric acid, or Wy 14643 resulted in an increase of UGT2B4 mRNA levels. Furthermore, treatment of HepG2 cells with Wy 14643 induced the glucuronidation of hyodeoxycholic acid, a specific bile acid UGT2B4 substrate. Analysis of UGT2B4 mRNA and protein levels in PPARα wild type and null mice revealed that PPARα regulates both basal and fibrate-induced expression of these enzymes in rodents also. Finally, a PPAR response element was identified in the UGT2B4 promoter by site-directed mutagenesis and electromobility shift assays. These results demonstrate that PPARα agonists may control the catabolism of cytotoxic bile acids and reinforce recent data indicating that PPARα, which has been largely implicated in the control of lipid and cholesterol metabolism, is also an important modulator of the metabolism of endobiotics and xenobiotics in human hepatocytes.

Glucuronide conjugation is a major metabolic pathway for numerous endogenous and exogenous compounds, including bile acids (BA), bilirubin, steroids, drugs, and environmental pollutants. This reaction consists in the transfer of the glucuronosyl group from UDP-glucuronic acid to the acceptor molecule (1). The addition of the glucuronosyl group on a compound results in a more water-soluble molecule, which can be excreted into bile or urine. Glucuronidation is catalyzed by enzymes belonging to the UDP-glucuronosyltransferase (UGT) family, and based on primary structure homology, UGT proteins have been divided into two major subfamilies, UGT1A and UGT2B (2). In humans, seven members of the UGT2B subfamily have been characterized: UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, and UGT2B28 (3, 4).

Among the UGT2B enzymes, UGT2B4 catalyzes the glucuronide conjugation of various molecules, including BAs, 5α-reduced androgens, catecholestrogens, and phenolic and monoterpenoid compounds (4–7). A certain degree of overlapping substrate specificity exists among the UGT2Bs, and these compounds are also conjugated by other UGT2B isoforms. However, various studies established the crucial role that UGT2B4 plays in hepatic BA glucuronide conjugation. Pilott et al. (7) carried out immunoprecipitation studies to demonstrate the strict substrate specificity of UGT2B4 for the 6α-hydroxylated BA hyodeoxycholic acid (HDCA) in human liver. Furthermore, no or low glucuronidation activity of HDCA was observed in colon where UGT2B4 is not expressed (8, 9). Finally, a recent study revealed that UGT2B4 expression is positively regulated by the BA sensor farnesoid X-receptor (FXR) and suggested that UGT2B4 induction by BAs may be part of a negative feedback mechanism by which BAs limit their biological activity and control their intracellular levels to avoid a pathophysiological accumulation (10).

An important consequence of BA glucuronidation is the introduction of an additional negative charge in the molecule that allows their transport by conjugate transporters such as the multidrug-resistance related proteins, MRP2 (ABCC2) and MRP3 (ABCC3), which are present in liver (11, 12), and favors their excretion in urine. Whereas BAs are biological detergents with numerous important functions, these compounds are inherently cytotoxic and perturbations in their normal synthesis, transport, or secretion can result in a variety of pathophysiological conditions including intrahepatic cholestasis (13). During their enterohepatic circulation, BAs undergo several metabolic alterations, including glucuronide conjugation at ring acid; LCA, lithocholic acid; PPAR, peroxisome proliferator-activated receptor α; RXR, retinoid X-receptor; PPRE, PPAR response elements; RT, reverse transcription; UDPGA, UDP-glucuronic acid; TLC, thin layer chromatography; ANOVA, analysis of variance; PXR, pregnane X-receptor; CMV, cytomegalovirus; CYP, cytochrome P450.
hydroxyl groups (7, 14). The most abundant glucuronide conjugate reported in human plasma is the primary BA chenodeoxycholic acid (CDCA) glucuronide followed by the secondary lithocholic acid (LCA) glucuronide (7, 15). In the urine of cholestatic patients, the proportion of BA glucuronide metabolites increases to up to 35% of total BAs (16, 17) and HDCA is exclusively found as a glucuronide derivative (18).

