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Regulation of Drug Transporters by the Farnesoid X Receptor in Mice

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Abstract: The farnesoid X receptor (FXR, NR1H4) regulates bile acid and lipid homeostasis by acting as an intracellular bile acid-sensing transcription factor, resulting in altered expression of enzymes and transporters involved in bile acid synthesis and transport. Here, we quantitatively analyzed the alterations in expression levels of drug transporters, mainly organic anion-transporting polypeptides (oatp), in wild-type and FXR-null mice to evaluate the role of FXR in their expression and regulation by cholic acid. Changes in the mRNA amounts in liver, kidney, small intestine, and testis in FXR-null mice fed with or without a supplement of 0.5% cholic acid in the diet were analyzed by semiquantitative RT-PCR. In FXR-null mice, the mRNA levels of oatp1, oatp2, oatp3, and octn1 were lower than those of wild-type mice in kidney and testis, while there was no difference in liver or small intestine. Cholic acid feeding led to significantly decreased levels of expression of oatp1 and oct1 and an increased level of expression of oatp2 in wild-type mouse liver. In FXR-null mice, oatp1 and other transporters were downregulated in liver, kidney, and testis, whereas small intestine ASBT, octn2, and pept1 were upregulated. Our results suggested that FXR is involved in the transcriptional regulation of oatp and other transporters in a tissue-specific manner. Furthermore, the effect of cholic acid treatment indicates the involvement of regulatory mechanism(s) other than FXR.

Keywords: Drug transporter; farnesoid X receptor; FXR-null mice; regulation

Introduction

The major metabolic pathway for the elimination of cholesterol is its conversion into bile acid in the liver. Bile acids are secreted from the liver into the small intestine, where they act as detergents to emulsify dietary lipids and fat-soluble vitamins. Approximately 95% of bile acids is recycled in the small intestine by enterocytes and returned to the liver via the enterohepatic circulation, whereas 5% escapes into the colon and is excreted from the body. Bile acids are physiological ligands for the farnesoid X receptor (FXR).[1,2] Among them, the most potent activator of FXR is chenodeoxycholic acid (CDCA), which is able to stimulate FXR-mediated transcriptional activation of target genes.

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FXR [NR1H4, originally isolated as RIP14 (retinoid X receptor-interacting protein-14)] is a member of the nuclear hormone receptor superfamily and is primarily expressed in the liver, kidney, and intestine.[3] FXR-null mice, in which cholesterol−bile acid regulation is disrupted, show an increase in serum total bile acid levels.[4] Cholic acid feeding results in severe liver damage in FXR-null mice, although no apparent toxicity was detected in FXR-null mice fed with a control diet. It was shown that FXR directly or indirectly reduces the level of expression of cholesterol 7α-hydroxylase (cyp7a1),[5] sterol 12α-hydroxylase,[6] Na^+/taurocholate cotransporting polypeptide (NTCP),[7] and apolipoprotein A-I[8] and activates expression of intestinal bile acid-binding protein (I-BABP),[9] phospholipid transfer protein,[10] bile salt export pump (BSEP),[11] dehydroepiandrosterone sulfotransferase,[12] and apolipoprotein C-II.[13] BSEP is the major hepatic bile acid transporter that mediates the transport of bile acids across the canalicular membrane, the rate-limiting step in overall hepatocellular bile salt excretion. Recent studies showed that BSEP transcription is robustly activated by FXR via an FXR response element in the BSEP promoter.[11] However, the Na^+-dependent bile acid transporter in hepatic basolateral membrane (Ntcp), which is also thought to be important in the regulation of bile acid concentration in liver, was not directly regulated by FXR.[4] Furthermore, it is unknown whether Na^+-independent organic anion transporting polypeptide (oatp) families that transport bile acids across the basolateral membrane were regulated by FXR.[14,15]

Cholestasis results in systemic and intrahepatic retention of potentially toxic bile acids that can cause liver injury, ultimately leading to biliary fibrosis and cirrhosis.[16] Downregulation of hepatocellular transport systems may contribute to impaired hepatobiliary excretion of bile acids and other biliary constituents (conjugated bilirubin and glutathione) during cholestasis.[17] Bile salts alter the expression of several genes, including those involved in the synthesis[18] and intestinal transport of bile acids, such as cyp7a1,[2] I-BABP,[1] and ileal sodium-dependent bile acid transporter (I-BAT, ASBT).[19] Recently, Fickert et al. have reported downregulation of oatp1 and Ntcp after cholic acid feeding in mice.[20] In addition, levels of Oatp2 mRNA and protein, and oatp4 protein, were increased by feeding mice cholic acid.[21] Accordingly, transcriptional regulation in response to cholic acid could differ among oatp family members,[20,21] and it remains unclear how these transporters are regulated by nuclear receptors, including FXR.

