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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Farnesoid X Receptor Agonist Treatment Alters Bile Acid Metabolism but Exacerbates Liver Damage in a Piglet Model of Short-Bowel Syndrome

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SUMMARY

The farnesoid X receptor agonist obeticholic acid has been shown to ameliorate cholestasis in liver disorders. However, in the context of liver disease secondary to bowel loss, obeticholic acid administration exacerbated liver injury and repressed the expression of intestinal farnesoid X receptor target genes.

BACKGROUND & AIMS: Options for the prevention of short-bowel syndrome–associated liver disease (SBS-ALDs) are limited and often ineffective. The farnesoid X receptor (FXR) is a newly emerging pharmaceutical target and FXR agonists have been shown to ameliorate cholestasis and metabolic disorders. The aim of this study was to assess the efficacy of obeticholic acid (OCA) treatment in preventing SBS-ALDs.

METHODS: Piglets underwent 75% small-bowel resection (SBS) or sham surgery (sham) and were assigned to either a daily dose of OCA (2.4 mg/kg/day) or were untreated. Clinical measures included weight gain and stool studies. Histologic features were assessed. Ultraperformance liquid chromatography tandem mass spectrometry was used to determine bile acid composition in end point bile and portal serum samples. Gene expression of key FXR targets was assessed in intestinal and hepatic tissues via quantitative polymerase chain reaction.

RESULTS: OCA-treated SBS piglets showed decreased stool fat and altered liver histology when compared with nontreated SBS piglets. OCA prevented SBS-associated taurine depletion, however, further analysis of bile and portal serum samples indicated that OCA did not prevent SBS-associated alterations in bile acid composition. The expression of FXR target genes involved in bile acid transport and synthesis increased within the liver of SBS piglets after OCA administration whereas, paradoxically, intestinal expression of FXR target genes were decreased by OCA administration.

CONCLUSIONS: Administration of OCA in SBS reduced fat malabsorption and altered bile acid composition, but did not prevent the development of SBS-ALDs. We postulate that extensive small resection impacts the ability of the remnant intestine to respond to FXR activation. *(Cell Mol Gastroenterol Hepatol 2017;4:65–74; http://dx.doi.org/10.1016/j.jcmgh.2017.02.008)*

Keywords: Short-Bowel Syndrome; Liver Disease; Intestinal Failure–Associated Liver Disease; Obeticholic Acid; Bile Acids; Farnesoid X Receptor.

See editorial on page 201.

Short-bowel syndrome (SBS) describes a condition of malabsorption and malnutrition resulting from the loss of absorptive surface area after small-bowel resection.[1] The prevention of severe liver disease in patients with SBS is one of the major challenges in the clinical management of these complex patients. SBS–associated liver disease (SBS-ALD) occurs in approximately 65% of infants after small-bowel resection[2] and is the cause of death in 3%–19% of infants with SBS.[3] Despite the high mortality associated with SBS-ALD, the cause is not well understood and treatment options are limited.

By using a preclinical piglet model of SBS we have focused our work on uncovering the molecular, metabolic, and microbial alterations underpinning the development of SBS-ALD. Recently, we described SBS-ALD–associated alterations in bile acid composition associated with disrupted farnesoid X receptor (FXR) signaling mechanisms.[4] FXR is a member of the nuclear hormone receptor family of transcription factors.[5] FXR is highly expressed in the intestine and liver where it regulates the expression of genes involved in bile acid synthesis, absorption, and transport, thereby facilitating the emulsification and absorption of

Abbreviations used in this paper: **FXR, farnesoid X receptor; OCA, obeticholic acid; CDCA, chenodeoxycholic acid; FGF19, fibroblast growth factor-19; HCA, hyocholic acid; LCA, lithocholic acid; DCA, deoxycholic acid; HDCA, hyodeoxycholic acid; SBS, short-bowel syndrome; SBS-ALD, short-bowel syndrome–associated liver disease; UDCA, ursodeoxycholic acid; UPLC, ultraperformance liquid chromatography.**

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both dietary fats and fat-soluble vitamins, while preventing the toxic accumulation of bile acids within the liver. Hence, there is increasing interest in the application of FXR agonists in the treatment of gastrointestinal disease. The most commonly studied FXR agonist is obeticholic acid (OCA), a potent semisynthetic analogue of the primary bile acid chenodeoxycholic acid, which selectively activates FXR.[6,7] OCA has shown hepatoprotective effects in patients with primary biliary cirrhosis,[8,9] diabetes-associated nonalcoholic fatty liver disease,[6] and nonalcoholic steaohepatitis.[10] A recent report also suggested efficacy in patients with primary bile acid diarrhea.[11]

Given the success of OCA administration in the prevention of liver disease in both mouse models and human disease, we postulated that administration of OCA to SBS piglets would prevent the development of SBS-ALD via preservation of bile acid composition and FXR signaling pathways within the liver and intestine. Although OCA efficacy has been shown in a variety of gastrointestinal settings, this study investigated OCA efficacy performed in the context of reduced bowel length and associated liver disease.

Materials and Methods

Animals

This study was approved by the Animal Ethics Committee of the Murdoch Childrens Research Institute. Weaned female 3-week-old piglets were transported to The University of Melbourne Centre for Animal Biotechnology (Landrace/large white cross; Aussie Pride Pork, Kialla, Australia) and acclimatized before surgery. Piglets were housed at a temperature of 22°C with a 12-hour light/dark cycle and fed a polymeric infant formula diet (Karicare De-Lact; Nutricia, Macquarie Park, Australia) supplemented to meet the daily requirements for piglets. The diets were isocaloric and isonitrogenous among the groups and were administered on a per-kilogram basis. Water was given twice daily. Piglets were housed separately throughout the study.

Clinical Assessment and Growth

Piglet weight was measured weekly before feeding. Stool samples were collected weekly and stool consistency was scored by the Royal Children's Hospital Laboratory Services (Parkville, Australia). The presence of fat globules within the stool was assessed semiquantitatively and given a score between 0 and 3.