Peroxisome proliferator-activated receptors (PPARs) belong to the family of nuclear receptors that are ligand-activated transcription factors. Three distinct types of PPARs have been identified as PPARα, PPARγ, and PPARδ. Each isotype is encoded by a distinct gene and shows different distribution patterns (19, 20). Upon ligand activation, PPARs regulate gene transcription by dimerizing with the retinoid X-receptor (RXR) and binding to PPAR response elements (PPREs) within the regulatory regions of target genes (19). These PPREs usually consist of a direct repeat of the hexanucleotide AGGTCA sequence separated by one or two nucleotides (DR1 or DR2) (19). Furthermore, PPARs can also negatively interfere with pro-inflammatory transcription factor pathways by a mechanism termed transrepression (21). PPARα is highly expressed in various tissues such as liver, muscle, kidney, and heart where it stimulates the β-oxidative degradation of fatty acids (22). Natural eicosanoids derived from arachidonic acid via the lipoxygenase pathway, such as 8-hydroxytetraenoic acid, 15-hydroxytetraenoic acid, and leukotriene B4 as well as oxidized phospholipids, activate PPARα.

Natural eicosanoids derived from arachidonic acid via the lipoxygenase pathway, determining the ratio of cholic acid/CDCA (27). For RNA analyses, 10⁶ HepG2 or HuH7 cells were treated with Wy 14643 at the indicated concentrations in the presence or absence of 75 µM CDCA for 24 h. In all of the experiments, controls were incubated with an identical volume of Me₂SO (vehicle).

**RNA Analysis—**Total RNA was isolated from mice liver, primary human hepatocytes, HepG2, and HuH7 cells using TRIzol (Invitrogen). Northern blot analyses were performed as described previously (38) using human UGT2B4 and 28 S cDNAs as probes. For quantitative RT-PCR analyses of UGT2B4 gene expression, RNA was reverse-transcribed using random hexamer primers and 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen). Reverse-transcribed cRNA was amplified with an identical volume of Me₂SO (vehicle).

**Plasmid Cloning and Site-directed Mutagenesis—**The B4p-2400-pGL3 construct was obtained as described previously (10). The B4p-2084, B4p-1214, B4p-1149, and B4p-524 reporter constructs were generated by PCR amplification using Ffu Turbo polymerase (Stratagene) and 100 pmol of the sense oligonucleotides: B4-2004, 5'-CATCAGACTCTCAGTAGTTG-3', B4-1214, 5'-TTTTAATTTTATCATCAGAG-3'; and B4-1149, 5'-TTATAGGAGCCGATCAGAGG-3'; and B4-524, 5'-CATTCTTGAAATATACCTAG-3'. The reverse primer was pGL3-512, 5'-TATGCGATGCTCAGTACGAGAG-3'. PCR amplification was performed in a volume of 25 µl containing 100 nM of each primer, 4 mM MgCl₂, the Brilliant Quantitative PCR Core reagent kit mixture (Stratagene), and SYBR Green 0.33X (Sigma). The conditions were 95 °C for 10 min followed by 40 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. UGT2B4 mRNA levels were normalized to 28 S mRNA (36).

**Experimental Procedures**

**Animal Studies—**Animal studies were performed in compliance with European Union specifications regarding the use of laboratory animals. Details of experimental conditions have been described previously (33). Male Sprague-Dawley (n = 6) and PPARα null (−/−) (n = 6) mice (34) (kind gift of Dr. F. Gonzalez, National Cancer Institute, Bethesda, MD) were fasted for 17 days with a standard mouse chow diet containing 0.2% (wt/wt) fenofibrate or not. At the end of the treatment period, the animals were fasted for 4 h and sacrificed and livers were removed immediately, weighed, rinsed in 0.9% (w/v) NaCl, frozen in liquid nitrogen, and stored at −80 °C until total RNA or microsome preparation.

**Materials—**UDP-glucuronic acid (UDPGA), leupeptin, pepstatin, phosphatidylcholine, and BAs were obtained from Sigma. Human hepatoblastoma HepG2 cells were from the American Type Culture Collection (Manassas, VA). Restriction enzymes and other molecular biology reagents were from New England Biolabs (distributed by Ozyme, Saint-Quentin, France). Stratagene (La Jolla, CA), Promega, and Roche Applied Science. Protein assay reagents were obtained from Bio-Rad. The B4-2400-pGL3 plasmid was obtained by cloning six copies of the pCMV-β-galactosidase expression vector, and with or without 30 ng of the pS85-PPARα plasmid. All of the samples were comple
Statistically significant differences between vehicle- and Wy 14643-treated cells are indicated by asterisks. For Western blot, 25 μg of microsomal proteins were separated on a 10% SDS-polyacrylamide gel. The gel was transferred onto a nitrocellulose membrane, which was then hybridized with the anti-UGT2B2 antibody (dilution, 1/2000). An anti-rabbit IgG antibody conjugated with peroxidase was used as secondary antibody (dilution, 1/10000), and the resulting immunocomplexes were visualized using the Western blot Chemiluminescence Reagent Plus as specified by the manufacturer (PerkinElmer Life Sciences).

