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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.

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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