Experimental Design

Piglets were allocated randomly to untreated or OCA-treated sham and SBS groups and acclimatized for 1 week within the animal facility. Piglets then underwent either a 75% proximal small-bowel resection (SBS group) or a transection and re-anastomosis (sham group) surgery. The 75% small-bowel resection included the removal of the small bowel from 90 cm distal to the ligament of Treitz to 225 cm proximal to the ileocecal valve. During the sham procedure, the intestine was transected and re-anastomosed at a site 225 cm proximal to the ileocecal valve. Piglets received intramuscular amoxicillin (70 mg/kg; CSL Limited, Melbourne, Australia) 24 hours before surgery and the day of surgery. Piglets received amoxicillin and rehydration salts (Sanofi-Aventis, South Melbourne, Australia) for 3 days after surgery in line with current clinical practice. Water and polymeric infant formula diet were re-reintroduced from the third day after surgery. OCA/0.5% methylcellulose (kindly provided by Intercept Pharmaceuticals, Inc, New York, New York) was administered daily via a single gavage dose of 2.4 mg/kg/day for 14 days from the time of surgery. The dose rate of 2.4 mg/kg/day was based on the dose rate of 10 mg/kg/day used in previous murine and rabbit studies[12] that was adjusted to account for piglet metabolism according to guidelines published by the US Food and Drug Administration.

Sample Collection

Animals were euthanized 2 weeks after surgery. Portal plasma and bile samples were obtained on the day they were killed and frozen at -80°C until required. Liver samples were collected from the right medial lobe and terminal ileum samples were obtained from a point 7 cm proximal to the ileocecal valve. Samples were placed in 4% paraformaldehyde (Australian Biostain Pty Ltd, Traralgon, Australia), optimal cutting temperature compound, or snap frozen in liquid nitrogen.

Hepatic Histology

To provide an overview of changes in hepatic morphology, an experienced veterinary pathologist examined H&E-stained 5-μm liver sections. Alterations in hepatic lobular structure including the number of hepatic lobules per field of view and hepatic lobule area were measured on size-standardized, H&E-stained, liver sections using ImageJ software (National Institutes of Health, Bethesda, MD).[13] Oil Red O staining[14] was performed on frozen optimal cutting temperature–embedded 10-μm liver sections to visualize hepatic fat accumulation. After staining, a minimum of 10 individual hepatic lobules were photographed per pig (Leica Biosystems, Mount Waverly, Australia) and optical density measurements were performed using ImageJ software. Results are expressed as a percentage of Oil Red O staining per field of view obtained from a minimum of 10 fields of view per pig. Trichrome staining was performed on 4-μm formalin-fixed liver sections and the slides were scanned at $\times$20 magnification (Dotslide; Olympus Corporation, Tokyo, Japan). Changes in endothelial wall thickness were detected using ImageJ software on a minimum of 10 vessels per pig.

Determination of Bile Acid Composition

Enodogenous bile acid composition and the concentration of exogenous obeticholic acid was determined in portal bile. Bile acid standards and bile acid derivative obeticholic acid were purchased from Sigma-Aldrich Corporation (St Louis, MO) or Steraloids Inc (Newport, RI). Deuterated cholic acid (D-2452) and deuterated chenodeoxycholic acid (D-2772) were purchased from CDN Isotopes, Inc (Pointe-Claire, Quebec, Canada). High-performance liquid chromatography–grade chemicals were obtained from Fisher Scientific (Fair Lawn, NJ). Bile acids were extracted from

portal serum and bile with 50% ice-cold methanol, followed by further extraction with acetonitrile (5% NH_4OH). Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS) was performed on the resultant supernatant using a modified method[15] and samples were analyzed on an Acquity UPLC system coupled to an LCT Premier mass spectrometer (Waters Corporation, Milford, MA). Analytes were quantified individually (3 technical reads per sample) against known bile acid and obeticholic standard curves. All quantifications were normalized relative to internal standards.

RNA Preparation and Quantitative Polymerase Chain Reaction

RNA was extracted from liver and terminal ileum epithelium using TRIzol (ThermoFischer Scientific, Waltham, MA) and RNA (0.1 μg) and reverse-transcribed into complementary DNA. Primers were designed using the Roche Universal Probe Library Assay Design Center (Table 1). Quantitative reverse-transcription polymerase chain reactions (10 μL) contained 2.5 μL diluted complementary DNA, 5 μL FastStart TaqMan Probe Master (Roche Diagnostics, Risch-Rotkreuz, Switzerland), 900 nmol/L of each primer, and 250 nmol/L of probe mix. All reactions were performed in triplicate on the LightCycler 480 System (Roche Diagnostics). The $2^{-\Delta\Delta Ct}$ method[16] was used to calculate the relative changes in gene expression determined from quantitative reverse-transcription polymerase chain reaction experiments using either HRPT1 (liver) or RPL32 (terminal ileum) as a housekeeping gene and relative to the sham control group.