Although oatps are thought to be involved in bile acid transport, they are also important as drug transporters,

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Table 1. Primer Sequences and Amplicon Size (base pairs) for the Genes of G3PDH, Transporters, and Nuclear Receptors

transporter gene	alternative name	forward primer (5′−3′)	reverse primer (5′−3′)	amplicon size
Slc10a2	ASBT	TCCTGGCCTATTGGATAGATG	CCAGTTTCCAAGGCTACTGTTC	450
Slc22a6	oat1	GAGGTGTCTTAGTCAAGCGA	GCCAGAAGTTCCTTCAGAGT	289
Slc22a7	oat2	CGTCTCACTGTGCTGCATGA	GGGTACAACTCGGACGTGAA	339
Slc22a8	oat3	CAGTCTTCATGGCAGGTATACTGG	GTACCCACCAGGACAACAAGG	390
Slc21a1	oatp1	AGCATTTGGCCTGTCCTTAT	TGTGTGCGTCACCGTAG	1054
Slc21a5	oatp2	ACTGAGTACCTTCTGTCTTTTGT	AAGGCAGCTTTAATGTTAAGGG	767
Slc21a7	oatp3	CTGTGAGAAGTCTGTTGGAATG	GCCTCCTGAGGATGAGAC	767
Slc21a10	oatp4	CAAATCAGCAGCTTCATTGGAT	TTCCCTTCTGGAAACCAGAG	511
Slc21a13	oatp5	ATGCTAACCTGTGATACCACT	GGAAAATCTTAAGTGCTACCCT	972
Slc22a1	oct1	CTCTGGCTACAGGAGAACGAC	CCTGGTACAGCACAGCACAA	403
Slc22a2	oct2	TGGCATCGTCACACCTTTCC	AGCTGGACACATCAGTGCAA	410
Slc22a3	oct3	TCAGAGTTGTACCCAACGACATT	TCTGCCACACTGATGCAACT	266
Slc22a4	octn1	ACATTGCCACCATAACCGTG	ACTGCCCATGAGGATGTAGG	476
Slc22a5	octn2	TTTCGTGGGTGTGCTGAT	GTGATGACCCTGATATTCCGT	585
	octn3	ACTGGTGCCTTCAGACCTAC	TTCAGTTCAATCAGCTTCTGGAC	463
Slc15a1	pept1	GCAGGTGGAAATCGATAAAACTC	TTCTCTGGCTTTTGGTTAGGAC	320
Slc15a2	pept2	AAAATGAGTCCAAGGAAACGCT	TTTTCCTCCCAGTATTGGTATGG	403
Slc26a6	pat1	GGGAGATTGAAGTGGAAGTGTACATC	AAGGCCAGACTGACTGCAATAC	391
NR1H4	FXR	CGGACATGCAGACCTGTTGGAAG	CCAGTGGGTTTCCTGAAGCC	586
	HNF1α	CTCTGTACACCTGGTACGTC	ACTCCGCCCTATTACACTCT	242
NR1I2	PXR	ACCTACATGTTCAAGGGCGTCATC	GCAGGATATGGCCGACTACACTC	415
NR1H3	LXR	GACCTCTGCAATTGAGGTCATGCT	AGGGCAAACACTTGCTCTGAATGG	413
NR2B1	RXRα	CTAAGATGCGTGACATGCAG	GTAAAGATGGCGAGAGTGGT	481
NR5A1	LRH	ATGTCTGCTAGTTTGGATAC	AATTCTGGTTCTCTATGCAC	447
G3PDH		TGAAGGTCGGTGTGAACGGATTTGGC	CATGTAGGCCATGAGGTCCACCAC	983

especially in the hepatic uptake of anionic drugs. Since alteration of the expression level of drug transporters may affect the effectiveness and/or toxicity of clinically used drugs, it is important to clarify the regulation mechanism of drug transporters.

In this study, we examined the possible involvement of FXR and the related factor choilc acid in regulation of the expression of drug transporters using wild-type and FXR-null mice. We found that FXR is involved in the transcriptional regulation of transporters, probably through tissue-specific mechanisms, and provide insight into both the role of FXR in bile acids homeostasis and the influence of cholic acid on transporter expression.

Experimental Section

Animal Treatment and Sample Collection. FXR-null mice were back-crossed to strain C57BL/6 for at least five generations,[4] and were housed under a standard 12 h light−12 h dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow (CE-2, Clea, Tokyo, Japan) and water ad libitum for acclimation. Experimental diets contained 0.5% (w/w) cholic acid (Sigma-Aldrich, St. Louis, MO) mixed with the control diet (CE-2) and were used for 5 days. Age-matched groups of 10−11-week-old animals were used for all experiments. Total RNA was prepared from livers, intestine, kidney, and testis using ISOGEN (Wako Pure Chemical Industries, Tokyo, Japan). The total RNA content was determined by measuring the absorbance at 260 nm (Eppendorf, Hamburg, Germany).