Glucuronidation Assay—HepG2 cells were resuspended in Tris-buffered saline containing 0.5 mM dithiothreitol and homogenized using a Brinkman Polytron. Enzyme assays were performed as described previously (5). 100 μg of cell homogenate were incubated with 25 μM [14C]UDP-glucuronic acid, 2 mM unlabeled UDPGA, and 200 μM HDCA in a final volume of 100 μl of glucuronidation assay buffer for 8 h (5). Assays were terminated by adding 100 μl of methanol, and the samples were centrifuged at 14,000 rpm for 2 min to remove the precipitated proteins. 100 μl of glucuronidation assays were applied onto a thin layer chromatography (TLC) plate (Merck) and migrated using a toluene:ethanol:water (80:20:1) mixture. The extent of HDCA glucuronidation was analyzed and quantified by PhosphorImager analysis.

Statistical Analyses—A nonparametric Mann-Whitney test was used to analyze for significant difference between the experimental groups. Analyses of variance (ANOVA) and Tukey post-hoc tests were used for analysis of the effects of simultaneous treatment with FXR and PPARα agonists.

RESULTS

PPARα Activators Induce UGT2B4 Expression in Human Hepatocytes

Primary human hepatocytes were treated with fenofibric acid (250 μM) for 24 h, and UGT2B4 mRNA levels were determined by Northern blot analysis. A significant increase in UGT2B4 mRNA was observed in fenofibric acid-treated cells.
compared with vehicle-treated cells (Fig. 1a). With the cDNA sequences of the different human UGT2B isoforms being >85% homologous, a UGT2B4-specific real time RT-PCR method was used to specifically quantify UGT2B4 mRNA levels in hepatocytes treated for 6, 12, 24, or 48 h with fenofibric acid (250 μM) (Fig. 1b). Fenofibric acid rapidly induced UGT2B4 expression, because a maximal 10-fold increase in the concentration of UGT2B4 transcripts was observed within 12 h (Fig. 1b).

To further characterize the PPARα-dependent regulation of UGT2B4 expression, human hepatoma HepG2 and Huh7 cells were incubated in the presence of increasing concentrations of the PPARα ligand Wy 14643 (Fig. 2, a and b). UGT2B4 mRNA levels were induced in a dose-dependent manner to a maximum of 2.7- and 2.3-fold activation in HepG2 and Huh7 cells, respectively (Fig. 2, a and b).

**PPARα Activators Induce UGT2B4 Activity in HepG2 Cells**

To determine whether PPARα activation of UGT2B4 expression modifies its activity, HepG2 cells were treated with Wy 14643 (75 μM) for 36 h and their glucuronidation activity was analyzed using the UGT2B4-specific substrate HDCA (Fig. 3a). Treatment with Wy 14643 provoked a 3-fold increase in HDCA glucuronidation (Fig. 3b), thus demonstrating that PPARα agonists induce UGT2B4 activity.

**PPARα Gene Disruption Abolishes Fibrate Induction of UGT2B mRNA and Protein Levels in Mouse Liver**

The PPARα-dependent induction of UGT2B2 expression was measured in male Sv129 homozygous wild type (+/+) and PPARα-null (−/−) mice by Northern blotting (Fig. 4a). In wild type mice, fenofibrate treatment resulted in an ~5-fold increase of UGT2B2 mRNA levels compared with vehicle-treated animals. Interestingly, UGT2B2 transcripts were undetectable by this method in both fenofibrate- and vehicle-treated PPARα-null mice. As control, 36B4 mRNA levels were similar in all of the groups. These data indicate that PPARα is a crucial regulator of basal and fibrate-activated murine UGT2B2 gene expression.

