Research Article

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Altered FXR signalling is associated with bile acid dysmetabolism in short bowel syndrome-associated liver disease

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Background & Aims: Despite the mortality associated with liver disease observed in patients with short bowel syndrome (SBS), mechanisms underlying the development of SBS-associated liver disease (SBS-ALD) are poorly understood. This study examines the impact of bacterially-mediated bile acid (BA) dysmetabolism on farnesoid X receptor (FXR) signalling pathways and clinical outcome in a piglet model of SBS-ALD.

Methods: 4-week old piglets underwent 75% small bowel resection (SBR) or sham operation. Liver histology and hepatic inflammatory gene expression were examined. Abundance of BA biotransforming bacteria was determined and metabolomic studies detailed the alterations in BA composition of stool, portal serum and bile samples. Gene expression of intestinal and hepatic FXR target genes and small heterodimer partner (SHP) transrepression targets were assessed.

Results: Histological evidence of SBS-ALD included liver bile duct proliferation, hepatocyte ballooning and fibrosis. Inflammatory gene expression was increased. Microbiota changes included a 10-fold decrease in *Clostridium* and a two-fold decrease in *Bacteroides* in SBS-ALD piglets. BA composition was altered and reflected a primary BA dominant composition. Intestinal and hepatic regulation of BA synthesis was characterised by a blunted intestinal FXR activation response and a failure of SHP to repress key hepatic targets.

Conclusions: We propose a pathological scenario in which microbial dysbiosis following SBR results in significant BA dysmetabolism and consequent outcomes including steatorrhoea, persistent diarrhoea and liver damage. Furthermore alterations in BA composition may have contributed to the observed disturbance in FXR-mediated signalling pathways. These findings provide an insight into the complex mechanisms mediating the development of liver disease in patients with SBS.

Keywords: Short bowel syndrome; Liver diseases; Intestinal failure associated liver disease; Farnesoid X receptor NR1H4; Bile acids and salts; Gut microbiota.
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Abbreviations: SBS, short bowel syndrome; SBS-ALD, short bowel syndrome-associated liver disease; PN, parenteral nutrition; SBR, small bowel resection; BA, bile acid; FXR, farnesoid X receptor; SHP, small heterodimer partner; FGF19, fibroblast growth factor 19; CYP7A1, cholesterol 7α-hydroxylase; CYP27A1, mitochondrial sterol 27 hydroxylase; CYP8B1, sterol 12α-hydroxylase; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; CA, cholic acid; RNA, ribonucleic acid; cDNA, complementary DNA; rRNA, ribosomal ribonucleic acid; OUT, operational taxonomic unit; UPLC-MS, ultraperformance liquid chromatography-mass spectrometry; SEM, standard error of the mean; UDCA, ursodeoxycholic acid; HDCA, hyodeoxycholic acid; HCA, hyocholic acid; LRH-1, liver receptor homolog-1; LXRα, liver X receptor alpha; HNF4α, hepatocyte nuclear factor 4 alpha.

Introduction

Short bowel syndrome (SBS) is associated with a high mortality rate (20–30%) and for survivors the care is complex and resource intensive [1–4]. Despite the significant mortality associated with short bowel syndrome-associated liver disease (SBS-ALD), the mechanisms underlying the development of SBS-ALD are poorly understood. Parenteral nutrition (PN) therapy is recognised as a key contributing factor, however not all patients receiving PN develop liver disease. Small bowel resection (SBR) is the only proven independent risk factor for the development of liver disease in children and adults with intestinal failure [4].

Following massive SBR, morphological and functional adaptation occurs in the remaining bowel over weeks to months in an effort to compensate for the loss of absorptive surface. However, in some patients malnutrition associated with malabsorption of fat and bile acids (BA) may persist for years [5]. Disturbances in BA composition have been observed in children with SBS and

with intractable diarrhoea [5]. BAs play a role in a number of important physiological functions, including the solubilisation of cholesterol, fat soluble vitamins and lipids in the intestine [6]. In addition, BAs also activate nuclear receptors and cell signalling pathways involved in lipid, glucose and BA metabolism [7]. Given these key functions it is not surprising that BA dysmetabolism is associated with fat malabsorption, diarrhoea, inflammation and liver injury observed in a range of gastrointestinal and hepatic diseases including SBS [5], inflammatory bowel disease [8], diarrhoea-predominant irritable bowel syndrome [9], cirrhosis [10] and non-alcoholic liver disease [11].

BA composition is determined by (1) the biosynthesis of primary BAs from cholesterol in the liver and (2) bacterial modifications within the large intestine including deconjugation by bacteria with bile salt hydrolase activity, providing the gateway for further bacterial modification to secondary and tertiary BAs from deconjugated BAs by 7α-dehydroxylating and 7α-dehydrogenating bacteria. Under physiological conditions, activation of FXR is the key regulator of BA synthesis by directly inducing target genes in both the liver and intestine, including hepatic small heterodimer partner (*SHP*) and intestinal fibroblast growth factor 19 (*FGF19*), which in turn inhibit BA synthesis via repression of *CYP7A1*, *CYP8B1*, and *CYP27A1* gene transcription [12]. FXR activation is strongly influenced by BA composition with the activating potency of specific BA species being chenodeoxycholic acid (CDCA) > deoxycholic acid (DCA) > lithocholic acid (LCA) > cholic acid (CA) [13]. Given the major influence of bacteria on BA composition, it is not surprising that bacterial dysbiosis has been recently linked to perturbations in FXR signalling [14].