Reverse Transcription Polymerase Chain Reaction. Messenger RNA levels of differentially expressed genes were analyzed using semiquantitative reverse transcription polymerase chain reaction (RT-PCR). Single-strand cDNAs were constructed using an oligo(dT) primer (Invitrogen Corp., Carlsbad, CA). These cDNAs provided templates for PCRs using specific primers (Table 1) at a denaturation temperature of 94 °C for 30 s, an annealing temperature of 58−62 °C for 30−60 s, and an elongation temperature of 72 °C for 30 s in the presence of deoxynucleotides (dNTPs) and Taq polymerase (Takara Shuzo Co., Ltd., Tokyo, Japan). The annealing time and temperature were changed as required, depending on the genes. The PCR cycle numbers were titrated for each primer pair to ensure that amplification was in a linear range. PCR products were analyzed via 2% agarose gel (w/v) electrophoresis and stained with ethidium bromide for visualization. mRNA levels were quantified by using light capture (ATTO Co., Tokyo, Japan). PCR amplification data were normalized with respect to glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The sets of primers specific for the nucleotide sequences of the transporters are shown Table 1. The quantitation of each gene was repeated at least three times using RNA sources isolated from independent animals, and the results were statistically analyzed by using a Student's *t* test.

Results

Alteration of Expression Levels of Transporters in the Liver of FXR-Null Mice. This study used four groups of mice, i.e., wild-type mice fed with control diet (WC), FXR-