To determine whether PPARα also regulates murine UGT2B2 protein levels, liver microsomes from wild type and PPARα-null mice were subjected to Western blot analysis using an anti-UGT2B2 antibody. In wild type mice, a pronounced increase in UGT2B2 protein levels was observed in fenofibrate-treated compared with vehicle-treated animals (Fig. 4b). As for their mRNAs, UGT2B2 protein levels were almost undetectable in liver microsomes from PPARα-null mice and treatment with fenofibrate failed to increase UGT2B2 protein concentration (Fig. 4b). In fact, longer exposure of the Western blot revealed the presence of low amounts of UGT2B2s in PPARα-null mice, which were not affected by fenofibrate treatment (data not shown). These results clearly demonstrate that, similar to human UGT2B4, murine UGT2B2 enzymes are positively regulated PPARα target genes.

**PPARα Activates the UGT2B4 Gene Promoter**

To decipher the molecular mechanisms of human UGT2B4 induction by PPARα activators, a 2.4-kb fragment of the UGT2B4 gene promoter cloned in front of the pGL3-luciferase reporter gene was transfected into HepG2 cells in the presence or absence of a PPARα expression plasmid. Transfected cells were subsequently treated with the PPARα ligand Wy 14643 (Fig. 5). Wy 14643 alone slightly induced UGT2B4 promoter activity, whereas co-transfection of PPARα significantly enhanced Wy 14643-induced promoter activity to ~3-fold in HepG2 cells (Fig. 5). To localize the region within the UGT2B4 promoter that confers transcriptional responsiveness to PPARα ligands, serial deletions from −2084 to −524 bp of the UGT2B4 promoter were also co-transfected with or without the expression vector for PPARα (Fig. 5). A marked increase in reporter activities of the two larger fragments (−2084 and −1214 bp) was observed in HepG2 cells treated with Wy 14643, and co-
transfection of PPARα further increased the activities of the two constructs (Fig. 5). Further 5' deletion (−1149 and −524 bp) constructs were no longer induced by Wy 14643-activated PPARα, indicating that the region between −1214 and −1149 bp mediates the effect of PPARα ligands on the UGT2B4 promoter. Identical results were obtained when these reported constructs were co-transfected with or without PPARα in HepG2 cells (data not shown).

Identification of a PPRE within the UGT2B4 Gene Promoter

Consensus DR1 sites have been previously reported to bind the PPARα/RXR heterodimer (19). A computer-assisted analysis (42) of the −1214/−1149 region of the UGT2B4 promoter revealed the presence of a degenerated DR1 sequence, TGAACCTTAATCT, at positions from −1193 to −1180. To test whether this site mediates the induction by PPARα, mutations were introduced in this site in the context of the −2400 bp UGT2B4 promoter constructs (Fig. 6a). Mutation of this site abolished the induction of UGT2B4 promoter activity by Wy 14643. Furthermore, the UGT2B4 DR1 site was cloned in multiple copies upstream of the thymidine kinase minimal promoter-driven luciferase reporter TKpGL3. The resulting constructs (100 ng) were co-transfected with the pCMV-β-galactosidase plasmid (50 ng) in HepG2 cells in the presence or absence of pSG5-hPPARα (30 ng). Cells were subsequently treated or not with Wy 14643 (50 μM) for 24 h. Values are expressed as fold induction of the controls (pGL3) set at 1 normalized to internal β-galactosidase activity as described under “Experimental Procedures.” Values represent the means ± S.D.
was further enhanced by the addition of the PPARα ligand (Fig. 6b). By contrast, no change in activity was observed when either the empty TKpGL3 vector (Fig. 6b) or the TKpGL3 vector containing three copies of the mutated DR1 (data not shown) was transfected. These results indicate that the −1193 to −1180 site in the UGT2B4 promoter is a positive PPRE.

To demonstrate that PPARα binds to the PPRE identified in the UGT2B4 gene promoter, EMSAs were performed using a probe spanning nucleotides from −1199 to −1175 (B4-PPREwt) in the presence of in vitro translated PPARα and RXR proteins (Fig. 7). As expected, neither RXR nor PPARα alone bound the probe (Fig. 7a, lanes 2 and 3). By contrast, a clear shift was observed when this oligonucleotide was incubated in the presence of both RXR and PPARα (Fig. 7a, lane 4). Furthermore, this complex was supershifted by the anti-PPARα antibody (lane 5), thus demonstrating that the PPARα/RXR heterodimer specifically binds the −1193 DR1 site. By contrast, no protein-DNA complex was observed when mutated probes in the 5' and 3' half-sites (B4-PPREmt5' and B4-PPREmt3', respectively) were tested (Fig. 7b, lanes 5–12). For competition experiments, increasing amounts (1-, 10-, and 50-fold excess) of unlabeled oligonucleotides encompassing either a consensus DR1 site (DR1cons), the B4-PPREwt, or the B4-PPREmt5' oligonucleotides in EMSA with unprogrammed reticulocyte lysate, RXR, and/or PPARα.

