Obeticholic Acid Decreases Intestinal Content of *Enterococcus* in Rats With Cirrhosis and Ascites

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The intestinal microbiome and bacterial translocation (BT), the passage of microorganisms from the gut lumen to mesenteric lymph nodes and other extra-intestinal sites, are main mechanisms implicated in liver injury and further decompensation in patients with cirrhosis. We hypothesized that obeticholic acid (OCA), a semisynthetic bile acid, would change the microbiome composition and reduce bacterial translocation in experimental cirrhosis. Rats with cirrhosis induced by carbon tetrachloride inhalation (a nonseptic model) with ascites present for at least 7 days were randomized to receive a 14-day course of OCA at a dose of 5 mg/kg/day (n = 34) or placebo (n = 34). Stool was collected at days 1 (randomization), 8, and 14 (sacrifice) for analysis of intestinal microbiome using the V4 hypervariable region of the bacterial 16S gene amplified by polymerase chain reaction. Bacteriological cultures of mesenteric lymph nodes, blood, and ascites were performed at end of study. Twenty-four animals in each group reached the end of study. Compared with placebo, rats treated with OCA had decreased relative abundance of *Enterococcus* in both ileum content ($P = 0.02$) and in stool ($P < 0.001$). BT from pathogenic bacteria was not different between groups. At end of treatment, rats on OCA had a significantly lower aspartate aminotransferase (AST) (266 vs. 369 IU/L; $P < 0.01$) and higher serum albumin (0.9 vs. 0.7 g/dL; $P < 0.01$) than rats on placebo. *Conclusion:* Although OCA did not appear to reduce BT by pathogenic bacteria, the reduction in intestinal content of *Enterococcus*, which has been associated with hepatocyte death, in OCA-treated animals is consistent with our observed improvements in AST and in liver function, as evidenced by higher serum albumin. (*Hepatology Communications* 2021;5:1507-1517).

The intestinal microbiome is altered in cirrhosis[(1)] and plays an important role in the development of complications of cirrhosis, primarily through the mechanism of bacterial translocation (BT) (i.e., the passage of bacteria from the intestinal lumen to mesenteric lymph nodes or other extra-intestinal sites).[(2)] BT has been implicated in the pathogenesis of bacterial infections in cirrhosis and the inflammatory state responsible for other complications such as hepatic encephalopathy[(3)] and multiorgan failure.[(4,5)] More recently, changes in the microbiome, particularly the overgrowth of intestinal *Enterococcus* and its translocation to the liver, have been shown to induce inflammation and hepatocyte damage.[(6)] This direct liver injury represents another mechanism by which the intestinal microbiome could affect the progression of cirrhosis.

Nonabsorbable antibiotics, such as norfloxacin, have been useful in preventing infections and its deleterious

Abbreviations: AST, aspartate aminotransferase; BT, bacterial translocation; CCl_4, carbon tetrachloride; MLN, mesenteric lymph node; OCA, obeticholic acid; OTU, operational taxonomic unit; PCR, polymerase chain reaction; PERMANOVA, Permutational analysis of variance.

Received November 10, 2020; accepted April 19, 2021.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1740/suppinfo.

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Supported by Intercept Pharmaceuticals, National Institutes of Health (R01AT010014), Yale Liver Center National Institutes of Health (P30 DK34989), and the Department of Infectious Diseases, The First Hospital of Shanxi Medical University, Taiyuan, Shanxi Province, China.

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DOI 10.1002/hep4.1740

Potential conflict of interest: Dr. Garcia-Tsao was a consultant for Intercept.

consequences by modifying the intestinal microbiome, and have been associated with a decreased mortality, particularly in patients with variceal hemorrhage.[7] However, norfloxacin is no longer available in the United States, with a recent study suggesting that its prophylactic efficacy may be decreasing, probably due to the development of multidrug-resistant organisms.[8,9] Therefore, nonantibiotic measures to prevent gut dysbiosis and BT are being sought.

