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AKR1B7 Is Induced by the Farnesoid X Receptor and Metabolizes Bile Acids*[S]

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Although bile acids are crucial for the absorption of lipophilic nutrients in the intestine, they are cytotoxic at high concentrations and can cause liver damage and promote colorectal carcinogenesis. The farnesoid X receptor (FXR), which is activated by bile acids and abundantly expressed in enterohepatic tissues, plays a crucial role in maintaining bile acids at safe concentrations. Here, we show that FXR induces expression of *Akr1b7* (aldo-keto reductase 1b7) in murine small intestine, colon, and liver by binding directly to a response element in the *Akr1b7* promoter. We further show that AKR1B7 metabolizes 3-keto bile acids to 3β-hydroxy bile acids that are less toxic to cultured cells than their 3α-hydroxy precursors. These findings reveal a feed-forward, protective pathway operative in murine enterohepatic tissues wherein FXR induces AKR1B7 to detoxify bile acids.

Bile acids are detergent molecules that are essential for the absorption of dietary fat. They are also endogenous ligands for the farnesoid X receptor/bile acid receptor (FXR),[6] which regulates bile acid homeostasis by modulating the expression of genes in liver and small intestine that control the synthesis and enterohepatic circulation of bile acids (reviewed in Refs. 1 and 2).

Bile acids are synthesized from cholesterol in the liver through a series of reactions that involve multiple hydroxylation steps, followed by generation of a carboxylic acid group and ultimately conjugation with an amino acid. Many of these reactions are carried out by cytochrome p450 enzymes and aldo-keto reductases (AKRs) (3). Conjugated bile acids, secreted by the liver, enter the intestine where they are deconjugated and dehydroxylated by bacterial enzymes (4). Dehydroxylation occurs primarily in the colon and results in the production of secondary bile acids that are relatively hydrophobic in comparison with the primary bile acids produced by the liver. Upon reabsorption from the intestine, bile acids are returned to the liver where they are reconjugated and in some cases rehydroxylated (5).

When present at high concentrations, bile acids induce cytotoxic effects (6). Secondary bile acids, in particular, cause membrane damage and produce reactive oxygen species resulting in DNA damage, apoptosis, and necrosis (7). Several nuclear receptors, including FXR, pregnane X receptor (PXR), constitutive androstane receptor, and vitamin D receptor, have been shown to protect against the cytotoxic effects of bile acids by increasing expression of binding proteins, transporters, and enzymes that detoxify bile acids (8).

In this report, we demonstrate that FXR induces aldo-keto reductase 1B7 (*Akr1b7*) in murine small intestine, colon, and liver and that AKR1B7 in turn catalyzes the conversion of 3-keto bile acids to 3β-hydroxy bile acids. Consistent with 3β-hydroxy bile acids having higher critical micelle concentrations (9), we also demonstrate that 3β-hydroxy bile acids are less cytotoxic than their 3α-hydroxy counterparts. These results suggest that FXR plays a protective role in enterohepatic tissues by inducing AKR1B7 levels, which leads to the detoxification of bile acids.

EXPERIMENTAL PROCEDURES

Animals and Animal Husbandry—Male C57BL/6 mice were purchased from Charles River Laboratories. Male $FXR^{+/+}$ and $FXR^{-/-}$ mice were obtained from heterozygous breeders on a pure 129 S background. All mice were housed in the same specific pathogen-free facility. Animals were maintained under a temperature-controlled environment and 12 h light/dark cycles with *ad libitum* access to water and irradiated rodent chow (TD.2916, Harlan-Teklad). Mice were euthanized by isofluorane inhalation and exsanguinated via the descending vena cava prior to tissue collection. For the

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[6] The abbreviations used are: FXR, farnesoid X receptor; PXR, pregnane X receptor; AKR, aldo-keto reductase; DCA, deoxycholic acid; FXRE, FXR response element; CDCA, chenodeoxycholic acid.

experiment involving collection of adrenals, mice were sacrificed by decapitation. All animal experiments were approved by the Institutional Animal Care and Research Advisory Committee at the University of Texas Southwestern Medical Center.

Animal Treatments—GW4064 (GlaxoSmithKline) (10) was administered by either oral gavage or intraperitoneal injection. For oral gavage experiments, GW4064 (100 mg/kg) in 1% Tween 80, 1% methylcellulose was administered 15 and 5 h before sacrifice. For intraperitoneal injection experiments, GW4064 (30 mg/kg) in dimethyl sulfoxide was administered 4 h before sacrifice. Deoxycholic acid (DCA, 0.2% (w/w), Steraloids) was admixed in the diet (custom diet TD.07410, Harlan-Teklad) for 10 days. Cholestyramine (2% (w/w)) was admixed in the diet (custom diet TD.07658, Harlan-Teklad) for 2 weeks. For the study involving multiple nuclear receptor agonists, mice were treated as follows: LG268 (30 mg/kg) in 0.25% Tween 80, 1% methylcellulose was administered by oral gavage 12 h before sacrifice. The following compounds were admixed in the diet at the concentration indicated (w/w) and provided *ad libitum* for 12 h before sacrifice: 0.05% pregnenolone-16α-carbonitrile (Sigma), 0.0015% 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (Sigma), 0.025% T0901317 (Cayman Chemical), 0.05% GW4064 (GlaxoSmithKline), 0.0025% GW7647 (GlaxoSmithKline), 0.0025% GW0742 (GlaxoSmithKline), 0.075% troglitazone (GlaxoSmithKline), 0.0000125% 4-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (Sigma). Vitamin D (1α,25-dihydroxycholecalciferol, 50 μg/kg, Sigma) in sterile saline was administered by intraperitoneal injection 4 h before sacrifice. In all cases, mice in the control group received the appropriate vehicle solutions and diets in a manner identical to the treatment groups.