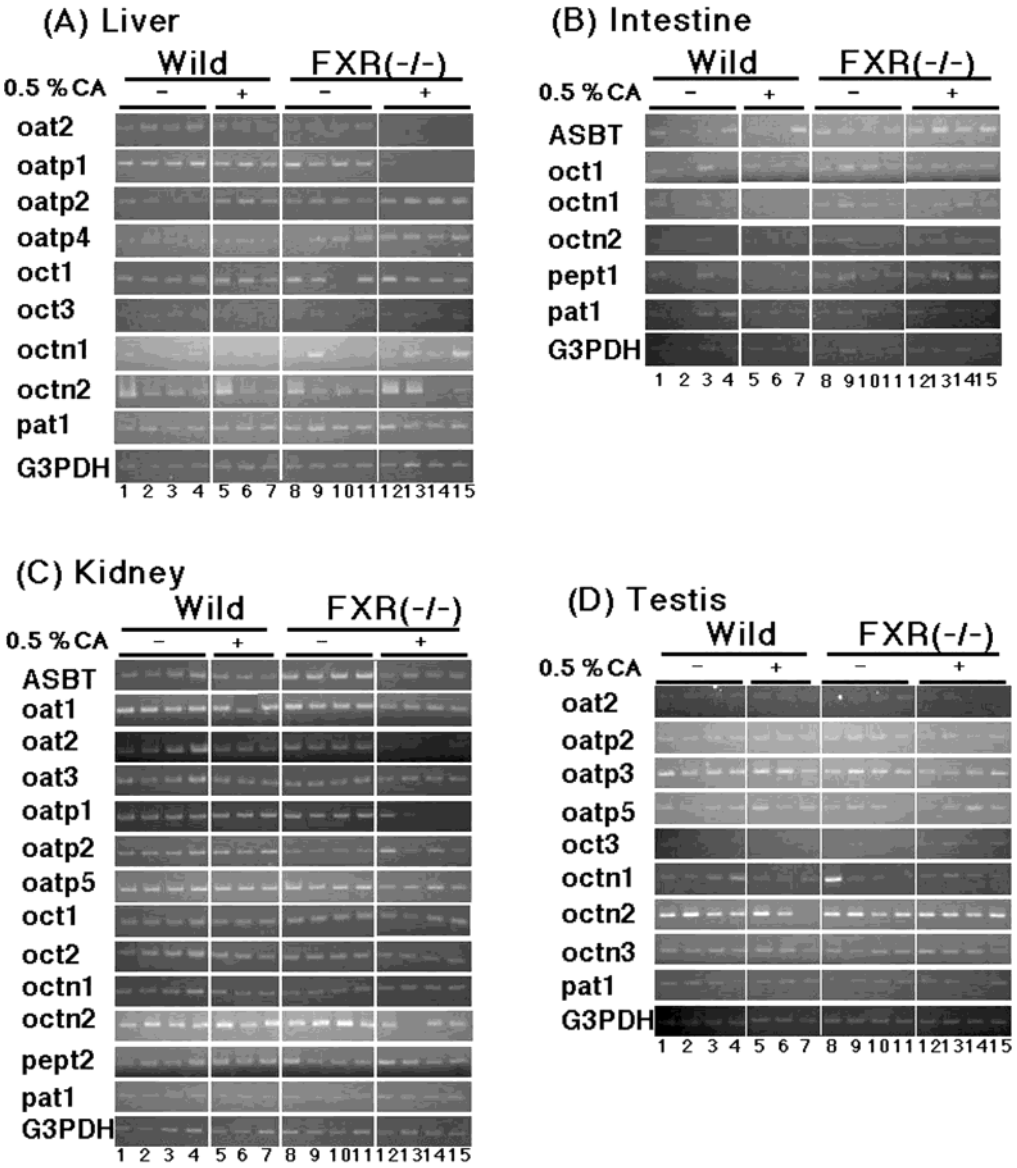

Figure 1. RT-PCR analysis of mRNA levels of transporter in mice tissues. The mRNA levels of transporter genes were measured by RT-PCR. mRNAs were prepared from wild-type and FXR-null male mice fed with a control diet or a diet supplemented with 0.5% cholic acid for 5 days. Specific primers as described in the Experimental Section were used to determine the levels of expression of each drug transporter mRNA in liver (A), small intestine (B), kidney (C), and testis (D). mRNAs from three or four separate mice were analyzed for each group [wild-type mice fed with control diet (lanes 1−4), wild-type mice fed with 0.5% cholic acid (lanes 5−7), FXR-null mice fed with control diet (lanes 8−11), and FXR-null mice fed with 0.5% cholic acid (lanes 12−15)].

null mice fed with control diet (FC), wild-type mice fed with 0.5% cholic acid (WCA), and FXR-null mice fed with 0.5% cholic acid (FCA). The effects of FXR on transcriptional regulation of drug transporters were evaluated directly by comparison of the mRNA levels between WC mice and FC mice. Then, since cholic acid is a ligand for FXR, the involvement of FXR was evaluated by comparison of mRNA levels between WC mice and WCA mice. Last, the effect of cholic acid on FXR was evaluated by comparison of mRNA levels between WC mice and FCA mice.

Hepatic levels of the specific mRNAs of transporters were compared between WC and FC mice by using RT-PCR. Three oatps (oatp1, oatp2, and oatp4) were expressed in liver, and no significant differences in the mRNA levels of these oatps were observed between FC and WC mice (Figure 1A and Table 2). mRNA levels of other transporters expressed in the liver, such as oat2, oct1, oct3, octn1, and octn2, showed no significant differences between FC and WC mice (Figure 1A and Table 2). These results suggested that none of the transporters studied in this experiment are regulated by FXR in the liver.

Cholic acid feeding led to a significant change in the expression of transporters in the liver. In WCA mice, the oatp2−mRNA level was higher and the mRNA levels of

Table 2. Expression Levels of mRNA for Each Transporter Gene Normalized to G3PDH in Wild-Type and FXR-Null Mice Fed a Control Diet or a Diet Supplemented with 0.5% Cholic Acid for 5 Days[a]

	liver			kidney			intestine			testis		
	WCA	FC	FCA	WCA	FC	FCA	WCA	FC	FCA	WCA	FC	FCA
ASBT		NT[d]		0.9	1.6[b]	0.8[c]	1.1	1.5	3.2[b,c]		NT[d]	
Oat1		NT[d]		0.6	0.7	0.4[c]		NT[d]			NT[d]	
Oat2	0.3	0.4	0[b,c]	0.9	0.7	0.1[b,c]		NT[d]		1.0	1.0	0.5[b,c]
Oat3		ND[e]		0.8	0.6[b]	0.6		NT[d]			NT[d]	
Oatp1	0.5[c]	0.7	0[b,c]	0.8	0.4[b]	0.2[b,c]		NT[d]			NT[d]	
Oatp2	2.3[c]	1.9	2.9	1.0	0.3[b]	0.6		NT[d]		0.8	1.0	0.8
Oatp3		NT[d]			NT[d]			ND[e]		0.8	0.6[b]	0.5[b]
Oatp4	0.7	1.3	1.3		NT[d]			NT[d]			NT[d]	
Oatp5		NT[d]		0.8	0.7	0.5[b,c]		NT[d]		0.9	0.6	0.8
Oct1	0.6[c]	0.6	0.4	1.0	1.0	1.0	1.0	1.0	0.7		NT[d]	
Oct2		NT[d]		0.9	0.6[b]	0.5		NT[d]			ND[e]	
Oct3	0.8	0.8	0.6		NT[d]			NT[d]		1.7	2.6	2.2
Octn1	0.5	0.9	1.0	1.0	0.6[b]	0.8	0.9	1.1	1.0	0.5	0.8	0.5
Octn2	0.3	0.6	0.5	1.1	1.0	0.6	0.6	1.3	1.4[b]	0.5	0.6	0.6
Octn3		NT[d]			ND[e]			NT[d]		0.9	0.7	0.7
Pept1		NT[d]			NT[d]		0.4	1.5	2.5[b]		NT[d]	
Pept2		NT[d]		0.9	1.6	0.8		NT[d]			NT[d]	
Pat1	0.8	1.2	0.8	0.9	1.6	0.8	0.6	0.8	1.0	0.9	0.6	0.7
Ntcp[4]	0.4[b]	0.9	0.9									
Bsep[4]	6.0[c]	0.3[b,c]	0.3[c]									
Mdr2[4]	3.3[c]	1.3	3.3[c]									