Bile acids are bacteriostatic, and the oral administration of different bile acids (cholic acid, deoxycholic acid, cholysarcosine, and cholylglycine) or whole bile have been shown to decrease BT in experimental obstructive jaundice[10] (compared with animals with obstructive jaundice who received saline) and in experimental cirrhosis with ascites[11] (compared with cirrhotic animals treated with placebo). In the latter, the oral administration of conjugated bile acids appeared to significantly reduce ileal bacterial content (particularly *Escherichia coli* and *Enterococcus*) to levels comparable to that of healthy rats.[11]

Obeticholic acid (OCA) is a potent semisynthetic bile acid that, in addition to having a bacteriostatic activity, activates the nuclear farnesoid X receptor that induces genes involved in enteroprotection. [12] In different experimental models of portal hypertension and cirrhosis, OCA has been associated with a decreased number of bacteria in mesenteric lymph nodes (MLNs) or decreased rate of BT,[13,14] with evidence of improved ileal barrier function[13] and amelioration of gut leakiness.[15]

In experimental studies investigating the effect of OCA on BT and the microbiome in cirrhosis, the models used were not ideal as either bile duct ligation[13] or intraperitoneal injection of carbon tetrachloride (CCl_4)[15] are potentially "septic" models.[16,17] In addition, the use of CCL_4 (or its vehicle) by gavage[14] could potentially induce changes in the intestinal microbiota that would be of significance.[18,19]

In this study, we aimed to investigate, in rats with cirrhosis and ascites induced by CCL_4 by *inhalation*, whether the oral administration of OCA (compared with placebo) was associated with (1) changes in intestinal microbiota (stool and intestinal content), (2) a decrease in the rate of BT, and/or (3) changes in liver tests/ascites.

Materials and Methods

ANIMALS

Male Sprague-Dawley rats with an initial weight of 100-180 g were housed individually in Plexiglas cages throughout the study at a constant room temperature of 21°C and a 12/12-hour light/dark cycle. Animals had free access to water and rat chow during the course of the study. The first week after their arrival, they underwent a bedding swap from the cages of control rats to equilibrate intestinal microbiome.

CIRRHOSIS MODEL

Cirrhosis was induced by CCl_4 inhalation as previously described.[4,5] Briefly, rats underwent exposure to CCl_4 (Sigma-Aldrich, St. Louis, MO) by inhalation in a Plexiglas chamber for 1-2 minutes the first week, 2-4 minutes the second week, and 4-7 minutes the third week; exposure times were generally doubled from the week prior for the first 3 weeks as long as the rats were consistently gaining 20 g of weight per week and staying awake during exposure. In subsequent weeks, time of exposure was based on the time it took animals to fall asleep (usually ≥ 5 minutes). Exposure was continued until ascites development

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(average of 9 weeks; range 8-15 weeks), after which CCl_4 exposure was reduced to once a week. Exposure to CCl_4 was discontinued 7 days before laparotomy and sample collection.

STUDY GROUPS

Animals with persistent ascites for 7 days were randomized to placebo (0.5% carboxymethylcellulose solution; Sigma-Aldrich) or to OCA, administered by orogastric tube at a dose of 5 mg/kg/day for 14 days. The dose selected was based on dose-finding studies, which showed lower mortality with this dose compared with a 100% mortality at a dose of 30 mg/kg/day, as well as doses used in previous studies.[13,14]

ASSESSMENT OF GUT MICROBIOME

Stool from each randomized animal was collected on day 1 (randomization) and on day 8 of treatment (OCA or placebo). On day 14 of treatment, animals were sacrificed and intestinal content was collected from the descending colon (considered as "stool") and from the ileum (considered "ileal content"). Bacterial DNA was isolated from stool samples and ileal content, and microbiome composition was determined using 16S sequencing.

Except for samples obtained at laparotomy, a small (~0.1 mL) sample of stool was collected directly from the rat and stored in a sterile 1-mL microcentrifuge tube at −80°C until ready for microbiome analysis. At laparotomy, intestinal content of ileum and descending colon (stool equivalent) were collected and stored in similar fashion.

DNA from stool samples was extracted using published techniques[20] and stored at −80°C. The V4 hypervariable region of the bacterial 16S gene was amplified by polymerase chain reaction (PCR) to determine the bacterial density and bacterial diversity in each sample. Bacterial load was estimated using quantitative real-time PCR and calculated from the number of copies of the 16S gene found per weight of stool. To analyze bacterial composition, bidirectional reads of the PCR-amplified V4 region were used. PCR products were cleaned and normalized using the SequalPrep kit (Invitrogen, Carlsbad, CA) and pooled into groups of about 280. Samples were sequenced with a paired-end 2 × 250 bp, dual 8-bp indexing protocol on an Illumina MiSeq instrument (San Diego, CA) running version 2 sequencing chemistry and CASAVA1.8.2 software at the Yale Center for Genome Analysis. Microbiome analysis was conducted using QIIME as previously described.[20-22]