RNA Extraction and Quantitative RT-PCR—Following euthanasia, intestines were flushed with PBS and frozen immediately in liquid nitrogen. Total RNA was extracted using RNA STAT-60^{TM} (IsoTex Diagnostics). Four micrograms of RNA from each sample were DNase-treated and reverse-transcribed using random hexamers. The resulting complementary DNA (cDNA) was analyzed by quantitative RT-PCR as described (11). Briefly, quantitative PCR reactions containing 25 ng of cDNA, 150 nmol of each primer, and SYBR® GreenER^{TM} PCR Master Mix (Invitrogen) were carried out in triplicate in 384-well format using an ABI PRISM® 7900HT instrument (Applied Biosystems). Relative mRNA levels were calculated using the comparative Ct method normalized to *U36b4*. The primer sequences used for gene expression analyses are listed below. They were designed using Primer Express® software (Applied Biosystems) and validated as described (11). Primers were as follows: *Akr1b7* (forward, 5′-ccactggccacagggatt-3′ and reverse, 5′-tttgcctttattgtctttgggtaa-3′), *Fxr* (forward, 5′-tccggacattcaaccatcac-3′ and reverse, 5′-tcactgcacatcccagatctc-3′), *Ostb* (forward, 5′-gacaagcatgttcctcctgaga-3′ and reverse, 5′-tgtcttgtggctgcttctttc-3′), and *U36b4* (forward, 5′-cgtcctcgttggagtgaca-3′ and reverse, 5′-cggtgcgtcagggattg-3′).

In Situ Hybridization—*In situ* hybridization was performed on formaldehyde-perfused, paraffin-embedded sections of intestine using ^{35}S-labeled sense and antisense probes against the FXR ligand binding domain (nucleotides 910–1367; GenBank^{TM} accession no. NM_009108.1). Slides were exposed at 4 °C for 14 days.

ChIP Analysis—Ilea from 5–6 wild-type and FXR$^{-/-}$ mice were pooled, frozen, and crushed. ChIP was performed using 300 mg of tissue and an FXR antibody (Santa Cruz Biotechnology, antibody sc-13063) as described (33). Quantitative PCR (QPCR) analysis was performed with either primers flanking the *Akr1b7* FXR response element (FXRE) site (forward, 5′-tccactcccagggcaatg-3′ and reverse, 5′-gtcatccaagatgaactggtaag-3′) or a nonspecific site 2 kb upstream of the *Akr1b7* start site (forward, 5′-atggcaaagtagtctgcaggat-3′ and reverse, 5′-ggagacagggtcaggaagtgaa-3′). ChIP assays were performed in triplicate.

Expression Plasmids—*Akr1b7* was cloned from mouse adrenal cDNA, and *Crad2* was a gift from Dr. David Russell, University of Texas Southwestern Medical Center. The coding sequence of both genes was subcloned into pCMX and p3xFLAG-CMV-10 vectors containing the constitutive CMV promoter. The promoter of *Akr1b7* extending from −1798 to +27 was cloned and inserted upstream of a luciferase reporter. Mutation of the FXRE was accomplished by substitution of a single guanosine with adenine in the upstream half-site. FXR, retinoid X receptor (RXR)α, and β-galactosidase expression plasmids and the FXRE-luciferase reporter plasmid have been described previously (12, 13). The coding regions of all plasmids were verified by DNA sequencing.

Cell Culture—HEK293 cells were cultured in 6-well plates at 37 °C and 5% CO_2 in DMEM (containing 4 g/liter glucose and L-glutamine, Invitrogen) supplemented with 10% charcoal-stripped, heat-inactivated FBS. Cells were transfected with 1 μg DNA and 6 μl FuGENE 6 (Roche Applied Science) per well. After 12–16 h, cells were treated with 25 μM bile acids. Culture media was collected 24 and 48 h after treatment.

Bile Acid Extraction and Analysis by LC/MS—Culture media was combined with 10 volumes of acetonitrile and centrifuged at 16,000 × *g*. Supernatant was dried at 50 °C in a vacuum oven. Bile acids were resuspended in 25% methanol. Bile acids were resolved by reverse-phase liquid chromatography (C8 precolumn, C18 analytical column) and quantified by mass spectrometry with electrospray ionization in negative ion mode. Samples were run in selected ion monitoring mode as described (14) with the following ions being monitored simultaneously: 373, 375, 387, 389, 391, 393, 401, 403, 405, 407, and 419.