Table 1. List of Primer Sequences and Universal ProbeLibrary Probe Combinations Used in This Study

Primer	Sequence 5' to 3'	UPL probe number
CYP7A1 forward	AGGGTGACGCCTTGAATTT	46
CYP7A1 reverse	GGGTCTCAGGACAAGTTGGA	
SHP (NR0B2) forward	AGTGCTGCCTGGAGTCCTTA	50
SHP reverse	CCTTTCAGGTAGGCGTATTCC	
MRP2 (ABCC2) forward	TCTTGGTGACACACAGCATTC	60
MRP2 reverse	TTCCCACAACCACAATCTCA	
BSEP (ABCB11) forward	GCCTGACCACGAGCATCT	3
BSEP reverse	AGGTCAGTTTCCAACCCTGAT	
OSTα (SLC51A) forward	CCTGTTTCTCATCCCTGACG	3
OSTα reverse	AGCAGCGCTCTCCTCAGA	
OSTβ (SLC51B) forward	CAGGAGCTGCTGGAAGAGAT	37
OSTβ reverse	GACCATGCTTATAATGACCACCA	
SULT2A1 forward	GCCTCATCAGTTCCCACCT	60
SULT2A1 reverse	GCCTTGGACTTGAAGAAAGC	
FGF19 forward	ACACCATCTGCCCGTCTCT	13
FGF19 reverse	CCCCTGCCTTTGTACAGC	
ILBP (FABP6) forward	GCAAGAAGTTCAAGGCCACT	77
ILBP reverse	GGTGGTAGTTGGGGCTGTT	
HPRT1 forward	CAGTCAACGGGCGATATAAAA	22
HPRT1 reverse	CAACAATCAAGACATTCTTTCCAG	
RPL32 forward	AACTGGCCATCAGGGTCAC	64
RPL32 reverse	CACAACTGGAACTCCTGTCTATTC	

IL, ileal lipid; UPL, universal probelibrary.

Protein Detection

Fibroblast growth factor-19 (FGF19) was detected in portal serum samples obtained at the time the piglets were killed after an overnight fast using a porcine-specific FGF19 enzyme-linked immunosorbent assay kit (Mybiosource, Inc, San Diego, CA).

Statistical Analysis

All authors had access to the data and reviewed and approved the final manuscript. All study results are reported as means ± SEM. Statistical analysis was performed using 1-way analysis of variance followed by the Tukey post hoc test (GraphPad Prism Software 6.0, La Jolla, CA). *P* values less than .05 were considered statistically significant.

Results

OCA Administration Reduced Fat Malabsorption but Altered Liver Morphology

Circulating levels of OCA within portal samples were quantified via UPLC-MS to confirm OCA uptake in treated animals. Circulating OCA was not detectable in untreated sham or SBS piglets. Circulating OCA was confirmed to be of similar levels in both treated SBS and sham animals (Figure 1*A*). SBS piglets showed common clinical manifestations of SBS including failure to gain weight, persistent diarrhea, and fat malabsorption (Figure 1*B–D*). OCA treatment did not influence weight gain or resolve diarrhea in SBS piglets, however, OCA-treated SBS piglets showed decreased stool fat, suggestive of improved fat absorption.

OCA treatment has been efficacious in preventing the development of liver disease in a range of animal models. Untreated, sham-operated piglets showed normal liver histology when compared with SBS piglets who showed decreased hepatic lobule area and small clusters of inflammatory cells together with mild-to-moderate vesicular zone 3 lipidosis (Figure 2). SBS-associated histologic alterations were observed to be exacerbated by OCA treatment with the number of hepatic lobules per field of view increased, and conversely hepatic lobule areas decreased in OCA-treated SBS piglets (Figure 2*A*). Oil Red O staining was quantified as an indication of fat droplet accumulation within the liver. Levels of fat droplet accumulation did not differ between untreated and treated sham groups (Figure 2*B*). Droplet accumulation was considered mild-to-moderate in SBS piglets and was confined to zone 3 and midzone, with sparing of the midportal parenchyma. Lipid accumulation was increased further in SBS piglets after OCA treatment,