[a] FXR-null mice fed with control diet (FC), wild-type mice fed with 0.5% cholic acid (WCA), and FXR-null mice fed with 0.5% cholic acid (FCA). The number shows the expression intensity relative to wild-type mice fed with control diet (WC). [b] Significantly different vs wild-type mice fed a control diet ($p < 0.05$). [c] Significantly different vs wild-type mice fed a control diet or FXR-null mice fed a control diet ($p < 0.05$). [d] Not tested. [e] Not detectable.

oatp1 and oct1 were lower than those of WC mice. Similarly, in FCA mice, the oatp2−mRNA level was higher and the oatp1− and oat2−mRNA levels were lower than those of WC mice. The differential changes in oatp1 and oatp2 induced by cholic acid were in good accordance with previous observations after bile salt feeding in wild-type mice and rats.[20,21]

Alteration of Expression of Transporters in the Small Intestine of FXR-Null Mice. The mRNA levels of transporters expressed in the intestine, such as ASBT, oct1, octn1, octn2, pept1, and pat1, did not show significant differences between FC and WC mice (Figure 1B and Table 2). These results suggested that none of these transporters was regulated by FXR in the intestine. In addition, none of the transporters expressed in the intestine showed any significant difference in mRNA levels between WC and WCA mice. However, the mRNA levels of ASBT, octn2, and pept1 transporters in FCA mice were higher than those of WC mice in the intestine (Figure 1B and Table 2).

Alteration of Expression of Transporters in the Kidney of FXR-Null Mice. Six transporters, ASBT, oat3, oatp1, oatp2, oct2, and octn1, were expressed in kidney, and significant differences in the mRNA levels of these transporters were observed between FC and WC mice (Figure 1C and Table 2). The ASBT−mRNA level was higher, but the mRNA levels of oat3, oatp1, oatp2, oct2, and octn1 transporters were lower in FC mice than in WC mice (Figure 1C and Table 2). In the kidney of wild-type mice, feeding of 0.5% cholic acid did not cause any change in the expression of mRNAs of ASBT, octn1, octn2, oct1, pat1, and pept1 (Figure 1C and Table 2). In addition, the mRNA levels of ASBT, oat1, oat2, oatp1, and oatp5 in kidney of FCA mice were lower than those of WC mice (Table 2). The observation suggests that FXR is partly involved in the regulation of drug transporters in kidney.

Alteration of Expression of Transporters in the Testis of FXR-Null Mice. Expression of FXR in the testis was examined by RT-PCR. As can be seen in Figure 2, a band corresponding to FXR was detected. Oatp3 was expressed in the testis, and a significant difference in mRNA levels of this transporter was observed between FC mice and WC mice (Figure 1D and Table 2). Cholic acid did not affect transcriptional regulation of the transporters expressed in testis in wild-type mice. Oat2 and oatp3 mRNA levels in testis of FCA mice were lower than those in testis of WC mice (Figure 1D and Table 2).

Expression of Nuclear Receptors in Several Tissues of FXR-Null Mice. The expression of nuclear receptors that might be involved in the transcriptional regulation of transporters, including RXR, LXR, PXR, HNF1α, LRH-1, CAR, and SHP, in liver, kidney, intestine, and testis was examined by RT-PCR. As shown in Figure 2, mRNAs of RXR, LXR, PXR, and SHP were detected in liver, kidney, intestine, and testis. LRH-1 mRNA was detected in liver and intestine, and mRNA of HNF1α and CAR was detected in liver, kidney, and intestine. A comparison of PXR mRNA levels in WC mice and FC mice revealed no obvious differences in liver (Figure 2B). However, WCA and FCA