Cotransfection and Luciferase Assay—HEK293 cells were grown at 37 °C, 5% CO_2 in 96-well plates in DMEM (containing 4 g/liter glucose and L-glutamine, Invitrogen) supplemented with 10% charcoal-stripped, heat-inactivated fetal bovine serum and transfected by calcium phosphate coprecipitation as described (13). Following 16-hour treatment with bile acids, luciferase and β-galactosidase activities were measured as described (12). Luciferase activity was normalized for transfection efficiency using β-galactosidase activity

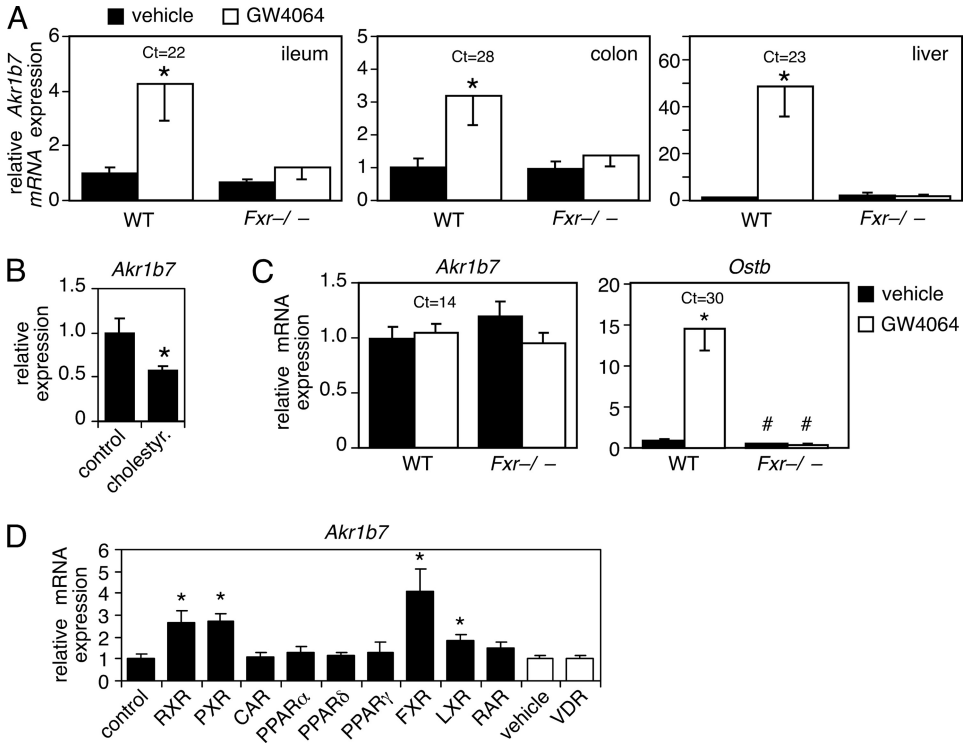

FIGURE 1. **Akr1b7 is regulated by FXR in enterohepatic tissues.** *A*, mice of the indicated genotype ($n = 5$/group) were treated for 4 h with GW4064 (intraperitoneal injection). *Akr1b7* expression was measured by QPCR in distal ileum, distal colon, and liver. QPCR Ct values are indicated for the GW4064-treated wild-type groups. *, $p < 0.05$ compared with control. *B*, wild-type mice ($n = 4$/group) were treated for 10 days with cholestyramine (*cholestyr.*) admixed in diet. *Akr1b7* expression in ileum was measured by QPCR. *, $p < 0.05$ compared with control. *C*, mice of the indicated genotype ($n = 5$/group) were treated for 4 h with GW4064 (intraperitoneal injection). *Akr1b7* and *Ostb* expression in adrenal was determined by QPCR. QPCR Ct values are indicated for the GW4064-treated wild-type groups. *, $p < 0.05$ compared with vehicle of the same genotype; #, $p < 0.05$ compared with wild-type mice of the same treatment group. *D*, wild-type mice ($n = 5$/group) were treated orally for 12 h (*black bars*) or intraperitoneally for 4 h (*white bars*) with the ligands for RXR (LG268), PXR (pregnenolone-16α-carbonitrile), constitutive androstane receptor (1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene), peroxisome proliferator-activated receptor (*PPAR*)α (GW7647), peroxisome proliferator-activated receptor β/δ (GW0742), peroxisome proliferator-activated receptor γ (troglitazone), FXR (GW4064), liver X receptor (*LXR*; T0901317), RAR (4-[(E)-2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid), or vitamin D receptor (1α,25-dihydroxycholecalciferol (vitamin D)) as described under "Experimental Procedures." *Akr1b7* expression in ileum was determined by QPCR and graphed relative to vehicle-treated control. *, $p < 0.05$ compared with control. All data represent the mean ± S.E.

and expressed as relative luciferase units. Data shown are mean ± S.D. of triplicates.

Cell Viability Assay—IEC-18 and HepG2 cells were grown in 96-well plates at 37 °C and 5% carbon dioxide in 50 μl DMEM (containing 4 g/liter glucose and L-glutamine, Invitrogen) supplemented with 10% charcoal-stripped, heat-inactivated FBS. IEC-18 cells were plated at a density of 1000 cells per well and untreated dilutions of 750, 500, 250, and 100 cells per well were also plated. HepG2 cells were plated at a density of 5000 cells per well and untreated dilutions of 4000, 3000, 2000, and 1000 cells per well were also plated. 24 h after plating, cells were treated by the addition of 50 μl culture medium containing bile acids. Three days after treatment, plates were assayed using CellTiter96™ (Promega) according to the manufacturer's instructions. Following determination of absorbance at 490 nm, cells in wells containing cell dilutions were trypsinized and counted using a hemocytometer. Cell counts and absorbance values were used to generate a standard curve allowing conversion of absorbance to cell number for all treatment conditions.

Statistical Analysis—Gene expression data are presented as mean ± S.E. and were analyzed by two-tailed, unpaired Student's *t* test. *p* values less than 0.05 were considered significant.