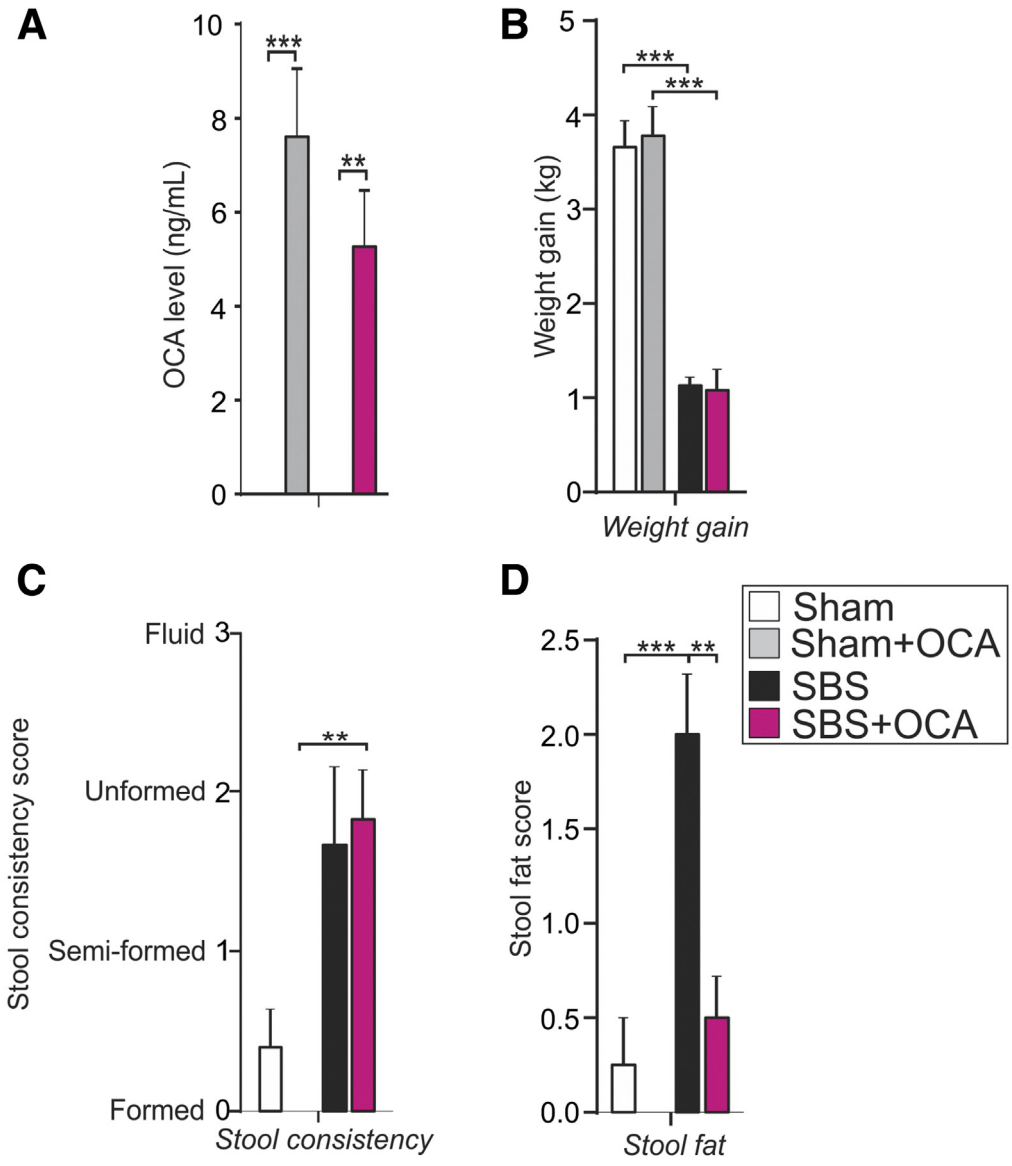

Figure 1. Clinical characteristics of untreated and OCA-treated sham and SBS piglets. (*A*) Portal OCA levels as measured by UPLC-MS, (*B*) weight gain, (*C*) stool consistency, and (*D*) stool fat score in untreated and OCA-treated sham and SBS piglets. Means ± SEM, $^{**}P < .01$, $^{***}P < .001$. N = 5–6/group.

however, these differences were only significant in OCA-treated sham compared with OCA-treated SBS piglets. Examination of H&E-stained hepatic sections showed evidence of lymphedema including the presence of capsule swelling, connective tissue edema, and swollen fibroblasts in OCA-treated SBS piglets (Figure 2*C*).

OCA Treatment Prevents the SBS-Associated Depletion of Taurine but Is Unable to Prevent Bile Acid Dysmetabolism

Bile samples were analyzed to assess changes in hepatic bile acid synthesis after enterohepatic recirculation. Low bile taurine levels were observed in untreated SBS piglets when compared with sham controls, but these levels normalized after OCA treatment (Figure 3*A*). The biliary bile acid profile of SBS piglets was disturbed significantly relative to that observed in sham controls, with increased levels of both conjugated and free primary bile acid hyocholic acid (HCA) and its conjugated precursor chenodeoxycholic acid (CDCA). Secondary bile acid species resulting from microbe and hepatic metabolism (conjugated and unconjugated lithocholic acid [LCA], conjugated deoxycholic acid [DCA], conjugated hyodeoxycholic acid [HDCA], and conjugated ursodeoxycholic acid [UDCA]) were decreased in SBS piglets. OCA treatment decreased conjugated CDCA levels in sham piglets alone, whereas conjugated LCA, DCA, and HDCA levels in SBS piglets increased, although these levels remained significantly decreased when compared with sham controls.

Measurement of portal bile acid composition was performed to determine the composition of bile acid in circulation and for transport to the liver. Portal taurine levels were unchanged by either surgery or OCA treatment (Figure 3*B*). Both unconjugated and conjugated HCA levels were increased substantially and significantly in SBS piglets,