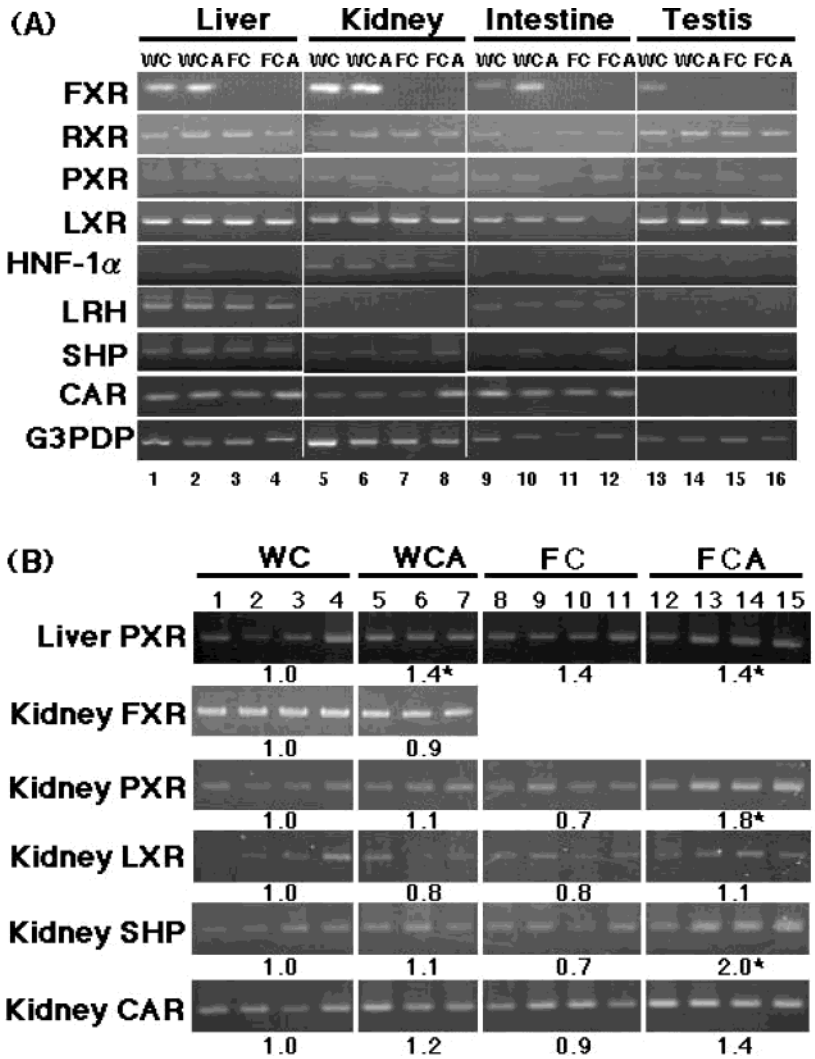

Figure 2. RT-PCR analysis of expression profiles of nuclear receptors in mice tissues. The expression profiles of nuclear receptor genes were examined by RT-PCR. mRNAs were prepared from wild-type and FXR-null male mice fed with a control diet or a diet supplemented with 0.5% cholic acid for 5 days. Specific primers as described in the Experimental Section were used to determine the levels of expression of each nuclear receptor mRNA in liver, small intestine, kidney, and testis. mRNAs from one mouse were analyzed for each group [wild-type mice fed with control diet (lanes 1, 5, 9, and 13), wild-type mice fed with 0.5% cholic acid (lanes 2, 6, 10, and 14), FXR-null mice fed with control diet (lanes 3, 7, 11, and 15), and FXR-null mice fed with 0.5% cholic acid (lanes 4, 8, 12, and 16)] (A). To determine the levels of expression of each nuclear receptor mRNA in kidney, we used by RT-PCR (B). mRNAs from three or four separate mice were analyzed for each group [wild-type mice fed with control diet (lanes 1−4), wild-type mice fed with 0.5% cholic acid (lanes 5−7), FXR-null mice fed with control diet (lanes 8−11), and FXR-null mice fed with 0.5% cholic acid (lanes 12−15)]. The number shows the expression intensity relative to wild-type mice fed a control diet (WC). Asterisks show values are significantly different vs those of wild-type mice ($p < 0.05$).

mice exhibited increased amounts of this transcript compared with WC mice (Figure 2B). In addition, since six transporters, ASBT, oat3, oatp1, oatp2, oct2, and octn1, showed significant differences in the mRNA levels between FC and WC mice (Figure 1C and Table 2), it was possible that mRNA levels of other nuclear receptors changed in kidney. mRNAs of FXR, LXR, PXR, CAR, and SHP were quantified using RT-PCR in kidney. A comparison of PXR, LXR, CAR, and SHP mRNA levels in WC mice and FC mice kidney revealed no significant differences. However, mRNA levels of PXR and SHP in WCA and FCA mice kidney were higher than those in WC mice kidney. In contrast, it is known that the level of expression of SHP mRNA in FC and FCA mice liver was lower than in WC mice liver.[4] In addition, LXR, SHP, and PXR mRNA levels in WCA mice liver were higher than those of WC mice liver but not in kidney.