RESULTS

Fxr Is Expressed in Colonic Mucosa—In mice, FXR is expressed throughout the small intestine and colon (15, 16). QPCR analysis showed that *Fxr* expression was highest in ileum with decreasing expression in the proximal and distal colon (supplemental Fig. S1*A*). *In situ* hybridization analysis revealed that *Fxr* expression was highest in the mucosa in both the small intestine and colon (supplemental Fig. S1, *B–D*). These results are consistent with a recent report that FXR protein is present in the epithelium of the colon (17).

Akr1b7 Is Regulated by FXR—Microarray profiling experiments were performed with colon from wild-type mice treated with the synthetic FXR agonist GW4064 (data not shown). Among the genes regulated by GW4064 was *Akr1b7*. QPCR analysis using colon from wild-type and $Fxr^{-/-}$ mice treated with GW4064 showed that *Akr1b7* was induced ~3-fold in an FXR-dependent manner (Fig. 1*A*). *Akr1b7* was induced by FXR not only in colon but also in ileum and liver (Fig. 1*A*). Conversely, *Akr1b7* expression was decreased in ileum of mice treated with the bile acid binding resin cholestyramine (Fig. 1*B*). In contrast to its regulation by FXR in enterohepatic tissues, *Akr1b7* was not induced by GW4064 in adrenal tissue (Fig. 1*C*), where *Akr1b7* is highly expressed

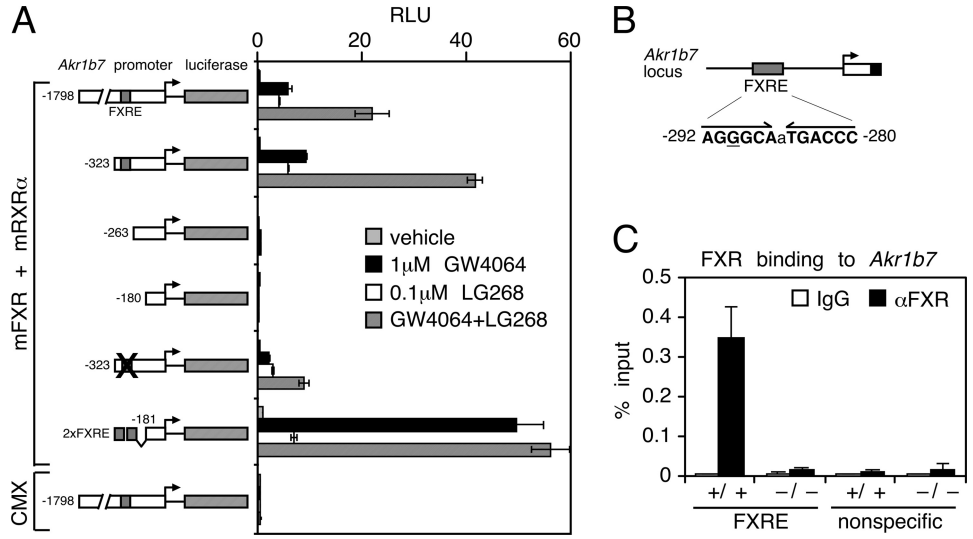

FIGURE 2. ***Akr1b7* is a direct transcriptional target of FXR.** *A*, the *Akr1b7* promoter was cloned upstream of the luciferase gene. *Numbers* indicate nucleotide position relative to the *Akr1b7* transcriptional start site. HEK293 cells were cotransfected with reporter constructs and either mouse (*m*) FXR/RXRα expression plasmids or control CMX plasmid as indicated. Following treatment with GW4064 (1 μM), LG268 (0.1 μM), GW4064+LG268, or vehicle as indicated, luciferase activity was quantified and normalized to β-galactosidase activity. Data are graphed relative to vehicle treatment and represent the mean ± S.D. of three replicates. *X* indicates mutated FXRE. *B*, graphic representation of the *Akr1b7* promoter showing the location of the FXRE relative to the transcriptional start site. The sequence of the inverted repeat FXRE is shown. Half-sites are in *boldface type*, and the nucleotide mutated in the promoter analysis shown in *A* is *underlined*. *C*, ChIP with either anti-FXR or IgG was performed using ileum from wild-type or *Fxr*$^{-/-}$ mice. QPCR analysis was used to quantify ChIP signal from the region flanking the FXRE and a nonspecific region 2 kb away from the FXRE. Results are graphed as percent input signal and represent the mean ± S.D. from three independent ChIP assays. *RLU*, relative luciferase units.

(18). We speculate that this high level of expression may account for why *Akr1b7* is not further induced by FXR agonism. In control experiments, the established FXR target gene, *Ostb* (organic solute transporter β), was induced by GW4064 in adrenal tissue (Fig. 1*C*). In additional specificity studies, neither closely related *Akr1b8* nor *Akr1c19*, which is also expressed in enterohepatic tissues (19), were induced by GW4064 in liver, ileum, or colon (supplemental Fig. S2).

Previous reports showed that *Akr1b7* is induced in murine intestine by agonists for liver X receptor, PXR, and constitutive androstane receptor (20, 21). Comparison with *Akr1b7* induction by a panel of nuclear receptor agonists revealed that the FXR agonist was among the most efficacious inducers (Fig. 1*D*). Significant induction was also seen with RXR, PXR, and liver X receptor agonists (Fig. 1*D*).

