A role for FXR and human FGF-19 in the repression of paraoxonase-1 gene expression by bile acids

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Abstract Paraoxonase-1 (PON1), an enzyme that metabolizes organophosphate insecticides, is secreted by the liver and transported in the blood complexed to HDL. In humans and mice, low plasma levels of PON1 have also been linked to the development of atherosclerosis. We previously reported that hepatic Pon1 expression was decreased when C57BL/6J mice were fed a high-fat, high-cholesterol diet supplemented with cholic acid (CA). In the current study, we used wild-type and farnesoid X receptor (FXR) null mice to demonstrate that this repression is dependent upon CA and FXR. PON1 mRNA levels were also repressed when HepG2 cells, derived from a human hepatoma, were incubated with natural or highly specific synthetic FXR agonists. In contrast, fibroblast growth factor-19 (FGF-19) mRNA levels were greatly induced by these same FXR agonists. Furthermore, treatment of HepG2 cells with recombinant human FGF-19 significantly decreased PON1 mRNA levels. Finally, deletion studies revealed that the proximal −230 to −96 bp region of the *PON1* promoter contains regulatory element(s) necessary for promoter activity and bile acid repression. These data demonstrate that human *PON1* expression is repressed by bile acids through the actions of FXR and FGF-19.—Shih, D. M., H. R. Kast-Woelbern, J. Wong, Y-R. Xia, P. A. Edwards, and A. J. Lusis. **A role for FXR and human FGF-19 in the repression of paraoxonase-1 gene expression by bile acids. *J. Lipid Res.* 2006.** 47: **384–392.**

Supplementary key words gene regulation • high density lipoprotein • atherosclerosis • mouse • c-Jun N-terminal kinase • farnesoid X receptor • fibroblast growth factor-19

Serum paraoxonase-1 (PON1) is a 45 kDa protein that is transported in the blood in association with HDL (1). The protein was initially identified by its ability to hydrolyze and detoxify organophosphate insecticides (2). Thus, PON1 may play an important role in organophosphate detoxification in vivo (3, 4). Subsequently, PON1 was shown to exhibit lactonase activities against various substrates, including homocysteine thiolactone (5–7). In addition, PON1 prevents LDL oxidation in vitro (8–10). Consistent with the latter observation, low circulating PON1 levels have been associated with increased risk for coronary artery disease (11–13). In animal studies, PON1-deficient mice exhibited enhanced susceptibility to atherosclerosis (14, 15), whereas transgenic mice expressing increased levels of human PON1 were more resistant to diet-induced atherosclerosis (16). These data suggest that high PON1 levels are atheroprotective in both humans and mice.

Circulating PON1 levels vary by >10-fold in human populations as a result of genetic and environmental factors (17). Although several genetic polymorphisms in the human *PON1* promoter have been shown to influence plasma PON1 levels (17), the polymorphism C-108T had the greatest influence. The C-108 allele is associated with a doubling in circulating PON1 levels, compared with the T-108 allele (17). The C-108T polymorphism is located within a consensus sequence for binding of the transcription factor Sp1 (17). Other factors that reduce PON1 levels include diets enriched in either *trans*-unsaturated fat (18) or oxidized lipids (19), smoking (20, 21), diabetes (22, 23), and inflammatory cytokines (24–26). On the other hand, light alcohol consumption (27, 28), pomegranate juice (29), fibrates (30), and simvastatin (31) have been shown to increase PON1 levels. Simvastatin appears to increase PON1 expression through the activation of sterol-regulatory element binding protein-2 (SREBP-2), which binds to sterol-regulatory element-like sites located within the proximal promoter region of the human *PON1*

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Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; CYP7A1, cholesterol 7α-hydroxylase; FGF-19, fibroblast growth factor-19; FGFR4, fibroblast growth factor receptor 4; FXR, farnesoid X receptor; HNF-4, hepatocyte nuclear factor 4; JNK, c-Jun N-terminal kinase; PON1, paraoxonase-1; RXR, retinoid X receptor; SHP, small heterodimer partner; SREBP-2, sterol-regulatory element binding protein-2.

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gene (31). Dietary polyphenols, such as quercetin, have been shown to increase *PON1* gene expression by an aryl hydrocarbon receptor-dependent mechanism (32, 33). Deletion and mutation studies also identified a xenobiotic-responsive element-like sequence located at −112 to −108 bp of the human *PON1* promoter that mediated the effect of quercetin (32).

Bile acids have been identified as the endogenous agonists for the nuclear hormone receptor farnesoid X receptor (FXR) (34–36). FXR forms a heterodimer with retinoid X receptor (RXR) and regulates the expression of genes involved in bile acid and lipid homeostasis (37, 38). Activation of the FXR/RXR heterodimer by bile acids induces the expression of a number of genes, including small heterodimer partner (SHP), a non-DNA binding transcriptional repressor. SHP, in turn, binds to and inhibits various transcription factors, including liver receptor homolog-1. Consequently, liver receptor homolog-1-dependent genes, such as cholesterol 7α-hydroxylase (CYP7A1), are repressed. Because CYP7A1 regulates bile acid synthesis, the net result of FXR activation is the repression of bile acid production (38). The expression of SREBP-1c, a transcription factor involved in regulating fatty acid synthesis and lipogenesis, is also repressed by treatment of FXR agonists via the activation of FXR and the induction of SHP (39, 40).

The human gene *fibroblast growth factor-19* (*FGF-19*) was recently identified as an FXR target gene (41). FGF-19 is a secreted growth factor that signals through the cell-surface receptor tyrosine kinase, fibroblast growth factor receptor 4 (FGFR4) (42). FGF-19 strongly inhibits the expression of hepatic CYP7A1 when added to primary cultures of human hepatocytes or after its administration to mice (41). This inhibition is reported to involve the c-Jun N-terminal kinase (JNK)-dependent pathway (41). Interestingly, FGFR4-deficient mice exhibited increased hepatic expression of CYP7A1, increased excretion of bile acids, and increased size of the bile acid pool (43). In contrast, transgenic mice overexpressing a constitutively active human FGFR4 in the liver exhibited repressed levels of CYP7A1 and decreased excretion of bile acids and the bile acid pool, compared with nontransgenic controls (44). These studies confirm that hepatocyte FGFR4 plays an important role in maintaining bile acid homeostasis. However, the regulation of CYP7A1 by bile acids can also occur via FXR-independent pathways that appear to involve the JNK pathway as well (45, 46).

We previously observed that PON1 plasma levels and hepatic mRNA levels are reduced in C57BL/6J mice fed an "atherogenic" diet containing high levels of cholesterol, fat, and 0.5% cholic acid (CA) (47). In this study, we identify CA as the major component in the atherogenic diet responsible for the reduction of PON1 expression. Further studies revealed that bile acids decrease the expression of the PON1 gene in mouse and in a human hepatoma cell line through the action of the nuclear receptor, FXR. Finally, the data presented here suggest that FGF-19 and the JNK pathway are involved in the repression of *PON1* by bile acids.

EXPERIMENTAL PROCEDURES

Materials

The FXR-specific agonist GW4064 was a gift from Patrick Maloney (GlaxoSmithKline, Research Triangle Park, NC). Chenodeoxycholic acid (CDCA) was from Sigma. The JNK inhibitor SP600125 was from Biomol Research Laboratories, Inc. (Plymouth, PA). The recombinant human FGF-19 was from R&D Systems (Minneapolis, MN). The sources of other reagents and cDNA plasmids have been described previously (47, 48).

Mice, diets, and the measurement of plasma PON1 activity

The FXR null ($FXR^{-/-}$) mouse was generated as described (49) and was backcrossed to a C57BL/6J background. In one set of experiments, female $FXR^{-/-}$ mice and wild-type littermates were fed a control chow diet or a chow diet supplemented with 1% sodium cholate (Teklad Research Diets, Madison, WI) for 2 days before euthanasia. Before euthanasia, mice were fasted for 16 h before collection of blood and tissues. Plasma PON1 levels were then determined by paraoxonase activity assay as described previously (14). In a second study, female C57BL/6J mice were fed either a chow diet or a chow diet supplemented with 0.25% CA (chow/CA), or high fat (15.75%) and high cholesterol (1.25%) (HF/HC), or the same high-fat, high-cholesterol diet supplemented with 0.25% CA (HF/HC/CA) (Teklad Research Diets) for 10 days before euthanasia.

Cell culture and treatment of HepG2 cells

HepG2 cells were maintained in 6 cm cell culture dishes with MEM (Cellgro; Mediatech, Inc., Herndon, VA) plus 10% fetal bovine serum (Omega Scientific, Tarzana, CA), 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 1 U/ml penicillin, 1 μg/ml streptomycin, and 0.25 μg/ml Fungizone. The HepG2 cells were grown to near confluence before treatment. The cells were then washed twice with PBS and incubated in medium containing 10% charcoal/dextran-treated fetal bovine serum (Hyclone, Logan, UT) and various ligands and inhibitors as described in the figure legends. CDCA or the FXR-specific ligand GW4064 was dissolved in DMSO, as 1,000× stocks, and added to the cells for 20–24 h of treatment. The control cells received 0.1% DMSO (vehicle). The JNK inhibitor SP600125 was dissolved in DMSO and added to the cells 30 min before the addition of CDCA, GW4064, or FGF-19. Where indicated, cells were treated for 6 h with recombinant human FGF-19 reconstituted in PBS containing 0.1% BSA or with buffer/0.1% BSA (control).