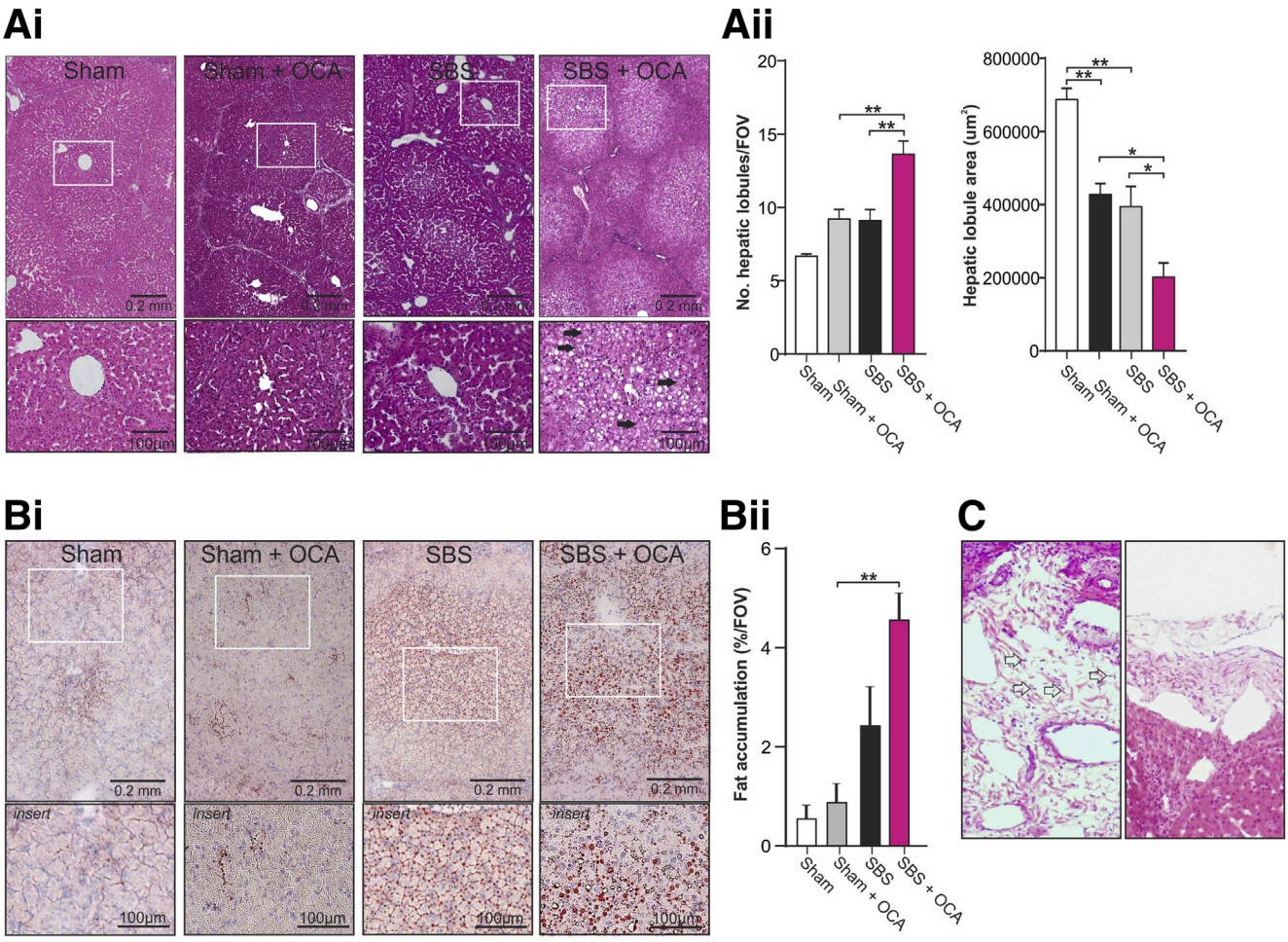

Figure 2. OCA treatment exacerbated SBS-associated liver injury. (*A*) Histologic evidence of fat droplet accumulation was observed in H&E-stained liver sections of SBS piglets with granulated hepatocytes apparent in OCA-treated SBS piglets (*block arrow*; i–ii). (*B*) Fat droplet accumulation in SBS piglets was confirmed further by Oil Red O staining (i and ii). (*C*) OCA-treated SBS piglets also were observed to show evidence of lymphedema including swelling of the capsule, the development of capsule tags, loose connective tissue around portal structures, and swollen fibroblasts (indicated by *arrows*). Means ± SEM, $^{*}P < .05$ and $^{**}P < .01$. N = 5–6/group.

whereas unconjugated DCA was almost undetectable and unconjugated LCA was reduced. SBS piglets had a decrease in portal conjugated UDCA when compared with sham controls, consistent with the reduced levels observed in the biliary bile. Conjugated LCA and HDCA were undetectable in portal samples from SBS piglets, but HDCA levels increased with OCA treatment. OCA treatment in SBS piglets resulted in a significant reduction in portal unconjugated HCA levels when compared with untreated SBS piglets, however, levels remained significantly increased compared with sham controls.

A Dichotomous Gene Response to OCA Treatment Between the Intestine and Liver in SBS

Given our previous findings of disturbed FXR signaling in SBS piglets[4] and the OCA-induced modulation of bile acid composition in SBS piglets, we investigated the relative expression of key intestinal and hepatic FXR target genes involved in the regulation of bile acid synthesis and transport. Small-bowel resection had no effect on gene expression levels of FXR targets in either the intestine or the liver (Figure 4*A* and *B*). In the liver, OCA treatment of sham and SBS piglets resulted in increased MRP2 gene expression, whereas small heterodimer partner (SHP), bile salt export pump (BSEP), organic solute transporter beta (OSTβ), and Sulfotransferase Family 2A Member 1 (SULT2A1) were increased only in OCA-treated SBS piglets when compared with untreated SBS piglets. Conversely, within the intestine, gene expression of the FXR targets FGF19, ileal lipid binding protein, and OSTβ were reduced in SBS piglets after OCA treatment. The ileal hormone FGF19 is secreted into the portal blood in response to FXR activation and although levels were increased after small-bowel resection, OCA treatment did not alter portal FGF19 levels in OCA-treated sham or SBS piglets (Figure 4*C*). FXR gene expression was measured in liver and intestine samples (Figure 4*D*). FXR gene expression was increased significantly within the liver of OCA-treated sham piglets and untreated SBS piglets when