To determine whether *Akr1b7* is a direct transcriptional target of FXR, cell-based reporter assays were performed in HEK293 cells. A reporter construct containing nucleotides −1798 to +27 of the *Akr1b7* promoter was induced by GW4064 and the RXR-selective agonist LG268 (Fig. 2*A*). Treatment with both GW4064 and LG268 caused a synergistic induction of reporter levels. No response to these ligands was observed in the absence of co-transfected FXR and RXRα expression plasmids (Fig. 2*A*). These data demonstrate that the *Akr1b7* is activated by the FXR/RXRα heterodimer. Analysis of *Akr1b7* promoter truncations localized an FXR/RXR responsive region between nucleotides −263 and −323. Analysis of this region revealed an inverted repeat with a one nucleotide spacer conforming to the consensus FXRE (Fig. 2*B*). Two copies of this site were sufficient to confer responsiveness to the FXR/RXRα heterodimer, and transactivation of the *Akr1b7* promoter was markedly reduced by mutating only a single nucleotide within this element (Fig. 2*A*). ChIP analysis confirmed that FXR bound to this region of the *Akr1b7* promoter in intestine (Fig. 2*C*). These results demonstrate that FXR binds directly to the *Akr1b7* promoter to regulate its transcription.

AKR1B7 Metabolizes 3-Keto Bile Acids—Because other aldo-keto reductases are involved in the conversion of cholesterol to bile acids in liver, we hypothesized that bile acids might be substrates for AKR1B7 in the intestine. To test this, *Akr1b7* was cloned and expressed in HEK293 cells. Following treatment with bile acids, culture medium was collected and analyzed for bile acid metabolites by LC/MS. It was found that a variety of 3-keto bile acids were reduced to their 3-hydroxy equivalents in cells expressing AKR1B7 (Table 1). Conversion of 3-keto lithocholic acid (3-keto-LCA) was apparent 24 h after treatment, and incubation for 48 h resulted in >50% of the substrate being metabolized (Fig. 3). Interestingly, reduction of the 3-keto group occurred in a stereospecific manner such that only 3β-hydroxy bile acids were formed. In contrast, an endogenous enzyme in HEK293 cells produced the 3α-hydroxy epimer (Fig. 3, *middle panels*). AKR1B7 did not oxidize the 3-hydroxy group of either 3α-hydroxy or 3β-hydroxy bile acids (Table 1), indicating that it selectively increases the rate of the reducing reaction. Furthermore, reduction of 6-keto, 7-keto, and 12-keto bile acids did not occur (Table 1), indicating specificity of AKR1B7 for the 3-keto group.

Some of the more abundant 3-keto bile acids present in colon, such as 3-keto-DCA and 3-keto-chenodeoxycholic acid (CDCA), are not commercially available. To test whether these bile acids are substrates for AKR1B7, heterologous coexpression of AKR1B7 with CRAD2 (*cis*-retinol/androgen dehydrogenase 2) was followed by treatment with DCA and CDCA. CRAD2 has been shown to oxidize the 3-hydroxy po-

TABLE 1

Bile acids metabolized by AKR1B7

HEK293 cells were transfected with an *Akr1b7* expression plasmid or control vector and treated with the indicated bile acids (25 μM). Bile acids were extracted from culture medium collected 24 and 48 h after treatment. Extracts were resolved by reverse phase chromatography and analyzed by mass spectrometry using selected ion monitoring in negative ion mode. Monitored ions included all combinations of mono-, di-, and trihydroxy and keto bile acids.

Bile acid tested	Detected metabolites
3-Keto-5β-cholanic acid (3-ketoLCA)	3β-Hydroxy-5β-cholanic acid
3,6-Diketo-5β-cholanic acid	Monohydroxy-monoketo-5β-cholanic acid[a]
3,7-Diketo-5β-cholanic acid	Monohydroxy-monoketo-5β-cholanic acid[a]
3,12-Diketo-5β-cholanic acid	Monohydroxy-monoketo-5β-cholanic acid[a]
3,7,12-Triketo-5β-cholanic acid (dehydro-CA)	Monohydroxy-diketo-5β-cholanic acid[a]
3-Keto-7α,12α-dihydroxy-5β-cholanic acid	3β,7α,12α-Dihydroxy-5β-cholanic acid
3α-Hydroxy-5β-cholanic acid (LCA)	None
3α,7α-Dihydroxy-5β-cholanic acid (CDCA)	None
3α,12α-Dihydroxy-5β-cholanic acid (DCA)	None
3α,7α,12α-Trihydroxy-5β-cholanic acid (CA)	None
3α-Hydroxy-6-keto-5β-cholanic acid	None
3α-Hydroxy-7-keto-5β-cholanic acid	None
3α-Hydroxy-12-keto-5β-cholanic acid	None
3β-Hydroxy-5β-cholanic acid	None
3β,12α-Dihydroxy-5β-cholanic acid	None

[a] No authentic standard is available for identification.