(7c). By contrast, the mutated B4-PPREmt3' did not compete for PPARα binding to the DR1. Taken together, these data demonstrate that PPARα binds to the PPRE site at nucleotides from −1193 to −1180 in the UGT2B4 gene promoter.

PPARα and FXR Activators Additively Induce UGT2B4 Expression

We previously reported that CDCA-activated FXR positively regulates the expression of UGT2B4 in human hepatocytes and HepG2 cells (10). To test whether ligand-activated FXR and PPARα can cooperate to regulate UGT2B4 expression, HepG2 cells were treated for 24 h with Wy 14643, CDCA, or both Wy 14643 and CDCA together. As expected, UGT2B4 mRNA levels were induced 2.6-fold by Wy 14643, whereas CDCA-induced UGT2B4 gene expression was 10-fold (Fig. 8). Interestingly, cells treated with both PPARα and FXR activators contained 14-fold higher concentrations of UGT2B4 transcripts, indicating that the two receptors coordinately regulate UGT2B4 gene expression.

DISCUSSION

In this study, we identify the human UGT2B4 enzyme as a positively regulated PPARα target gene. UGT2B4 induction by fibrates occurs via PPARα binding to a PPRE in the UGT2B4 promoter. Furthermore, we show that fenofibrate induces hepatic UGT2B mRNA and protein levels only in Sv129 wild type
mice, whereas a drastically lowered expression of UGT2Bs is observed in livers from PPARα-null mice treated or not with fenofibrate. This observation demonstrates that PPARα is a crucial regulator of both human UGT2B4 and murine UGT2B enzyme expression. Interestingly, PPARα gene disruption also critically reduced the basal expression of mitochondrial fatty acid-metabolizing enzymes such as very long chain acyl-CoA dehydrogenase, long chain acyl-CoA dehydrogenase, and long chain acyl-CoA synthetase enzymes (43). Thus, the present findings demonstrate that in mice, PPARα plays a crucial role in the constitutive expression of not only mitochondrial fatty acid-metabolizing enzymes but also microsomal UGT2B enzymes.

Considering the major role that UGT2B4 plays in BA glucuronidation, we hypothesized that UGT2B4 induction following PPARα activation may affect BA glucuronidation in HepG2 cells. Indeed, we observed that Wy 14643-dependent PPARα activation provoked a 2-fold increase of HDCA-glucuronidation activity. HDCA is a 6α-hydroxylated metabolite of LCA, which is primarily excreted as a glucuronide derivative in urine (18, 44). Because of its high degree of lipophilicity, LCA is a potent cholestatic agent and possesses an elevated cytotoxicity (45, 46). However, conjugation of LCA with sulfate, a conjugation reaction catalyzed by the dehydroepiandrosterone sulfotransferase (SULT2A1) enzyme, allows an increased hydrosolubility of LCA and facilitates its biliary excretion (47–49). In addition to sulfation, LCA is efficiently 6α-hydroxylated into HDCA by the hepatic CYP3A4 enzyme, and this modification facilitates its glucuronidation by UGT2B4 at the 6α-hydroxy position prior to renal excretion (50). Thus, glucuronidation of HDCA has been proposed as an alternative mechanism for reducing the hepatic toxicity of monohydroxylated LCA (44, 50). Recent studies indicate that the BA sensors pregnane X-receptor (PXR) and FXR play important roles in LCA detoxification. As such, activation of PXR induces both SULT2A1 and CYP3A4 expression, whereas BA-activated FXR stimulates SULT2A1 and UGT2B4 expression (10, 44, 49, 51). Results from the present study prove that PPARα also participates in the control of LCA detoxification in addition to PXR and FXR (Fig. 9). Furthermore, PPARα, FXR, and PXR inhibit CYP7A1 expression (26, 52, 53), thus suggesting that the three receptors may cooperate to control BA homeostasis and detoxification by both reducing BA synthesis and inducing their metabolism (Fig. 9). Recently, PPARα was identified as a FXR target gene, thus providing molecular evidence for a cross-talk between the FXR and PPARα transcriptional pathways in humans (54). Considering that UGT2B4 expression is also up-regulated upon CDCA activation of FXR (10), we investigated whether this cross-talk between FXR and PPARα can affect UGT2B4 expression in HepG2 cells. We observed that upon ligand activation, PPARα and FXR act in concert to stimulate BA glucuronidation. Overall, these results demonstrate that FXR and PPARα control not only the same BA-metabolizing enzyme but also share cooperative activity to induce BA glucuronidation catalyzed by UGT2B4. It would be interesting to determine whether a sim-