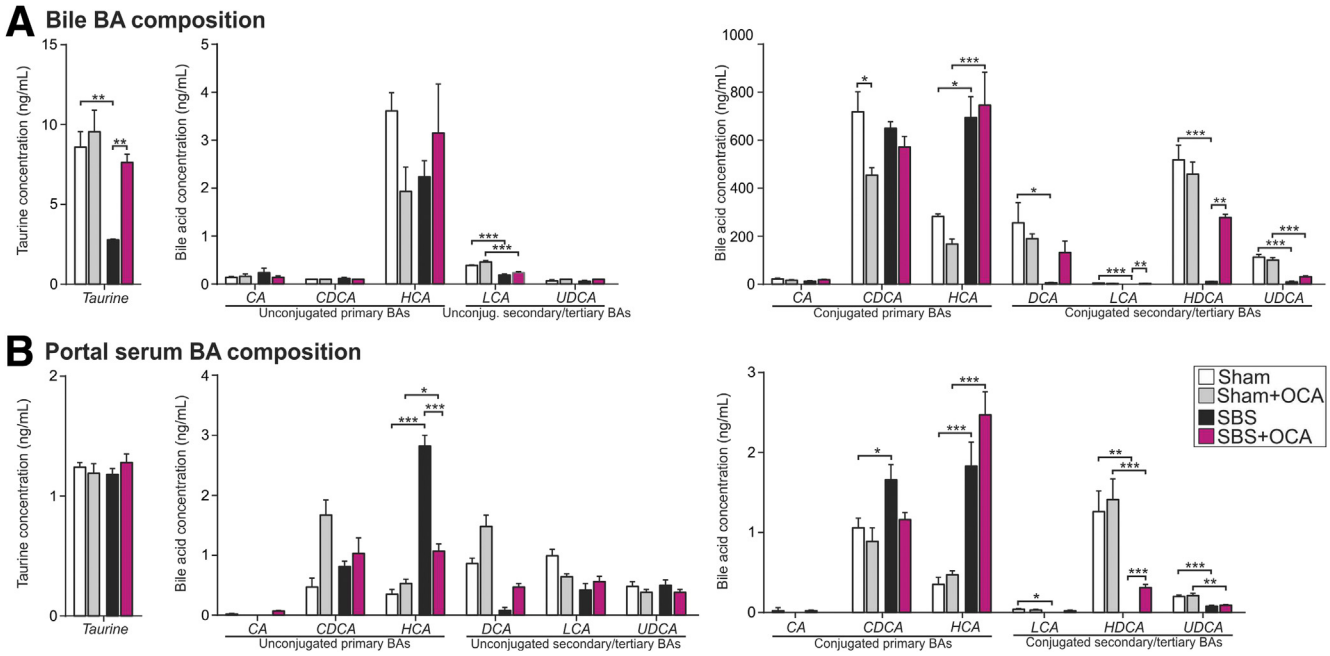

Figure 3. SBS-associated alterations in bile acid composition were not prevented by OCA treatment. Concentration of taurine, unconjugated bile acid species, and conjugated bile acid species with (*A*) bile and (*B*) portal samples. Means ± SEM, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. N = 5–6/group. BA, bile acid; CA, cholic acid.

compared with nontreated sham piglets. Hepatic FXR gene expression was comparable between untreated and OCA-treated SBS piglets. Within the intestine, FXR gene expression was comparable between untreated and OCA-treated sham piglets and between both sham groups and OCA-treated SBS piglets. In contrast, intestinal FXR gene expression was 11-fold higher in untreated SBS piglets when compared with untreated sham piglets.

Discussion

FXR agonists have been implicated in hepatoprotective functions and show multiple metabolic benefits, however, accumulating evidence has suggested that the effects of chronic FXR activation are likely to be scenario-dependent. Although chronic FXR activation is beneficial in primary biliary cirrhosis,[8] diabetes-associated nonalcoholic fatty liver disease,[6] and nonalcoholic steatohepatitis,[10] the chronic activation of FXR in a perinatal mouse model led to unexpected partial perinatal lethality and spontaneous liver toxicity.[5] In this study we examined FXR agonist administration in a novel pathologic setting: that of liver disease associated with short-bowel syndrome. Administration of the FXR agonist OCA to SBS piglets prevented steatorrhea and normalized bile taurine levels, but did not prevent bile acid dysmetabolism or the development of SBS-ALD. Furthermore, although the expression of FXR target genes was increased in the liver of SBS piglets treated with OCA, paradoxically the expression of FXR target genes in the intestine was decreased.

OCA treatment was effective in reducing fat malabsorption, which may offer clinical benefit in patients with SBS. Fat malabsorption is one of the most troublesome symptoms experienced by patients with SBS. Not only does it cause discomfort, but also results in a waste of ingested energy and fat-soluble vitamins, and can be a key contributor to malnutrition experienced by patients with SBS. A treatment that enhances absorptive status has the potential to convert a parenteral nutrition–dependent SBS patient to enteral independence. However, clinical benefit provided by OCA treatment on enhancing intestinal fat absorption is outweighed by the impact of increased hepatic fat accumulation and lymphedema observed after OCA treatment.

The results from our study are consistent with a study in patients with secondary bile acid diarrhea after extensive intestinal resection.[11] We were unable to replicate the results described in patients with primary bile acid diarrhea, in whom OCA administration improved stool frequency and form, and stimulated FGF19 secretion.[11] Because taurine promotes bile flow and biliary conjugation, it is possible that prevention of low biliary taurine levels resulting from OCA treatment in SBS may reduce the risk of development of SBS-ALD. OCA treatment also partially reversed disturbances in levels of conjugated bile acids and the portal bile acid transporter BSEP in SBS piglets. Low levels of conjugated bile acids have been associated with malnutrition, but whether this may contribute to malnutrition experienced in patients with SBS-ALD is not known.[17] The prevention or modification of disturbances in the bile acid profile and biliary taurine levels, and a reduction in fat malabsorption after OCA treatment, may have the potential to offer clinical benefit to patients with SBS.

OCA treatment has been effective in preventing liver disease in a range of animal models, including those mimicking cholestasis,[18] steatosis,[19] and cirrhosis.[20] However, in the current study we report evidence of hepatic lipidosis, hepatic metabolite toxicity, and extensive lymphedema within the