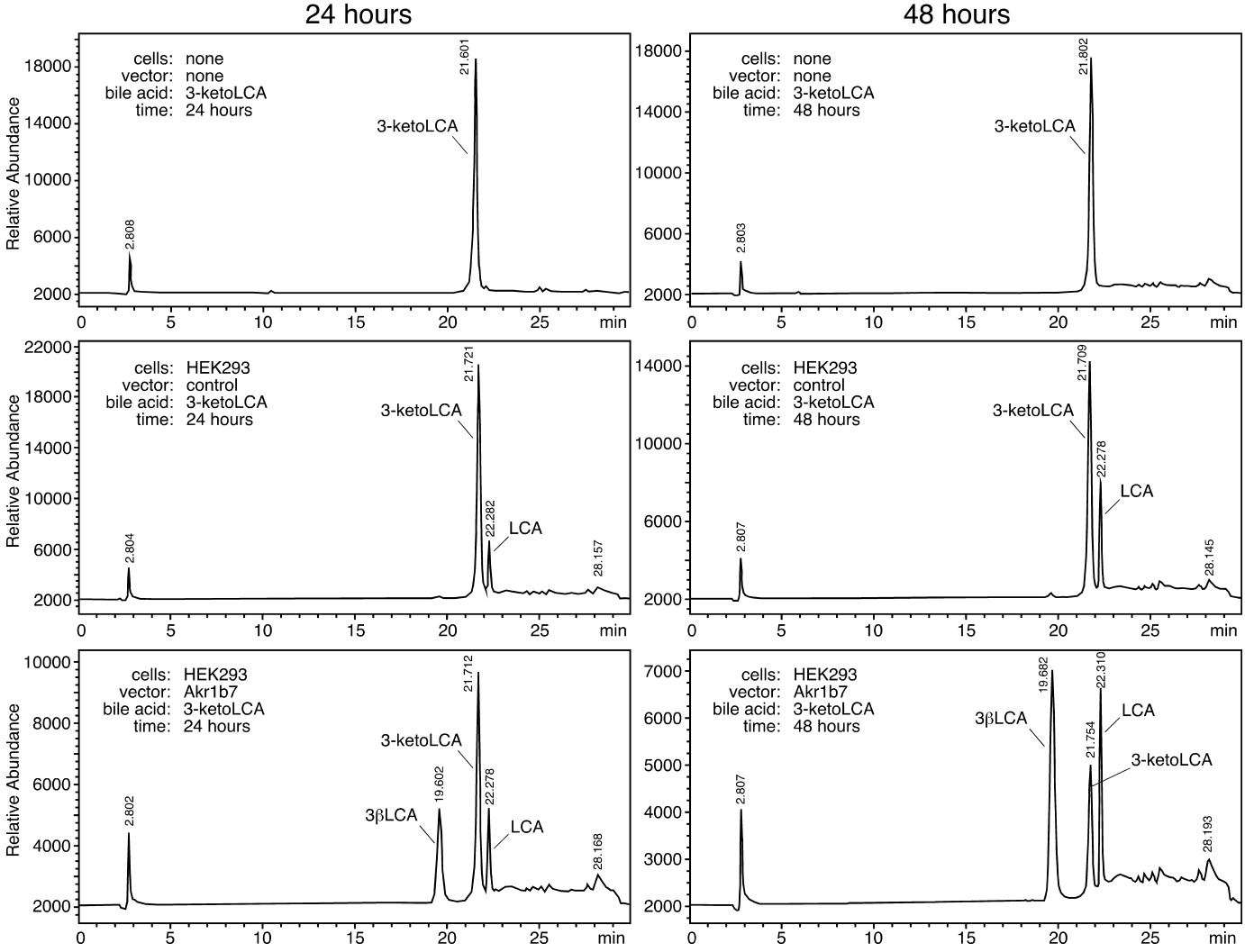

FIGURE 3. **AKR1B7 metabolizes 3-keto-LCA to 3β-hydroxy-LCA.** HEK293 cells were transfected with an *Akr1b7* expression vector (*bottom panels*) or control vector (*middle panels*) and treated with 25 μM 3-keto-LCA. Control treatments were done with no cells (*upper panels*). Bile acids were extracted from culture medium collected at 24 h (*left panels*) or 48 h (*right panels*) after treatment. Extracts were resolved by reverse phase chromatography and analyzed by mass spectrometry using selected ion monitoring in negative ion mode. Monitored ions included all combinations of mono-, di-, and tri-hydroxy and keto bile acids.

sition of bile acids, generating 3-keto bile acids (22). Cells expressing AKR1B7 did not metabolize DCA, whereas cells expressing CRAD2 produced 3-keto-DCA and smaller amounts of 3β-hydroxy-DCA (Fig. 4). Co-expression of AKR1B7 and CRAD2 resulted in production of mainly 3β-hydroxy-DCA (Fig. 4). In ancillary studies, we have found that in addition to