**FIG. 8.** PPARα and FXR induce UGT2B4 gene expression in an additive manner. HepG2 cells were treated for 24 h with Wy 14643 (75 μM), CDCA (75 μM), or both Wy 14643 and CDCA. UGT2B4 mRNA levels were measured by real-time RT-PCR and expressed relative to control set as 1. Values are means ± S.D. (n = 6). Values followed by different letters are statistically significantly different from each other (ANOVA followed by Mann-Whitney test, p < 0.01).

**FIG. 9.** PPARα participates with FXR and PXR in the control of bile acid homeostasis and detoxification. By inhibiting CYP7A1 expression and inducing CYP3A4, SULT2A1, and UGT2B4 enzyme expression, FXR, FXR, and PPARα form a cluster of ligand-activated transcription factors that control bile acid homeostasis and reduce bile acid toxicity. *HDCA-G*, HDCA-glucuronide; *LCA-S*, LCA-sulfate; *SULT2A1*, sulfotransferase 2A1.
UGT2B4 is considered to be the specific BA-conjugating UGT enzyme in a human liver, although it also participates to the glucuronide conjugation of a wide variety of endogenous or xenobiotic compounds. As such, various C19-steroids such as androstane-3α,17β-diol are substrates for UGT2B4 (5, 6). In the Helsinki Heart Study population, gemfibrozil treatment resulted in a 3-fold elevation of plasma 3α,17β-diol glucuronide levels (31), which may reflect an increased expression and activity of UGT2B4 in these patients. A recent study in non-human primates revealed that UGT enzymes expressed in androgen target tissues glucuronidate, preferentially C19-steroids (55), suggesting that UGTs participate in the control of intracellular levels of the active androgen. It would be of interest to determine whether PPARs activation also affects androgen glucuronidation in a tissue such as the prostate where both PPARs and UGT2B4 are expressed (5, 56). Based on the present study, it is tempting to speculate that fibrate treatment may induce androgen glucuronidation and that PPARs can be a potential regulator of androgen levels in such a tissue.

UGT2B4 is also involved in the inactivation of various xenobiotics, such as phenolic and monoterprenoid compounds (4, 5, 7). Interestingly, Kok et al. (29) reported that ciprofibrate induces the hepatic expression of the multidrug resistance (Mdr2) gene in a PPARα-dependent manner in mice. P-glycoprotein, the Mdr2 gene product, is a hepatocyte transporter located on the canalicular membrane (57), which has a broad substrate specificity that encompasses glucuronide conjugates of a variety of endobiotics and xenobiotics (29, 57–59). Thus, by stimulating both glucuronidation and transport, PPARα appears to be a key factor for the elimination of many endogenous and exogenous glucuronic derivatives from the liver, at least, in rodents. The role of different nuclear receptors, such as PXR and constitutive androstane receptor, in the control of xenobiotic metabolism has been more extensively studied, whereas PPARα received less attention regarding xenobiotic metabolism and regulation enzyme expression has been fully characterized, whereas PPARs receives less attention regarding xenobiotic metabolic regulation enzyme expression regulation. Nevertheless, the present findings added to previous reports indicate that PPARα is also an important xenobiotic sensor that regulates both phase I (CYP1A2, 2A1, 2B1, and 2B2) and phase II (glutathione S-transferases A1, glutathione S-transferase M2, UGT1A9, and UGT2B4) enzymes (60–63).

In conclusion, the present study illustrates for the first time the implication of PPARs in the control of BA glucuronidation and more generally reinforces the role of this nuclear receptor as a regulator of endobiotic and xenobiotic metabolism.

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