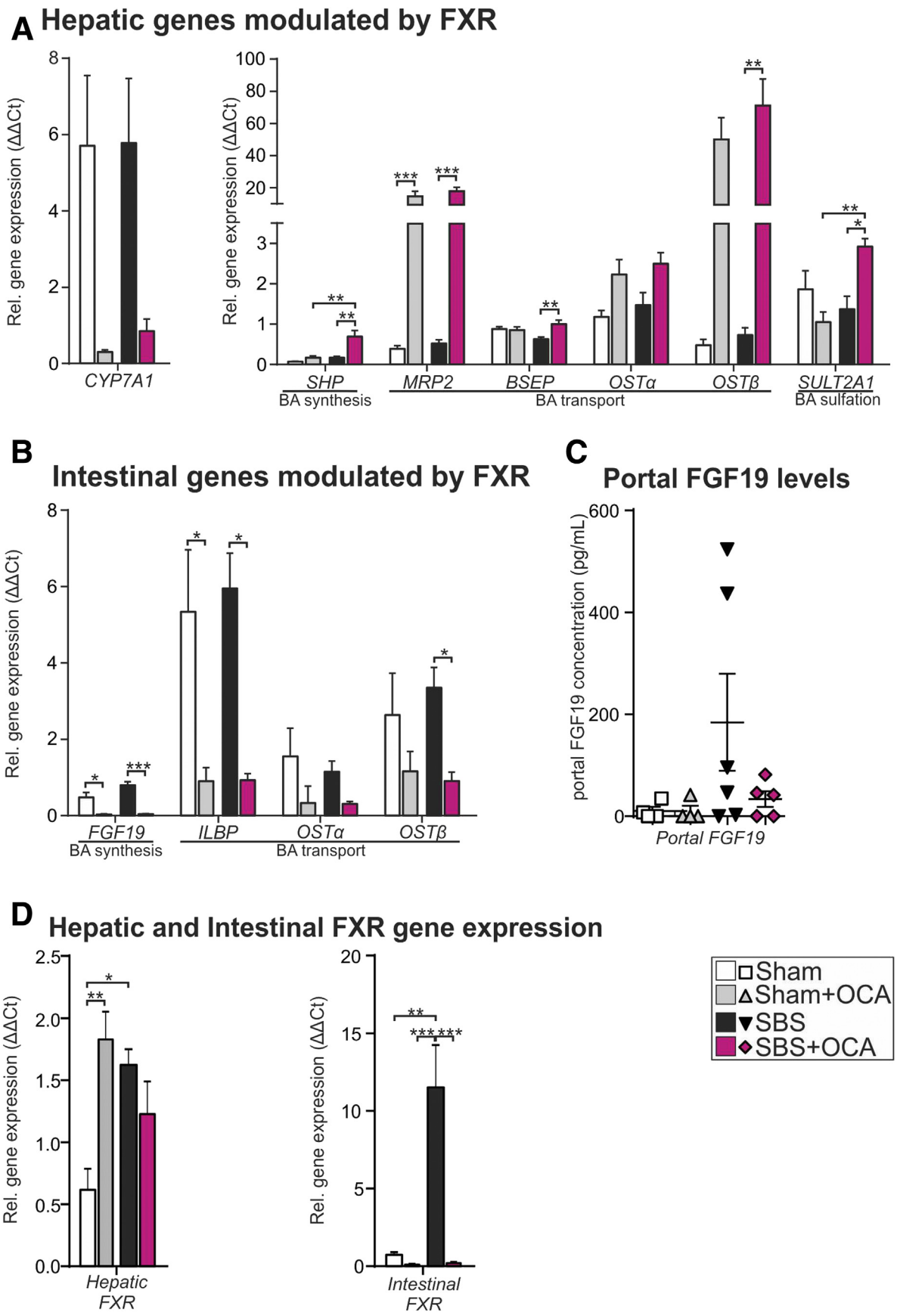

Figure 4. OCA treatment resulted in increased gene expression of FXR targets within the liver, but paradoxically a decrease in FXR target gene expression in the intestine of SBS piglets. The relative gene expression of FXR gene targets within the (*A*) liver and (*B*) intestine from untreated and OCA-treated sham and SBS piglets, and (*C*) portal FGF19 concentration. Means ± SEM, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$. N = 5–6/group. BA, bile acid.