Information regarding the impact of small bowel resection on the colonic microbiota is limited, however a study of children with SBS suggested that impaired BA metabolism with a switch to primary BA dominance occurred following small bowel resection [5]. The impact of such changes in BA composition on FXR signalling and development of SBS-ALD is not known. We have established a novel large animal model of SBS using the weanling piglet. These animals develop liver disease following SBR, independent of PN administration, thereby challenging the view that SBS-ALD occurs as a direct consequence of PN administration.

We have previously reported a decrease in colonic microbial diversity in the SBS-ALD model [15]. These changes might predict to influence BA composition and consequently FXR activation via alterations in the proportion of bacteria responsible for BA deconjugation and conversion of primary BAs into secondary BAs. The aims of the current study were to: (1) determine if the SBS-ALD model exhibits alterations in the abundance of bacteria responsible for biotransformation of BAs, (2) determine if alterations in the colonic microbiota are reflected in changes in BA composition and (3) examine potential alterations in the hepatic and intestinal FXR signalling pathways. BA composition and correspondingly the colonic microbiota were significantly altered following SBR. In particular, SBS piglets exhibited decreased levels of 7α-dehydroxylating bacteria following SBR, which corresponded to an increase in the levels of potentially hepatotoxic primary BAs returning to the liver, and concurrently a significant decrease in secondary BA levels. Abnormal FXR signalling within both the intestine and liver accompanied alterations in BA composition. These findings provide a unique insight into the development of liver disease in patients with SBS.

Materials and methods

Animals and experimental design

This study was approved by the animal Ethics Committee of the Murdoch Childrens Research Institute. To authenticate the robustness of the model, weaned female 3-week-old piglets were randomly assigned and transported to either the Royal Children's Hospital animal research laboratory (Landrace/Large White cross; Victorian Institute of Agriculture Science, Australia) or the University of Melbourne Centre for Animal Biotechnology (Landrace/Large White cross; Aussie Pride Pork, Australia) and acclimatized prior to surgery. There were a total of 10–12 piglets per operation group. Piglets were housed at a temperature of 22 °C with a 12 h light/dark cycle and fed a supplemented polymeric infant formula diet (Karicare De-Lact, Nutricia, Macquarie Park, Australia). The surgical procedures and peri-operative and post-operative care used in this experiment have been described previously [16–18]. Briefly, 4-week-old piglets underwent either a 75% proximal small bowel resection (SBR) or transection and re-anastomosis (sham) operation. The 75% SBR included the removal of the small bowel from 90 cm distal to the ligament of Treitz to 225 cm proximal to the ileocaecal valve. During the sham procedure, the intestine was transected and re-anastomosed at a site 225 cm proximal to the ileocecal valve.

Sample collection

Animals were euthanized six weeks post-surgery. Portal plasma, circulating serum, bile, colonic content and stool samples were obtained on the day of sacrifice and frozen at −80 °C until required. Liver samples were collected from the right medial lobe and terminal ileum samples obtained from a point 5 cm proximal to the ileocecal valve. Samples were placed in 4% paraformaldehyde (Australian Biostain Pty Ltd, Traralgon, Australia), O.C.T. compound or snap frozen in liquid nitrogen.

Clinical assessment

Piglets were weighed bi-weekly and weight gain was recorded. Stool consistency and presence of fat globules were assessed by the Royal Children's Hospital laboratory service (Melbourne, Australia). In brief, stool was given a consistency score based on 0 = formed, 1 = semi-formed, 2 = unformed and 3 = fluid. The presence of fat globules within the stool was semi-quantitatively assessed and given a score between 0 and 3.

Hepatic histology

Histological examination was performed on trichrome stained 4 μm formalin-fixed liver sections. To measure fibrosis, Sirius Red staining [19] was performed on 4 μm formalin-fixed liver sections and an adapted quantitation method [20] was used to determine the percentage of Sirius Red staining in each liver section. Oil Red O staining [21] was performed on frozen O.C.T-embedded 10 μm liver sections to visualize hepatic fat accumulation. Post staining, a minimum of 10 individual hepatic lobules were photographed/pig (Leica Microsystems, Germany) and optical density measurements were performed individually for hepatic zones 1–3 using the Image J software [22].

RNA preparation and quantitative PCR

RNA was extracted from liver and terminal ileum epithelium using TRIzol, and 0.1 μg RNA was reverse-transcribed into complementary DNA (cDNA). Primers were designed using the Roche Universal ProbeLibrary Assay Design Center (Supplementary Table 1). qRT-PCR reactions (10 μl) contained 2.5 μl diluted cDNA, 5 μl FastStart TaqMan Probe Master (Roche), 900 nM of each primer and 250 nM of probe mix. All reactions were performed in triplicate on the LightCycler 480 System (Roche). The $2^{-\Delta\Delta Ct}$ method [23] was used to calculate relative changes in gene expression, determined from qRT-PCR experiments using either *HRPT1* (liver) or *RPL32* (terminal ileum) as a housekeeping gene and relative to a non-operation control group (n = 9).