During data processing, operational taxonomic units (OTUs) not present in at least 10% of stool samples were excluded. OTU abundances were converted into pairwise dissimilarities (Bray-Curtis index). Multidimensional scaling analysis was used to visualize microbiome similarities in ordination plots. Permutational analysis of variance (PERMANOVA) was used to test for the significance of microbiome differences. Negative binomial tests (DESEq2 R package) were performed for differential abundance analysis. Stool from Day 1 and Day 8 of treatment as well as stool from the descending colon at Day 14 of treatment (laparotomy) was analyzed to determine the effect of treatment, time, and treatment:time interaction: $\log_{10}$(gene abundance) ~ treatment + time + treatment:time. We used a design formula that models the treatment difference at the initial time point, the difference over time, and any treatment-specific differences over time. This effectively identifies OTUs that react in a treatment condition–specific manner over time, but does not explicitly model random effects. Ileum intestinal content at day 14 of treatment was analyzed to determine the effect of treatment with OCA versus placebo. *P* values were calculated with likelihood ratio tests. An OTU was considered significant if its false discovery rate–adjusted *P* value was below 0.05. Alpha diversity was calculated using Shannon's diversity index.

LAPAROTOMY/SAMPLE COLLECTION

At the end of the treatment period, animals underwent laparotomy performed under general anesthesia (isoflurane using the open-drop method, followed by ketamine HCl, 75 mg/kg body weight and xylazine 5 mg/kg intramuscularly) and strict aseptic conditions. Samples were collected in the following order: ascites fluid (when present), blood, mesenteric lymph nodes, liver, segments of ileum, proximal colon and cecum, intestinal content from ileum (ileal content), and from descending colon (stool). Ascites fluid was used for culture analysis. Blood was collected from a large systemic vein, 3-6 mL of which were used for

culture analysis and the rest for serum laboratory analysis. The mesenteric lymph nodes were placed in sterile saline solution and homogenized for bacteriological cultures. Intestinal content from the ileum and descending colon were obtained and processed as described previously. The liver, as well as samples of ileum, cecum, proximal colon and distal colon, were stored in formalin for histopathological analysis.

ASSESSMENT OF BACTERIAL TRANSLOCATION

Ascites fluid and blood were immediately inoculated into blood culture bottles and incubated at 37°C. MLN homogenate was plated on three solid culture media (blood agar, MacConkey agar, and Columbia CNA agar [Thermo Fisher Scientific, Waltham, MA], as well as in BHI broth (Thermo Fisher Scientific) and incubated at 37°C. Anaerobic conditions were created by placing culture plates in an environmental chamber bag (Becton Dickinson GasPak EZ Pouch System; BD, Franklin Lakes, NJ) that removes oxygen. Solid culture media and broth were examined and colonies counted after 24, 48, and 72 hours. Gram staining was used to confirm growth, and organisms were speciated using mass spectrometry. For fluid and blood cultures, growth value (a measure of CO_2 production by bacteria) was monitored with the Bactec instrument twice daily for the first 2 days and daily for the following 5 days. If growth value reached at least 35, gram stain and subcultures onto solid plates were performed. Positive cultures (for any bacteria) at any site were considered indicative of BT. Bacterial translocation to MLNs, blood, ascites, and any site in OCA-treated rats and placebo were compared using Fisher's exact test.

ASSESSMENT OF LIVER FUNCTION

A sample of blood collected from the systemic vein was allowed to clot at room temperature. Serum was collected by centrifugation and sent for chemistry tests (specifically, aspartate aminotransferase [AST], alanine aminotransferase [ALT], albumin, and bilirubin). Differences of liver function test values between OCA-treated rats and placebo were analyzed using the Mann-Whitney U test.