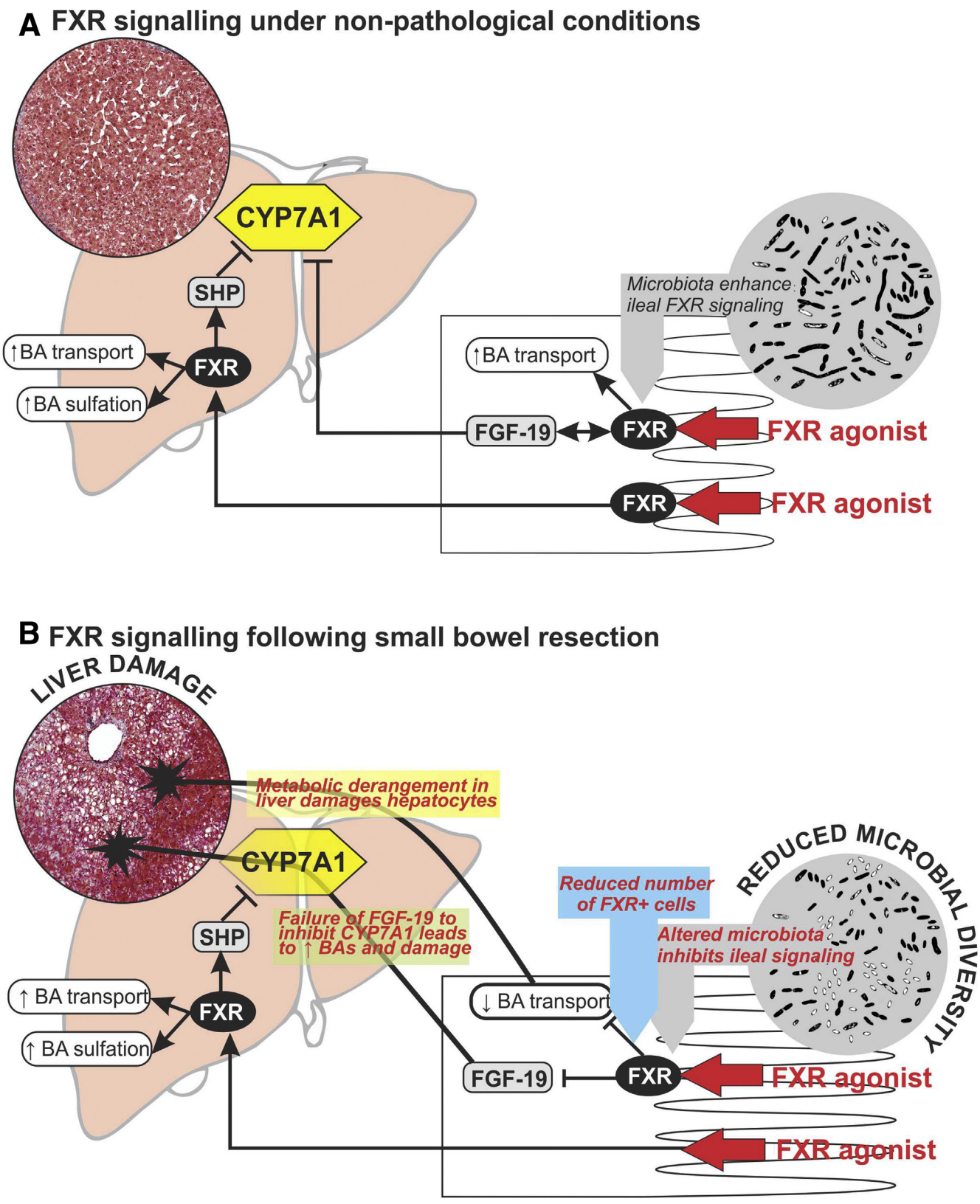

Figure 5. The potential influence of SBS-associated microbial dysbiosis on OCA efficacy within the intestine. BA, bile acid; CYP7A1, cytochrome P450 7A1.

liver of SBS piglets treated with OCA. A major difference between previous studies and this study is the underlying etiology of the liver disease. Prior studies have examined OCA treatment in models in which liver disease has been introduced by a chemical, surgical, or genetic cause with an intact intestine. In contrast, in this study, liver disease has occurred in the setting of a significant disruption of the gut–liver axis after the removal of 75% of the small intestine. Suppressed FXR activation within the remnant intestine of SBS piglets treated with OCA may explain the liver injury observed. Although hepatic and intestinal FXR traditionally have been considered to cooperate to down-regulate CYP7A1 expression, evidence emerging from tissue-specific knockout and transgenic FXR activation models suggest that it is intestinal FXR activation that protects the liver from bile acid–associated damage. These studies have shown that intestinal FXR activation confers protection against hepatocarcinogenesis[21] and intrahepatic and extrahepatic cholestasis[22] via restoration of the Fgf15/Fibroblast Growth Factor Receptor 4 enterohepatic signaling axis and consequent up-regulation of bile acid detoxification and efflux pathways. Conversely, Modica et al[22] have suggested that activation of the canalicular bile acid transport systems by hepatic FXR alone is unlikely to provide hepatoprotection. This is consistent with the current study in which hepatic FXR targets were activated in SBS piglets treated with OCA, but failed to prevent liver disease. In the setting of SBS, intestinal FXR activation would be hampered further by removal of a large portion of FXR containing cells in the resected intestine. Hence, we postulate that after extensive small-bowel resection, OCA is unable to generate sufficient FXR/FGF19 signaling to prevent SBS-ALD.

FXR activation also is influenced by the composition of the bile acid pool.[23] In this study we observed a significant disturbance in bile acid composition in SBS piglets. This was characterized by an increase in the primary bile acid HCA within the gallbladder and is consistent with previous findings from our group.[4] HCA is a hydrophilic bile acid derived from CDCA in the liver as a means of bile acid detoxification,[24] and the increase in HCA observed in our study may reflect an adaptive response to bile acid dysmetabolism associated with SBS. However, OCA treatment failed to normalize HCA levels in our model and levels of this bile acid remained significantly higher than sham controls despite OCA administration. We also observed decreases in the secondary/tertiary bile acids LCA, DCA, HDCA, and UDCA associated with SBS. The conversion of primary to secondary/tertiary bile acids is performed by the colonic microbiota, therefore these changes likely reflect SBS-associated microbial dysbiosis, as has been reported previously in this model.[25] Shifts in the secondary bile acid pool may be physiologically relevant because these bile acids are significant agonists (DCA and LCA) or antagonists (UDCA) of FXR and also influence other host bile acid receptors.[26] After OCA treatment of SBS piglets, significant improvements in HDCA and LCA levels were observed in systemic bile acid profiles (both gallbladder and portal serum) despite a reduction in the expression of intestinal bile acid transport systems. It is plausible that OCA treatment impacts the intestinal microbiota and subsequently the secondary bile acid pool. This hypothesis is supported by findings from a murine model of high-fat diet–induced nonalcoholic fatty liver disease, for which antibiotic treatment altered bile acid composition and inhibited FXR signaling in the ileum, but not in the liver.[27] Similarly, altering microbial composition via probiotic administration[28] or administration of a high-fat diet[29,30] is associated with reduced activity of the FXR/FGF15 signaling axis. Further studies are required to confirm if the disturbed intestinal FXR response observed in SBS piglets in response to OCA is owing to microbial dysbiosis and consequent bile acid dysmetabolism or owing to alternative activation of CYP3A4, which may impact on bile acid metabolism.

In conclusion, this study assessed the impact of treatment with OCA, an FXR agonist, on the development of SBS-ALD in a preclinical model of SBS. Importantly, we have shown that small-bowel resection significantly hampers the intestinal response to OCA, but not the hepatic response. Our results suggest that there are fewer intestinal-based bile acid targets and/or alterations in the resident microbiota[4,25] after extensive resection of the small intestine and that this influences the ability of the intestine to respond to FXR activation by OCA (Figure 5). Despite a lack of FXR activation by OCA among the intestinal target genes studied, there was an up-regulation of hepatic FXR target genes, including those involved in limiting bile acid synthesis and facilitating bile acid transport, but this failed to prevent the development of SBS-ALD. Although there may be clinical benefit of OCA on reducing fat malabsorption, it did not prevent the development of SBS-ALD, thereby limiting the potential therapeutic benefit of OCA in patients with SBS.

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Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and statistical analysis (P.P.-F.). Acquisition of data; critical revision of the manuscript for important intellectual content (S.L.). Acquisition of data; critical revision of the manuscript for important intellectual content (S.A.J.) Analysis and interpretation of the data; critical revision of the manuscript for important intellectual content (J.C.). Analysis and interpretation of the data; critical revision of the manuscript for important intellectual content; and administrative, technical or material support (P.J.F.). Critical revision of the manuscript for important intellectual content; obtained funding; and study supervision (J.E.B.).

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