Northern blot analysis and real-time RT-PCR

Total RNA was isolated from liver or cells using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Northern blot analyses were performed as described previously (47, 48). For real-time quantitative RT-PCR, 1 μg of total RNA from each sample was treated with 1 unit of DNase I, amplification grade (Invitrogen), according to the manufacturer's protocol. The DNase I-treated total RNA was then reverse-transcribed into first-strand cDNA using the ThermoScript RT-PCR system (Invitrogen) according to the manufacturer's protocol. Real-time RT-PCR was then performed using the Quanti-Tect SYBR Green PCR kit (Qiagen, Valencia, CA) in an ABI Prism 7700 cycler. Serial dilutions of cDNA pooled from the samples were used as standards. Both samples and standards were assayed in duplicate. The primer pairs used for the amplification of various cDNAs were as follows: human PON1, CTA TGA CTC AGA GAA TCC TCC TGC ATC AG and CAT GGG TGC AAA TCG GTC TGT TAG AGC; human SHP, AGT GGC TTC AAT GCT

GTC TGG AGT C and AAG CAT GTC CCC AAG AAG GCC A; human FGF-19, CAC GGG CTC TCC AGC TGC TTC CTG CG and TCC TCC TCG AAA GCA CAG TCT TCC TCC G; human CYP7A1, TAG GAA CCC AGA AGC AAT GAA AGC AGC and GGA TGT TGA GGG AGG CAC TGG AAA GC; human SREBP-1c, GGA TTG CAC TTT CGA AGA CAT G and AGG ATG CTC AGT GGC ACT G; human SREBP-2, GCT GAA GCT GGC AAA TCA AAA GAA C and TCA TCC AAT AGA GGG CTT CCT GGC T; human phospholipid transfer protein, GAG GAA GAG CGG ATG GTG TAT GTG G and GGC AAT GGT GAC GCT AGC AGT GAC A; and human GAPDH, GTC ATC ATC TCT GCC CCC TCT GCT G and CGA CGC CTG CTT CAC CAC CTT CTT G. The PCR conditions for all cDNAs except FGF-19 were 95°C for 15 min, 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 1 min, and 4°C hold. The PCR conditions for FGF-19 were 95°C for 15 min, 40 cycles of 95°C for 15 s, 62°C for 30 s, and 72°C for 1 min, and 4°C hold. After PCR, melting curve analysis and gel electrophoresis of the products were performed to ensure the specificity of the assay.

Human *PON1* promoter-luciferase reporter gene constructs and transient transfection studies

A 1.5 kb DNA fragment from −1,487 to −2 bp (where +1 is the start of translation) of the human *PON1* promoter region was amplified by PCR using a bacterial artificial chromosome clone containing the human *PON1* gene (GenBank accession number AC004022). The sequence of the upper primer, hPON1(−1,487), was 5′-AGG GTA CCG CAT ACT CAA CTC ATA GCC ACA T-3′, and the sequence of the lower primer, hPON1(−2), was 5′-TTC TCG AGT CGG GGA TAG ACA AAG GGA TCG A-3′. This fragment was cloned into the *Kpn*I/*Xho*I sites of the promoterless luciferase reporter gene construct pGL3-basic (Promega, Madison, WI), and the sequence of the promoter was verified by DNA sequencing. A series of shorter human *PON1* promoter-luciferase constructs were then constructed using one of the following upper primers, hPON1(−831), hPON1(−523), hPON1(−230), and hPON1(−96), in combination with the lower primer, hPON1(−2). These shorter promoter DNA fragments were verified by sequencing and cloned into the *Kpn*I/*Xho*I sites of pGL3-basic as well. The DNA sequences of hPON1(−831), hPON1(−523), hPON1(−230), and hPON1(−96) were AGG GTA CCT TCT TCA GCA GAG GGT ATT CCT T, AGG GTA CCG GTG TTG AGA GAA AAA TGC TTG A, AGG GTA CCT GGA AGG AGC AAA ATG GGA CTT T, and AGG GTA CCA TCG GCG CTG CCC CAG CA, respectively.

For transfection studies, HepG2 cells were plated onto 48-well cell culture plates at a density of 1.3×10^5/well. After 24 h, the cells were transfected using 50 ng of a control plasmid expressing β-galactosidase and 100 ng of the promoter/luciferase reporter gene construct in the presence or absence of plasmids expressing FXRα2 and RXRα by the calcium phosphate precipitate method. The cells were incubated with the DNA precipitate for 3.5 h, and at the end of incubation, the cells were washed once with PBS and then treated in medium containing vehicle (0.1% DMSO) or ligands for 20–24 h before harvest. Luciferase activities in each well were then determined and normalized for β-galactosidase activity.

Statistical analysis

Student's *t*-test was used for statistical analysis.

RESULTS

PON1 suppression by bile acids is dependent upon FXR

We previously observed that PON1 plasma levels and hepatic mRNA levels are decreased in C57BL/6J mice fed an atherogenic diet containing high levels of cholesterol, fat, and 0.5% CA (47). Because bile acids alone have been shown to downregulate *CYP7A1* gene expression through the activation of FXR (38), we hypothesized that PON1 expression may be repressed by bile acids through a similar mechanism. To test this hypothesis, wild-type and $FXR^{-/-}$ mice were fed for 2 days either a normal chow diet or the same diet supplemented with 1% CA. We observed that hepatic PON1 mRNA (**Fig. 1A**) and plasma PON1 activity levels (Fig. 1B) of the chow-fed $FXR^{-/-}$ mice were the same as those of the chow-fed wild-type mice, indicating that the loss of FXR did not affect PON1 expression when bile acid levels were low. In response to dietary CA, PON1 mRNA levels were decreased by 37% in wild-type mice (Fig. 1A). In contrast, PON1 mRNA levels were unaffected in the CA-fed $FXR^{-/-}$ mice (Fig. 1A). In agreement with the hepatic PON1 mRNA levels, plasma PON1 activity levels were also decreased by 35% in wild-type mice,

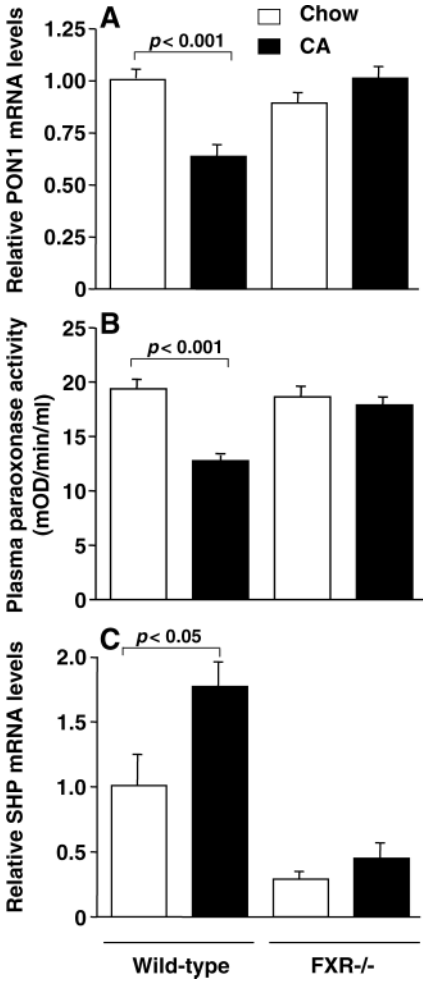

Fig. 1. Cholic acid (CA) feeding decreases hepatic paraoxonase-1 (PON1) mRNA levels and plasma PON1 levels in wild-type mice but not in farnesoid X receptor-null ($FXR^{-/-}$) mice. Hepatic PON1 (A) and small heterodimer partner (SHP; C) mRNA levels, and plasma PON activity levels (B), were determined in mice fed for 2 days either a chow diet or a chow diet supplemented with 1% CA. Values are shown as means ± SEM (n = 6 mice/group). OD, optical density.

but not in FXR$^{-/-}$ mice, in response to dietary CA (Fig. 1B). As expected, hepatic SHP mRNA levels were induced in wild-type mice but not in FXR$^{-/-}$ mice (Fig. 1C).

In a separate study, wild-type mice were fed for 10 days a chow diet, a chow diet supplemented with either 0.25% CA (chow/CA) or high fat/high cholesterol (HF/HC), or the same high-fat, high-cholesterol diet supplemented with 0.25% CA (HF/HC/CA). FXR$^{-/-}$ mice were not included in the study because long-term feeding of diets containing CA is toxic (50). The data in **Fig. 2A** demonstrate that hepatic PON1 mRNA levels were decreased by 32–36% after administration of the CA-supplemented diet. As expected, hepatic SHP mRNA levels were increased significantly (2.5-fold) when the diet contained CA (Fig. 2B). Interestingly, PON1 mRNA levels were repressed (22%) and SHP mRNA levels were induced 1.7-fold in mice fed the HF/HC diet in the absence of CA (Fig. 2A, B). We hypothesize that the changes in PON1 and SHP expression in mice fed the HF/HC diet resulted from an increase in the bile acid pool and activation of FXR. Mice fed the HF/HC/CA diet exhibited the greatest repression of PON1 (50%) and induction of SHP (3.6-fold) compared with the chow-fed mice (Fig. 2).

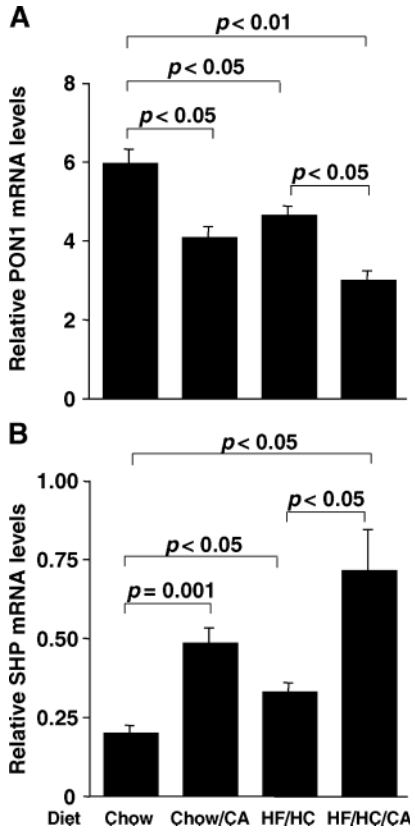

Fig. 2. Both CA and high-fat, high-cholesterol supplementation reduced hepatic PON1 expression in wild-type mice. Hepatic PON1 (A) and SHP (B) mRNA levels were determined in wild-type mice fed the indicated diets for 10 days: chow diet, chow diet supplemented with 0.25% CA (chow/CA) or high fat (15.75%) and high cholesterol (1.25%) (HF/HC), or the same high-fat, high-cholesterol diet supplemented with 0.25% CA (HF/HC/CA). Values shown are means ± SEM (n = 4 or 5 mice/group).

Bile acids and FXR ligands reduce PON1 mRNA in HepG2 cells

To examine the regulation of *PON1* in detail, we used the human hepatoma cell line HepG2. **Figure 3A** shows that treatment of HepG2 cells for 20 h with CDCA (100 μM) or GW4064 (1 μM), a highly specific FXR agonist, reduced the expression of PON1 mRNA levels by ~65%. As expected, SHP (Fig. 3B) and FGF-19 (Fig. 3C) mRNA levels were induced, whereas SREBP-1c mRNA levels were repressed (Fig. 3D), after incubation of the cells with either CDCA or GW4046. In contrast, SREBP-2 mRNA levels were unaffected by treatment of FXR agonists (data not shown). Together, the data in Figs. 1 and 3 suggest that the repression of PON1 mRNA levels by bile acids is dependent upon FXR and that SHP or FGF-19 may play a role in this repression.

FGF-19 reduces PON1 mRNA levels in HepG2 cells

To determine whether FGF-19 has a direct effect on *PON1* expression, HepG2 cells were treated with recombinant human FGF-19 for 6 h in the presence or absence of a JNK-specific inhibitor, SP600125. As shown in **Fig. 4A** (lanes 1–3), FGF-19 repressed PON1 mRNA levels in a dose-dependent manner. This repression was attenuated when the cells were pretreated with SP600125 (Fig. 4A, lane 5). In contrast, PON1 mRNA levels were unaffected after incubation of the cells for 6 h with SP600125 in the absence of FGF-19 (Fig. 4A, lane 4). Figure 4B demonstrates that CYP7A1 mRNA levels were also repressed by the FGF-19 treatment by a mechanism that was attenuated

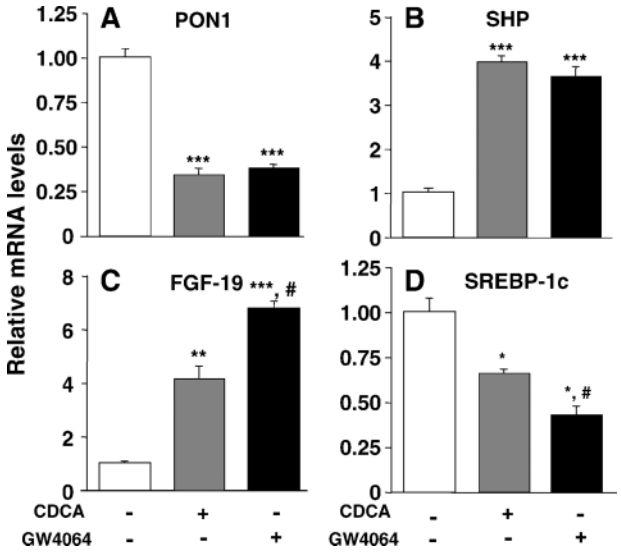

Fig. 3. Gene expression in HepG2 cells after activation of FXR. The mRNA levels of PON1 (A), SHP (B), fibroblast growth factor-19 (FGF-19; C), and sterol-regulatory element binding protein-1c (SREBP-1c; D) are shown. HepG2 cells were treated in quadruplicate with 0.1% DMSO, chenodeoxycholic acid (CDCA; 100 μM), or GW4064 (1 μM) for 20 h before analysis of RNA by quantitative RT-PCR. Values are shown as means ± SEM. * $P < 0.05$, ** $P < 0.001$, and *** $P < 0.0001$ versus the DMSO group; # $P < 0.05$ versus the CDCA group.

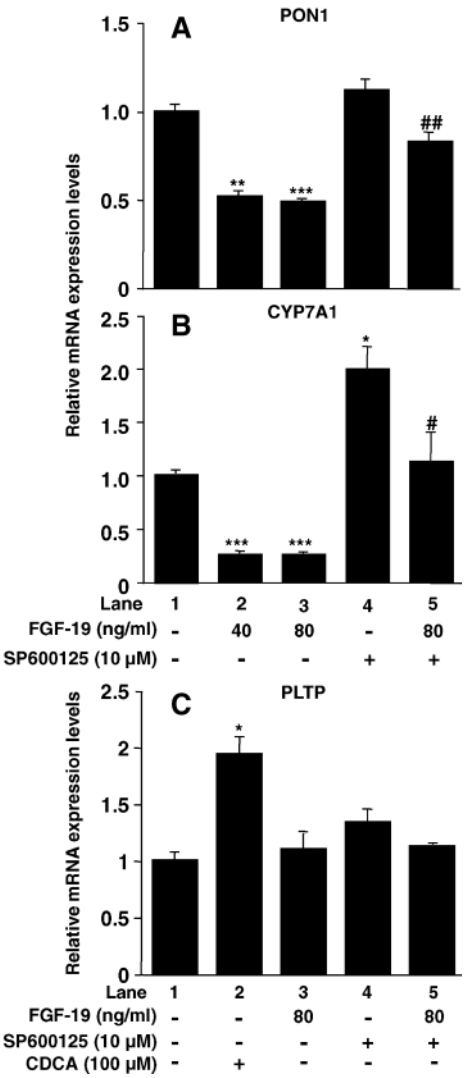

Fig. 4. Effects of FGF-19 on PON1, cholesterol 7α-hydroxylase (CYP7A1), and phospholipid transfer protein (PLTP) mRNA expression in HepG2 cells. HepG2 cells, in quadruplicate, were pretreated for 30 min with or without 10 μM SP600125 before the addition of vehicle, FGF-19, or CDCA, as indicated. After 6 h of treatment, the mRNA levels of PON1 (A), CYP7A1 (B), and PLTP (C) in HepG2 cells were examined by quantitative RT-PCR. Values are shown as means ± SEM. * $P < 0.01$, ** $P < 0.001$, and *** $P < 0.0001$ versus the control (DMSO; lane 1) group. # $P < 0.05$ and ## $P < 0.001$ versus the FGF-19 (80 ng/ml; lane 3) group.

by SP600125. In agreement with a previous study using human primary hepatocytes (41), treatment with SP600125 increased CYP7A1 mRNA levels in HepG2 cells (Fig. 4B, lane 4 vs. lane 1). Together, these data suggest that FGF-19 represses PON1 and CYP7A1 expression by a JNK-dependent pathway. In contrast, the expression of phospholipid transfer protein gene, an FXR target gene activated by FXR agonists (51), was increased by CDCA but unchanged by FGF-19 or SP600125 treatment (Fig. 4C), demonstrating the specificity of FGF-19's effect on PON1 and CYP7A1 gene expression.

The JNK pathway mediates PON1 inhibition

The inhibitory effect of bile acids on CYP7A1 gene expression in HepG2 cells and primary rat hepatocytes has been shown to be mediated in part by the JNK pathway (45, 46). We observed that HepG2 cells treated for 20 h with a JNK inhibitor, SP600125, exhibited a small increase in PON1 mRNA levels compared with nontreated cells (**Fig. 5**, lanes 1 and 2). In contrast, a shorter incubation time of 6 h with SP600125 did not change PON1 mRNA levels in HepG2 cells (Fig. 4A). Importantly, addition of SP600125 partially reversed the inhibitory effects of both CDCA and GW4064 on PON1 expression (Fig. 5, lane 4 vs. lane 3 and lane 6 vs. lane 5).

The proximal promoter of the human *PON1* gene is responsive to bile acids

To examine whether the proximal promoter of the human *PON1* gene contains *cis*-regulatory elements that are necessary for repression by bile acids, we generated a series of promoter-reporter plasmids. These constructs were transfected into HepG2 cells in the absence or presence of expression vectors for FXRα2 and RXRα, and the cells were then incubated for 24 h in the presence or absence of bile acids or GW4064.

In the absence of FXRα2 and RXRα, the pGL3-hPON1-1487 reporter was inhibited by <25% by either CDCA or GW4064 (**Fig. 6A**, lanes 7–9). This decrease likely results

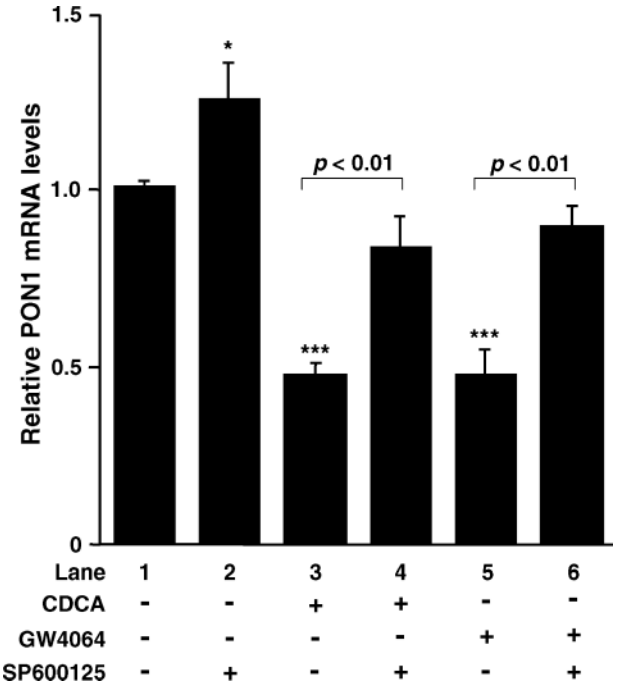

Fig. 5. Effects of CDCA, GW4064, and SP600125 on PON1 expression in HepG2 cells. HepG2 cells, in quadruplicate, were pretreated with or without 10 μM SP600125 for 30 min before the addition of vehicle, 100 μM CDCA, or 1 μM GW4064. After 20 h, RNA was isolated and PON1 mRNA levels were determined by quantitative RT-PCR and normalized to GAPDH. Values shown are means ± SEM of three independent experiments. * $P < 0.05$ and *** $P < 0.0001$ versus the control (DMSO; lane 1) group.

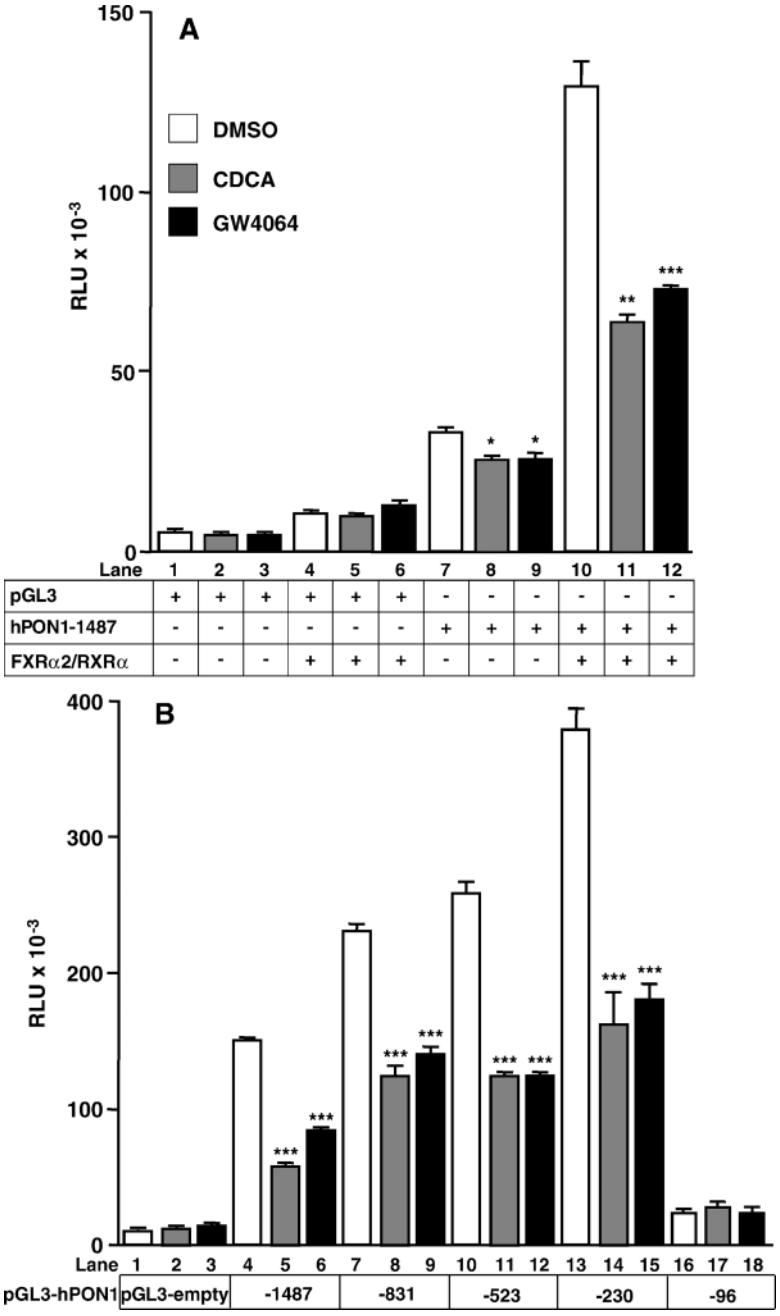

Fig. 6. Mapping of the human *PON1* promoter using deleted fragments. HepG2 cells were cotransfected with pGL3-hPON1-1487 or pGL3 in the absence or presence of expression vectors for FXRα2 and retinoid X receptor α (RXRα) (A) or cotransfected with the indicated human PON1 promoter-reporter plasmids or pGL3 and expression vectors for FXRα2 and RXRα (B). Cells were then treated with 0.1% DMSO, 100 μM CDCA, or 1 μM GW4064 for 24 h before determination of luciferase and β-galactosidase activities. Luciferase activity was normalized to β-galactosidase activity in each well. Values shown are means ± SEM (n = 4). Results from one representative experiment are shown. * $P < 0.05$, ** $P < 0.001$, and *** $P < 0.0001$ versus the DMSO group. RLU, relative light units.

from the activation of endogenous FXR that is expressed in HepG2 cells (52). However, when cells were also transfected with plasmids encoding FXRα2 and RXRα, luciferase activities declined by 44–51% after treatment with CDCA or GW4064 (Fig. 6A, lanes 10–12). In contrast, the promoterless control plasmid, pGL3, did not respond to CDCA or GW4046 in the absence or presence of FXRα2 and RXRα (Fig. 6A, lanes 1–6). A small increase in the activity of the empty pGL3 plasmid was observed when it was cotransfected with FXRα2 and RXRα (Fig. 6A). We attribute this induction to an intrinsic property of the pGL3 vector. As shown in Fig. 6B, lanes 4–15, the expression levels of human PON1 promoter-reporter constructs pGL3-hPON1-1487, pGL3-hPON1-831, pGL3-hPON1-523, and pGL3-hPON1-230 were all decreased by 40–63% when cells were transfected with plasmids encoding FXRα2 and RXRα and then incubated with CDCA or GW4064. In contrast, the shortest construct, pGL3-hPON1-96, exhibited weak promoter activity and did not respond to FXR agonists (Fig. 6B, lanes 16–18). Together, these data indicate that the −230 to −96 bp DNA region of the human *PON1* promoter contains *cis*-acting element(s) that are involved in the bile acid response and that the effect of bile acids on the *PON1* promoter is mediated by the action of FXR/RXR.

DISCUSSION

PON1 expression is an important factor in determining both susceptibility to atherosclerosis (11–14, 16) and resis-

tance to certain insecticides (3, 4, 14). In this study, we explored the regulation of PON1 expression by bile acids. Our studies resulted in several conclusions. First, they demonstrate the inhibitory effect of bile acids on PON1 expression in vitro and in vivo. Second, studies with $FXR^{-/-}$ mice and FXR agonists demonstrate that the inhibitory effect is mediated by the nuclear receptor FXR. Third, FGF-19 is involved in the repression of PON1 expression by bile acids. Fourth, the repression of PON1 mRNA levels by FXR agonists is dependent on JNK. Finally, we localized the regulatory element of the *PON1* promoter that is required for the FXR-dependent repression to between −230 and −96 bp.

There are two sterol-regulatory element-like regions (−104 to −95 bp and −138 to −130 bp) located in the proximal promoter of the human *PON1* gene. SREBP-2 has been shown to bind to the human *PON1* promoter, and activation of SREBP-2 increases PON1 expression in HepG2 cells (31). However, we did not observe a change in SREBP-2 mRNA levels in HepG2 cells after administration of FXR agonists (data not shown). Furthermore, the proteolytic activation of SREBP-2 in HepG2 cells is not affected by bile acids (53). Nonetheless, we cannot rule out the possibility that posttranslational modification of SREBP-2 or induction of a corepressor, after administration of FXR agonists, may be involved in the reduction of PON1 expression.

In contrast, the expression of SREBP-1c is decreased in HepG2 cells after administration of FXR agonists (Fig. 3D). Previous studies have also demonstrated that repression of murine SREBP-1c expression by bile acids involves the FXR-SHP pathway (39, 40). However, to date, no role for SREBP-1c in modulating PON1 expression has been reported. In unpublished studies, we have shown that treatment of HepG2 cells or wild-type mice with a liver X receptor-specific agonist (T0901317) induces, as expected, SREBP-1c mRNA and many genes involved in fatty acid synthesis and lipogenesis (54), but it has no effect on PON1 mRNA levels.

Human FGF-19 and its putative murine homolog, FGF-15, share 50% identity at the amino acid level (55). Inagaki et al. (56) recently demonstrated that murine FGF-15 is induced in the small intestine in response to FXR agonists. They concluded that intestinally derived FGF-15 is secreted and acts on hepatic FGFR4 to repress the hepatic expression of Cyp7a1. Inagaki et al. (56) also reported that infusion of FGF-15-expressing adenovirus into wild-type mice repressed hepatic *Cyp7a1* mRNA but did not alter SHP mRNA levels. In contrast, administration of FGF-15-expressing adenovirus into $SHP^{-/-}$ mice failed to repress hepatic *Cyp7a1* mRNA (56). Based on these studies, the authors proposed that binding of intestinally derived FGF-15 to FGFR4 in the liver led to activation of the JNK pathway, which in turn increased the activity, but not mRNA level, of SHP; the result was the repression of *Cyp7a1* expression. As discussed below, we propose that a similar mechanism may be involved in the repression of *Pon1* in the liver after the activation of intestinal FXR.

The current study demonstrates that FXR-dependent repression of PON1 requires nucleotides located between −230 and −96 bp of the *PON1* promoter. This 135 bp sequence does not contain an farnesoid X-receptor-responsive element, but it does contain two sterol-regulatory elements. Using the Transcription Element Search System (http://www.cbil.upenn.edu/tess/), we identified a putative binding site for the hepatocyte nuclear factor 4 (HNF-4) (−206 to −194 bp) that exhibited 85% homology to the HNF-4 consensus sequence (57). A previous study (58) that used chromatin immunoprecipitation followed by microarray analysis concluded that HNF-4 bound to the human PON1 promoter (−700 to +200) and likely regulated the expression of the gene. Interestingly, HNF-4 has been shown to interact with SREBP-2 and activate the expression of sterol Δ^8-isomerase (59), an enzyme participating in the late stage of cholesterol biosynthesis. In other studies, SHP was shown to bind and inhibit the transcriptional activity of HNF-4 (60).

Because both HNF-4-like and SREBP-2 binding sites are present in the −230 to −96 bp region of the PON1 promoter, we hypothesize that FGF-19/FGF-15, released after the activation of intestinal FXR, binds the hepatic receptor FGFR4 to activate the JNK pathway. Further studies are needed to test the hypothesis that activation of the JNK pathway and changes in SHP activity result in the inhibition of HNF-4 activity and PON1 expression.

Expression of apolipoprotein A-I, the major apolipoprotein on HDL, is also repressed in an FXR-dependent manner after treatment of mice, human primary hepatocytes, and HepG2 cells with bile acids (52, 61). Conversely, cholestyramine, a bile acid sequestrant that interrupts the enterohepatic circulation, increases hepatic apolipoprotein A-I mRNA levels in animals (62) and serum apolipoprotein A-I protein levels in both animals (62) and humans (63). Based on these observations, it seems likely that changes in bile acid pools that result from cholestyramine treatment likely induce PON1 expression in humans. Furthermore, FXR antagonist may have the beneficial effects of increasing both apolipoprotein A-I and PON1 levels in the circulation, resulting in the attenuation of atherosclerosis.

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