ASSESSMENT OF CIRRHOSIS AND INTESTINAL INFLAMMATION

A slice of liver and sections from colon and ileum were taken and preserved as formalin-fixed paraffin-embedded tissue. Sections from PPFE tissues were obtained and stained with hematoxylin and eosin (H&E) stain. Liver sections were also stained with Klatskin trichrome stain. The liver histology was evaluated with regard to any changes in the hepatocytes, inflammation, and fibrosis. The H&E-stained section of ileum and colon were evaluated for any histologic changes, especially degree of inflammation. All histologic samples were evaluated by a gastrointestinal pathologist (D.J.) in a blinded fashion

Results

Among 80 rats that underwent cirrhosis induction with CCL_4, 68 (85%) developed ascites after 8-15 weeks and were randomized to receive placebo (n = 34) or OCA (n = 34) (Fig. 1). Twenty rats (10 from each group) died during treatment, leaving 48 rats for analysis (24 placebo and 24 OCA) (Fig. 1). Stool from rats that had stool samples collected on days 1 and 8 of randomization were included in the microbiome analysis, even when they did not survive to day 14.

MICROBIOME ANALYSIS

Multidimensional scaling was used to compare the microbiomes of samples collected in the ileum and colon (Supporting Fig. S1). As shown in Fig. 2, there were no differences in the mean relative abundance of the 20 most abundant genera between OCA and placebo-treated animals, neither for stool (PERMANOVA R^2 = 0.01, P = 0.32) (Fig 2A) nor for ileal content (PERMANOVA R^2 = 0.03, P = 0.27) (Fig. 2B). However, as shown in Fig. 3, differential abundance testing identified genera/families that were significantly different in the stool (Fig. 3A) or in the ileum (Fig. 3B). The most notable of these was the relative abundance of *Enterococcus,* which was significantly lower in both stool (P < 0.001) and ileal content (P = 0.02) of OCA-treated animals.

There were no significant differences in alpha diversity in either stool or ileum between rats treated with OCA and those treated with placebo (Supporting Fig. S2).

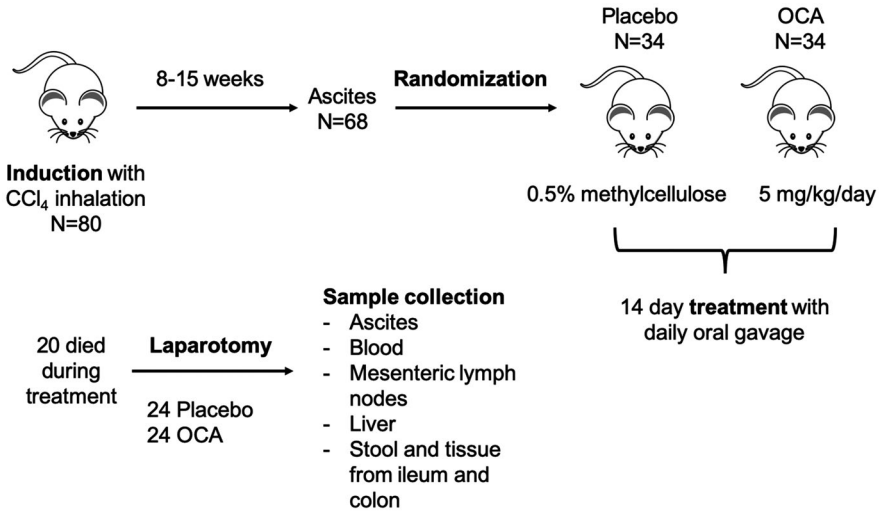

FIG. 1. Study flow.

BACTERIAL TRANSLOCATION

As indicated in Table 1, 11 of 24 (46%) OCA-treated animals had positive cultures of either MLNs, ascites or blood, a rate significantly lower than that observed in the placebo group (19 of 24 or 79%) (P = 0.04). As also detailed in the table, many of the bacteria isolated were nonpathogenic. When the analysis is confined to pathogenic bacteria (bolded in the table), BT to any extra-intestinal site was not different between groups (10 of 24 or 42% placebo, 6 of 24 or 25% OCA; P = 0.36), and BT to only MLNs was identical in both groups (6 of 24 or 25% for each group).

Of note, there was no ascites present for collection at the time of laparotomy in three placebo rats and in five OCA rats.

LIVER TESTS

Liver tests were obtained from serum of all rats at the time of laparotomy. Albumin levels were significantly higher (0.9 vs. 0.7 g/dL; P < 0.01), and AST levels were significantly lower (266 vs. 369 IU/L; P < 0.01) in the OCA-treated animals compared with placebo. There was a trend toward lower total bilirubin (0.6 vs. 0.9; P = 0.08) and ALT (118 vs. 151.5; P = 0.19) in the OCA-treated animals compared with placebo-treated animals (Supporting Table S1).