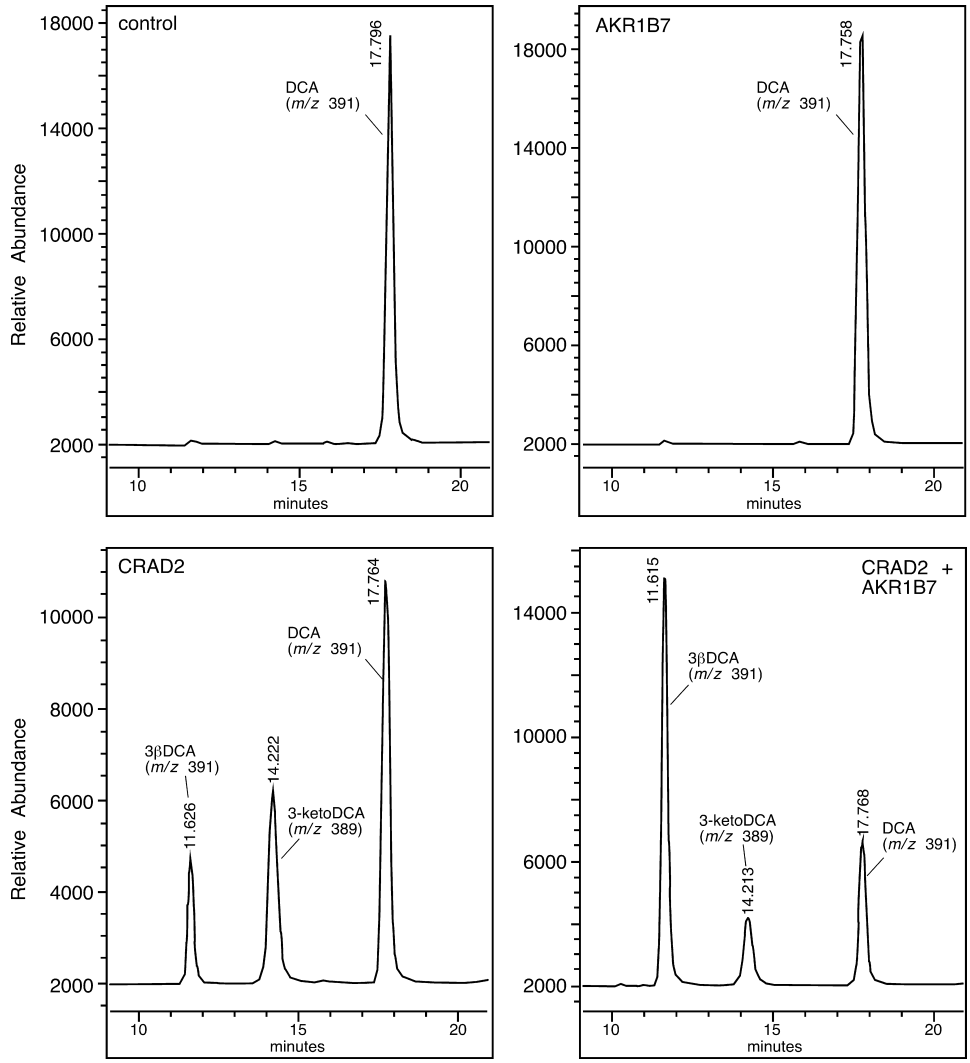

FIGURE 4. **CRAD2 and AKR1B7 convert DCA to 3β-hydroxy-DCA.** HEK293 cells were transfected with *Akr1b7* and *Crad2* expression vectors as indicated and treated for 48 h with 25 μM DCA. Bile acids were extracted from culture medium, resolved by reverse phase chromatography and analyzed by mass spectrometry using selected ion monitoring in negative ion mode. Monitored ions included all combinations of mono-, di-, and tri-hydroxy and keto bile acids. Mass-to-charge ratio (*m/z*) of the major ion for each peak is shown.

oxidation of the 3α-hydroxy group, CRAD2 also oxidizes 3β-hydroxy and reduces 3-keto groups, with conversion to the α-hydroxy epimer highly favored (data not shown). These data suggest that some of the 3-keto-DCA and DCA still present following AKR1B7/CRAD2 co-expression may be formed by concomitant conversion of 3β-hydroxy-DCA to these species by CRAD2. Thus, our assay underestimates the efficacy with which AKR1B7 converts 3-keto-DCA to 3β-hydroxy-DCA. Comparable results were obtained for conversion of CDCA to 3β-hydroxy-CDCA by co-expression of AKR1B7 with CRAD2 (data not shown). These data indicate that AKR1B7, together with an enzyme such as CRAD2, converts 3α-hydroxy bile acids to 3β-hydroxy bile acids.

3β-Hydroxy Bile Acids Are Ligands for FXR—Although 3β-hydroxy bile acids represent ~30% of bile acids in normal human cecal contents, not much is known about their biological function (23). In the absence of *Hsd3b7* (3β-hydroxy-steroid dehydrogenase type 7), mice do not produce 3α-hydroxy bile acids (24). Instead, 3β-hydroxy bile acids are produced and secreted by the liver. Although comparable levels of bile acids are formed, severe lipid malabsorption in these mice results in death at an early age. These results indicate that the conformation of the 3α-hydroxy group is essential for the detergent properties of bile acids *in vivo*. It was also found that FXR target gene expression was reduced in *Hsd3b7*$^{-/-}$ mice (24). To test whether 3β-hydroxy bile acids are ligands for FXR, a reporter-gene assay was performed. Interestingly, decreased induction was seen for 3β-hydroxy bile acids with a 12-hydroxy group (CA and DCA), whereas slightly higher induction occurred with bile acids without this group (LCA and CDCA) (supplemental Fig. S3). These results suggest that the two groups of bile acids bind FXR in slightly different conformations. Moreover, these results together with the finding that FXR target genes are dysregulated in *Hsd3b7*$^{-/-}$ mice support the idea that CA and possibly DCA serve as major physiological FXR ligands (25).

3β-Hydroxy Bile Acids Are Less Toxic Than 3α-Hydroxy Bile Acids—At high micromolar concentrations, bile acids are cytotoxic (6, 7). Given that 3β-hydroxy bile acids are poor detergents (9, 24), we hypothesized that they might be less

TABLE 2

Toxicity of taurine-conjugated, unconjugated, and 3β bile acids

IEC-18 and HepG2 cells were treated with bile acids for 72 h. The cell number was determined by MTS assay. The bile acid concentration that decreased cell number by one-half (EC_{50}) is shown. UDCA, ursodeoxycholic acid.

	EC_{50}	
	μM	μM
Bile acid	IEC-18	HepG2
LCA	65	65
3β-LCA	80	140
3-Keto-LCA	87	68
DCA	80	167
3β-DCA	381	>500
CDCA	124	199
3β-CDCA	343	>500
UDCA	>500	>500
CA	>500	>500
3β-CA	>500	>500
3-Keto-CA	>500	>500
Tauro-LCA	>500	>500
Tauro-DCA	>500	>500
Tauro-CDCA	>500	>500
Tauro-CA	>500	>500

toxic than their 3α-hydroxy epimers. MTS assays were used to quantitate the viability of HepG2 (human hepatocarcinoma) and IEC-18 (immortalized rat intestinal epithelium) cells treated with bile acids. These experiments showed that 3β-hydroxy-DCA and 3β-hydroxy-CDCA were markedly less toxic than DCA and CDCA, respectively (Table 2 and supplemental Fig. S4). Altering the conformation of the hydroxy group in the monohydroxylated bile acid, LCA, had a less striking effect (Table 2). Taurine-conjugated bile acids and the trihydroxylated bile acid, CA, had little effect on cell viability at the concentrations tested in these studies (Table 2 and supplemental Fig. S4). Thus, the conversion of 3α-hydroxy bile acids to 3β-hydroxy bile acids may be a mechanism for bile acid detoxification, particularly for unconjugated dihydroxy bile acids produced by bacterial metabolism in the gut.

DISCUSSION

The aldo-keto reductase AKR1B7 was previously proposed to function in the detoxification of lipid peroxides and toxic byproducts of steroid hormone synthesis. Biochemical analysis identified isocaproaldehyde, a product of side chain cleavage of cholesterol, and 4-hydroxynonenal, a lipid peroxidation product, as preferred substrates for AKR1B7 (26). Given its high level of expression in steroidigenic tissues such as adrenal and its substrate specificity, a role for AKR1B7 in the detoxification of byproducts of steroid biosynthesis has been proposed (27). In this report, we show that *Akr1b7* is directly and robustly induced by FXR in murine small intestine, colon, and liver. We further show that AKR1B7 catalyzes the reduction of 3-keto bile acids to their 3β-hydroxy derivatives. Finally, we demonstrate that these 3β-hydroxy derivatives are less cytotoxic than their 3α-hydroxy epimers. Thus, we propose a novel function for AKR1B7 in the detoxification of bile acids.

The endogenous source and prevalence of substrates for AKR1B7 is an important question that has not been addressed in this study. Intestinal bacteria are known to produce 3-keto bile acids (28). Alternatively, an intestinal enzyme with a function similar to CRAD2 may be the source. Indeed, our data demonstrate that AKR1B7 and CRAD2 together are capable of efficient conversion of 3α-hydroxy bile acids to 3β-hydroxy bile acids. Unlike AKR1B7, CRAD2 appears to increase the rate of both oxidation and reduction of the hydroxy/keto group at position 3. Although the biological function of CRAD2 is not known, its high expression in the liver suggests that it may function there in conjunction with AKR1B7 to prevent the accumulation of 3α-hydroxy bile acids to toxic levels.

Notably, elimination of FXR in mice increases their susceptibility to liver and colorectal tumor formation (17, 29, 30), indicating a protective role for FXR against cancer. Although it remains to be determined whether 3β-hydroxy-DCA and other 3β-hydroxy bile acids are less potent tumor promoters than their 3α-hydroxy and 3-keto precursors, we speculate that the induction of AKR1B7 may contribute to the antineoplastic actions of FXR. In this regard, it is interesting that the therapeutic bile acid, ursodeoxycholic acid, which has a hydroxyl group on the β-side of the steroid nucleus, is less cytotoxic than its CDCA epimer and prevents tumorigenesis in chemical-induced colon cancer models in rats (31, 32). Unlike humans and rats, a large proportion of bile acids in mice have β-hydroxyl groups, including α-, β-, and ω-muricholates. The presence of muricholates may help explain why the incidence of spontaneous colon cancer is low in mice.

Is there a pathway equivalent to FXR-AKR1B7 in humans? The most closely related human AKR family member is AKR1B10, which shares 89% amino acid identity to AKR1B7 and is also expressed in liver and intestine. We failed to observe induction of *AKR1B10* in response to GW4064 in HepG2 human hepatoma cells, which express both *FXR* and *AKR1B10* mRNAs (data not shown). Consistent with these results, the FXRE is not conserved in the *AKR1B10* promoter. Similarly, PXR regulates *Akr1b7* in murine liver and intestine but not *AKR1B10* in human cell lines (20). Although our data suggest that a pathway analogous to FXR-AKR1B7 may not be present in humans, there are several alternative explanations. First, FXR may induce *AKR1B10 in vivo*, but this regulation is not recapitulated in HepG2 cells. Second, there may be AKR family members other than AKR1B10 that are induced by FXR and metabolize 3-keto bile acids to 3β-hydroxy bile acids. A third possibility is that AKR1B10 or other human AKR family members have this enzymatic activity but are not regulated by FXR. We note that *AKR1B10* is already expressed at high basal levels in HepG2 cells (QPCR Ct value ~ 20), which may obviate the need for further induction in response to stress. Additional studies will be required to distinguish among these different scenarios.

In summary, we provide evidence for a novel feed-forward regulatory mechanism wherein FXR induces AKR1B7 to detoxify 3-keto bile acids in murine enterohepatic tissues. These findings add a new and unexpected dimension to the role of FXR in maintaining bile acid homeostasis.

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