Discussion

The purpose of this study was to characterize the role of FXR and bile acids in the regulation of the expression of drug transporter genes by using FXR-null mice and mice fed with a high cholic acid diet. First, the relative mRNA levels of 18 different SLC transporter genes, which are possibly involved in drug absorption and disposition as well

Table 3. Summary of Transporters Regulated by FXR

species	transporter	tissue distribution	evidence for regulation by FXR	ref
human	BSEP	liver	promoter binds and is transactivated by FXR	11
	OATP8	liver	promoter binds and is transactivated by FXR	30
mouse	Asbt	intestine, kidney	increased mRNA levels in the intestine and kidney of FXR-null mice	23, this study
	oat3	kidney	reduced mRNA levels in the kidney of FXR-null mice	this study
	oatp1	liver, kidney	reduced mRNA levels in the kidney of FXR-null mice	this study
	oatp2	liver, kidney, testis	reduced mRNA levels in the kidney of FXR-null mice	this study
	oatp3	testis	reduced mRNA levels in the kidney of FXR-null mice	this study
	oct2	kidney	reduced mRNA levels in the kidney of FXR-null mice	this study
	octn1	liver, kidney, intestine, testis	reduced mRNA levels in the kidney of FXR-null mice	this study
	Bsep	liver	reduced mRNA levels in the liver of FXR-null mice	4
rat	Mrp2	liver, kidney, intestine	promoter binds and is transactivated by FXR	26

as bile acid regulation, were directly compared in wild-type and FXR-null mice by means of a semiquantitative RT-PCR method. In addition, we determined the effect of cholic acid on expression of drug transporters in liver, intestine, kidney, and testis, since cholic acid is a ligand of FXR. Our results indicate that FXR mainly regulates transporters, such as ASBT, oat3, oatp1, oatp2, oatp3, oct2, and octn1, expressed in kidney and testis, whereas the transporter expression in liver or intestine was unchanged (Table 3).

Cholic acid feeding led to significantly decreased levels of expression of oatp1 and oct1 and increased levels of expression of oatp2 in the liver of WC mice. Expression of transporters that are expressed in other tissues (intestine, kidney, and testis) was not affected by cholic acid in WC mice (Table 2). However, in FXR-null mice, cholic acid feeding caused significantly decreased levels of expression of oatp1 and oat2 in liver, ASBT, oat1, oat2, oatp1, and oatp5 in kidney, and oat2 and oatp3 in testis, and increased levels of expression of hepatic oatp2 and intestinal ASBT, octn2, and pept1 (Table 2). Sinal et al. reported that the total serum bile acid concentration in FC mice was 8 times greater than that of WC mice. In FCA mice, the serum bile acid concentration was 23-fold greater than that of WC mice.[4] In wild-type mice, cholic acid exists only in liver and regulates several genes. However, disruption of bile acid homeostasis in FXR-null mice results in elevated levels of serum bile acids and possible increases in the levels of cholic acid in various other tissues, such as intestine, kidney, and testis, and thus may affect the transcriptional regulation of various genes, since the serum concentration of cholic acid in FCA mice is significantly higher than that of WC mice.

The oatp gene superfamily and its members mediate transport of a wide spectrum of amphipathic organic solutes, including bile salts.[14] It is possible that oatp1, oatp2, and oatp4 are involved in the hepatic uptake of bile acids. In this study, FXR did not directly regulate expression of oatp1, oatp2, or oatp4 in liver. In addition, NTCP, which is localized in the liver basolateral membrane and takes up bile acids, was not directly regulated by FXR.[4] In contrast, FXR regulates expression of Bsep, which is involved in the process of exporting bile acid from liver to bile (Table 3).[15] Since oatps are likely to participate in the hepatic uptake of bile acids in an Na^+-independent manner for bile acid homeostasis, FXR could regulate the process of export of bile acids from the liver by BSEP, but not the uptake from blood to liver by NTCP and oatp. The oatps expressed in liver, including oatp1, oatp2, and oatp4, may be differentially regulated under pathophysiogical conditions such as cholestasis, because oatp1 and oatp4 mRNA levels were downregulated, whereas oatp2 mRNA was upregulated in WCA mouse liver in comparison with WC mouse liver (Table 2). Recently, Fickert et al.[20] and Rost et al.[21] reported the effects of bile salts on oatp expression in liver. The mRNA levels of oatp1 and oatp4 were downregulated, and oatp2 mRNA was upregulated by bile salts.[20,21] Oatp2 gene expression is induced by pregnenolone 16α-carbonitrile, a ligand of PXR, in rats.[22] In the study presented here, PXR mRNA levels were increased in WCA and FCA mice compared with WC mice, by approximately 1.4-fold in each case (Figure 2B). Thus, mouse oatp2 appears to be regulated by PXR, as well as rat oatp2.

In intestine, the expression of ASBT, octn2, and pept1 transporters was upregulated in FCA mice. ASBT was upregulated by liver receptor homologue-1 (LRH-1).[23] LRH-1 was inactivated by small heterodimer partner (SHP), which is regulated by FXR, and the level of expression of SHP was decreased in FXR-null mice.[4] Accordingly, this result suggested that octn2 and pept1 might be upregulated by LRH-1 in intestine.

In kidney, ASBT, oat3, oatp1, oatp2, oct2, and octn1 transporters were regulated by FXR (Figures 1C and 2). Among these affected transporters, ASBT, oatp1, oatp2, and oat3[24] can transport bile acids. On the other hand, oct2 and

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octn1 mediate the transport of organic cations, and are unlikely to accept bile acids as substrates. Accordingly, FXR is also likely to regulate transcriptionally the expression of transporters that are not involved in the regulation of bile acids and/or lipid homeostasis in the kidney.

FXR is a bile acid-activated receptor that alters transcription by binding as a heterodimer with RXR to response elements (FXREs) within regulatory regions of target genes. There are several types of consensus sequence for FXREs, such as IR-1, IR0, ER8, and IR8. FXR can also weakly bind DR4 and DR5 sequences.[25] We have been looking for FXREs that are binding sites of FXR in the promoter region of seven transporters (ASBT, oat3, oatp1, oatp2, oatp3, oct2, and octn1). Oat3 possesses an ER8-like consensus sequence in the promoter region, while the other transporters, ASBT, oatp1, oatp2, oatp3, oct2, and octn1, do not have consensus sites for FXREs such as IR-1, IR0, ER8, and IR8 in their promoter regions when examined up to −10 kb upstream of the transcription initiation site. It is possible that there are other unknown consensus sequences recognized by FXR in these transporter promoter regions or that the recognized regions are distant from the start site of transcription. However, it is not easy to determine by scanning the sequence due to the impression of the consensus sequence at present. Bsep possesses an IR-1 consensus sequence in the promoter region and is regulated by FXR in vitro.[11] The mRNA level of Bsep was decreased in FXR-null mice compared with wild-type mice.[4] The multidrug resistance-associated protein 2 (Mrp2) gene has an ER-8 sequence and is regulated by FXR in vitro.[26] However, since Mrp2 mRNA levels were similar in wild-type and FXR-null mice,[27,28] it is possible that Mrp2 is regulated by FXR, but transcriptional regulation of Mrp2 is mainly controlled by other nuclear receptors, such as PXR or CAR.[26]

Members of the nuclear receptor superfamily of ligand-activated transcription factors have critical roles in many aspects of development and adult physiology, including cholesterol homeostasis, bile acid biosynthesis and transport, and xenobiotic metabolism. Recently, two orphan nuclear receptors, FXR and PXR, were shown to be activated by an overlapping spectrum of bile acids,[1,29] and it is important to evaluate the nuclear receptors that might be associated with FXR. In this study, the expressions of some nuclear receptors (HNF1α, PXR, LXR, RXR, LRH-1, CAR, and SHP) associated with FXR or bile salts were examined in liver, intestine, kidney, and testis by RT-PCR. The expressions of various transporters are known to be regulated by one or more orphan nuclear receptors; for example, Mrp2 expression is regulated by FXR, PXR, and CAR,[26] and OATP8 expression is regulated by FXR and HNF1α.[30] In this study, the expression of transporters, such as oatp1, oatp5, oat1, and oat2, was largely changed in FCA mice (Table 2); however, these effects were independent of FXR, and they might associate with other nuclear receptors. This study quantified mRNA levels of FXR, HNF1α, PXR, LXR, RXR, LRH-1, CAR, and SHP in four groups of mice kidneys (Figure 2B). A comparison of mRNA levels of these nuclear receptors in WC mice and FC mice kidney revealed no obvious differences. However, CAR and SHP mRNA levels in FC mice liver were different from those in WC mice liver.[4,31] The differential effect of FXR knockout between liver and kidney suggested that FXR did not regulate expression of SHP in kidney, and there are different transcriptional regulation mechanisms in the two tissues. In addition, LXR, SHP, and PXR mRNA levels in WCA mice liver were higher than those of WC mice liver but not in kidney. These results suggested that regulatory mechanisms of cholic acid were different in liver and kidney.

In summary, this study provides evidence that seven transporters, ASBT, oat3, oatp1, oatp2, oatp3, oct2, and octn1, were regulated by FXR in a tissue-specific manner, suggesting the presence of tissue-specific cofactors for FXR. It is possible that changes in the mRNA level in the FXR-null mice may be due not only to differences in transcription but also to differences in mRNA stability. Accordingly, more research will be required to determine whether FXR regulates seven transporters directly. Furthermore, it was suggested that FXR might have other functions besides regulation of

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bile salts and/or cholesterol, because some of the seven transporters (octn1 and oct2) are not involved in the transport of bile acids or cholesterol. Further, the effect of cholic acid treatment suggests the involvement of other regulatory mechanisms besides FXR in controlling expression of the tested transporters. In addition, LXR, SHP, and PXR mRNA levels in WCA mice liver were higher than those of WC mice liver but not in kidney. These results suggested that regulatory mechanisms for cholic acid were different in liver and kidney.

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