HISTOLOGICAL ANALYSIS

Cirrhosis was confirmed histologically in all 48 animals using trichrome stain. Histology of all ileum samples were normal. Cecal submucosal edema and inflammation (Fig. 4) were observed in 19 animals (9 OCA and 10 placebo). In rats with positive bacterial cultures at any site, cecal submucosal edema and inflammation (shaded areas in Table 1) were not different between OCA-treated (6 of 11 or 55%) and placebo-treated (10 of 19 or 53%) animals. However, submucosal edema and inflammation were more likely to be present in animals with positive cultures in any site (16 of 30 or 53%) than in those without any evidence of BT (3 of 18 or 17%). This difference was more marked when comparing rats with BT by pathogenic bacteria (11 of 16 or 69%) versus rats without any BT (3 of 18 or 17%).

Discussion

Our study was aimed at investigating the effects of OCA on the intestinal microbiome of rats with cirrhosis and ascites and to assess its effects on BT, as this would constitute proof-of-concept regarding potential beneficial effects of OCA in decompensated cirrhosis.

Although there was no significant effect of OCA treatment on the relative abundances of the most abundant genera in stool or ileal content, a significantly

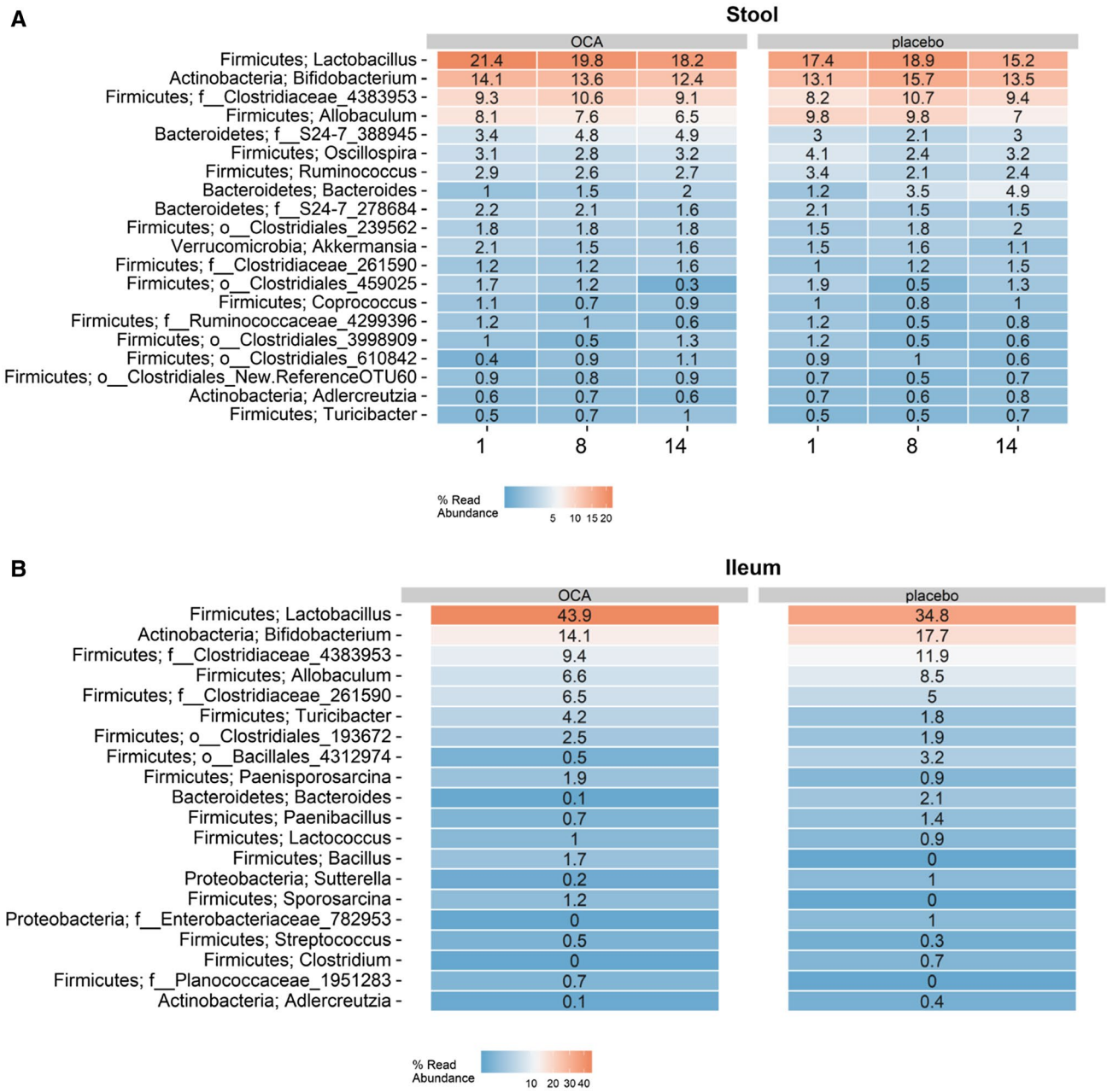

FIG. 2. OTU sequences were classified (by genera) and ordered by abundance by study group. Data represent the results of experiments in 24 placebo-treated animals and in 24 OCA-treated cirrhotic rats. (A) Mean relative abundance of the 20 most common genera in stool by study group at three time points: at randomization, day 8 of treatment, and after sacrifice. No significant differences between OCA and placebo were observed among any of the timepoints (PERMANOVA $R^2 = 0.01$, $P = 0.32$ in a model incorporating treatment, time, and the interaction between treatment and time). (B) Mean relative abundance of the 20 most common genera in ileum content collected after sacrifice (day 14 of treatment) by study group. No significant differences were observed between OCA and placebo (PERMANOVA $R^2 = 0.03$; $P = 0.27$).

lower relative abundance of *Enterococcus* in stool and ileal content at time of sacrifice (day 14 of treatment) was observed in OCA-treated animals compared with placebo-treated animals. This effect was already observed in stool obtained at day 8 of therapy, indicating that the finding is not spurious.

The OCA-driven decrease in intestinal *Enterococcus* in our study is of significance in light of recent findings

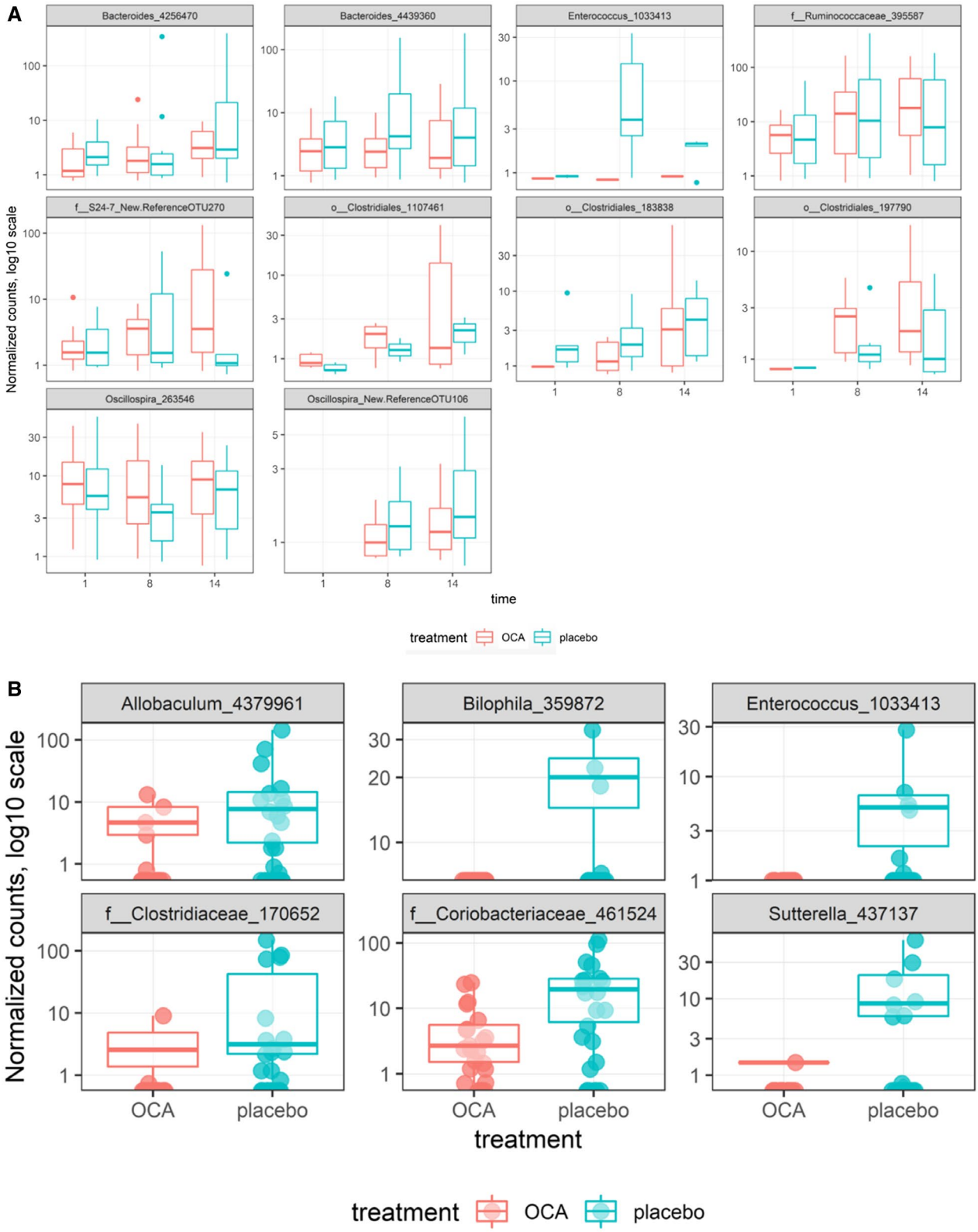
A
Bacteroides_4256470
Bacteroides_4439360
Enterococcus_1033413
f__Ruminococcaceae_395587
f__S24-7_New.ReferenceOTU270
o__Clostridiales_1107461
o__Clostridiales_183838
o__Clostridiales_197790
Oscillospira_263546
Oscillospira_New.ReferenceOTU106
Normalized counts, log10 scale
time
treatment OCA placebo
B
Allobaculum_4379961
Bilophila_359872
Enterococcus_1033413
f__Clostridiaceae_170652
f__Coriobacteriaceae_461524
Sutterella_437137
Normalized counts, log10 scale
OCA
placebo
treatment
treatment OCA placebo

FIG. 3. OTU sequences for the bacterial genera/family/order with the lowest false discovery rate–adjusted *P* values ($P < 0.05$) for significant change over time by study group. Data represent the results of experiments in 24 placebo-treated animals and in 24 OCA-treated cirrhotic rats. Note: Names without a prefix are genus; names with a "f" prefix are family; and names with an "o" prefix are an order. (A) Differential abundance testing of stool at three time points (at randomization [first set of bars], day 8 of treatment [second set of bars], and after sacrifice [third set of bars]) comparing OCA (red bars) versus placebo (blue bars) for the 10 bacterial genera/family/order that were most different between study groups in a model incorporating treatment, time, and the interaction between treatment and time. The most striking difference between OCA and placebo is observed in the genus Enterococcus (first row, third panel). No values are shown for day 1 in third row, second panel, because there were no OTUs affiliated with Oscillospora at this time point. (B) Differential abundance testing of ileal content at sacrifice comparing OCA (red bars) versus placebo (blue bars) for the six bacterial genus/family that were most different between study groups. The most striking difference between OCA and placebo is observed in the genus Enterococcus (first row, third panel).

in mice with experimental alcoholic liver disease and in patients with alcohol-associated liver disease, which show that overgrowth of intestinal *Enterococcus* and its translocation to the liver induces ethanol-induced liver inflammation and hepatocyte damage.[6] The mechanism is through the secretion of a cytolysin, a two-subunit exotoxin secreted by *Enterococcus faecalis* (*E. faecalis*),[23,24] which causes hepatocyte death and liver injury. Compared with controls, patients with alcoholic hepatitis have increased fecal numbers of *E. faecalis*, and the presence of cytolysin-positive (cytolytic) *E. faecalis* correlates with liver disease severity and mortality in patients with alcoholic hepatitis. Furthermore, using humanized mice colonized with bacteria from feces of patients with alcoholic hepatitis, bacteriophages specifically targeted against cytolytic *E. faecalis* decreased cytolysin in the liver and abolished ethanol-induced liver disease in humanized mice.[25] Additionally, a recent study in patients with cirrhosis showed that intestinal *Enterococcus* increases in parallel with severity of cirrhosis and Model for End-Stage Liver Disease score.[26] