Liver steatosis induced by small bowel resection is prevented by oral vancomycin

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Background. *Intestinal failure–associated liver disease causes significant mortality in patients with short bowel syndrome. Steatosis, a major component of intestinal failure–associated liver disease has been shown to persist even after weaning from parenteral nutrition. We sought to determine whether steatosis occurs in our murine model of short bowel syndrome and whether steatosis was affected by manipulation of the intestinal microbiome.*
Methods. *Male C57BL6 mice underwent 50% small bowel resection and orogastric gavage with vancomycin or vehicle for 10 weeks. DNA was extracted from stool samples then sequenced using 16s rRNA. Liver lipid content was analyzed. Bile acids were measured in liver and stool.*
Results. *Compared with unoperated mice, small bowel resection resulted in significant changes in the fecal microbiome and was associated with a >25-fold increase in steatosis. Oral vancomycin profoundly altered the gut microbiome and was associated with a 15-fold reduction in hepatic lipid content after resection. There was a 17-fold reduction in fecal secondary bile acids after vancomycin treatment.*
Conclusion. *Massive small bowel resection in mice is associated with development of steatosis and prevented by oral vancomycin. These findings implicate a critical role for gut bacteria in intestinal failure–associated liver disease pathogenesis and illuminate a novel, operative model for future investigation into this important morbidity. (Surgery 2016;■:■-■.)*

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SHORT BOWEL SYNDROME (SBS) results from a massive loss of small bowel associated with the treatment of several conditions, which include necrotizing enterocolitis and midgut volvulus, in the pediatric population. The mortality of children with SBS ranges from 30–40% making SBS one of the most lethal diseases of infancy.[1] Intestinal failure–associated liver disease (IFALD) is a major complication of SBS. IFALD affects 40–60% of children with intestinal failure on parenteral nutrition and has been reported to account for up to 60% of the long-term mortality from SBS.[1,2] The economic impact of comprehensive care and complications arising from SBS is significant at >6 billion US dollars annually.[3,4]

The ability of the gut microbiome to modulate liver injury is well established in several experimental models of nonalcoholic fatty liver disease (NAFLD).[5,6] A central role for the intestinal microbiome has been further secured by experiments whereby lean and obese phenotypes of twin humans have been transferred to mice via fecal transplantation.[7] Increased abundance of Firmicutes (primarily Gram-positive bacteria) is one of the more prevalent findings involving an obese phenotype.[8]

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Similar to obesity, we have identified a remarkably similar pattern of increased Firmicutes abundance in the small intestine after SBR.[9] Using our murine SBR model, we have established that mice exhibit a unique body composition profile in which fat is replaced preferentially over lean muscle during recovery.[10] The initial purpose of this study was to test the hypothesis that modulating the microbiome with vancomycin to selectively eliminate Gram-positive microbiota would alter the resection-associated changes in body composition. Although vancomycin did not affect resection-associated body composition, significant differences in hepatic histology were observed, which led to further investigation.

METHODS

Animals. C57BL6 mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 7 weeks of age. Mice were housed on arrival in a facility with a 12-hour light/dark cycle. Male mice were used exclusively in order to limit hormonal confounders when analyzing the enteric microbiome. This study was approved by the Washington University Animal Studies Committee (Protocol 20130308) in accordance with the National Institute of Health laboratory animal care and use guidelines.

Diets and operation. Eight-week-old male mice were placed on a standard liquid diet (LD; PMI Micro-Stabilized Rodent Liquid Diet LD 101, Testdiet Inc., St. Louis, MO) for 24 hours prior to operation. The mice were randomized into 2 groups treated with either vancomycin (2 mg per mouse) or the equivalent volume of water (40 μL) by orogastric gavage 4 hours prior to the operation. The mice then underwent a 50% proximal SBR as we have described previously.[11] Mice were maintained on standard LD for the remainder of the experiment. This SBR model does not involve parenteral nutrition, thereby eliminating multiple confounding variables including rates of glucose infusion, amino acid composition, different types of fat (Ω3 versus Ω6), and percent of enteral intake.

Experimental design and sample collection. SBR mice were treated with vancomycin or water every 3 days for 10 weeks. Fecal samples were collected and sequenced prior to SBR and at 3 weeks after SBR. Body composition measurements were obtained every 3 days for the first 15 days and weekly after that for a total of 5 weeks. Food intake and weight were measured daily. An unoperated group of male C57BL6 mice was maintained on the same LD for 10 weeks without additional intervention to provide unoperated controls.

After 10 weeks, the small intestine was removed and prepared for isolation of enterocytes as described previously.[12] Blood was obtained via cardiac puncture. Liver was flash frozen and stored at −80°C. Liver and small bowel 1 cm distal to the anastomosis were collected and preserved in 10% buffered formalin.

RNA isolation. RNA isolation and real-time PCR were performed on isolated enterocytes and whole bowel from the terminal ileum and liver tissue as described previously.[13] CYP7a1, Fgf15, and Nr0b2 (SHP) primers were used (Life Technologies; Carlsbad, CA).

Lipid quantification. Fecal fat quantification and fat absorption were measured using chloroform: a methanol extraction protocol as described previously.[13] Liver sections were stained with hematoxylin and eosin and analyzed using NIS Elements software (Version 4.3; Nikon Instruments, Melville, NY to obtain the percent lipid content using 4 random fields at 4× magnification per sample.

DNA isolation from stool. DNA was extracted from frozen fecal samples per the manufacturer's protocol for the QIAamp DNA stool mini kit (Qiagen, Germantown, MD). In addition, fecal pellets were placed in sterile tubes with buffer ASL, 425–600 μm acid-washed glass beads (Sigma-Aldrich, St. Louis, MO), and 2.3 mm zirconium/silica disruption beads (Research Products International Corp, Mt. Prospect, IL) and homogenized using a bead beater.

PCR amplification and sequencing of bacterial 16S rRNA genes. Fourteen PCR amplicons, representing all 9 16S variable regions, were constructed using the Fluidigm Access Array System (Fluidigm Corporation, San Francisco, CA). Five ng/μL of DNA were input into each reaction. The sample inlets consisted of 1X High Fidelity FastStart Reaction Buffer without $MgCl_2$ (Roche Holding AG, Basel, Switzerland), 4.5 nM $MgCl_2$ (Roche), 5% DMSO (Roche), 200 μM PCR Grade Nucleotide Mix (Roche), 0.05 U/μL 5 U/μL FastStart High Fidelity Enzyme Blend (Roche), 1X Access Array Loading Reagent (Fluidigm), 1 μL DNA, and water. Primers were added to the assay inlets at 200 nM forward and reverse primers with 1X Access Array Loading Reagent. PCR amplification was performed on the BioMark HD system from Fluidigm. Each sample was harvested and indexed using unique, 10 base pair sequences with 14 rounds of PCR to incorporate each index sequence. Samples were pooled into 48 sample libraries, cleaned using

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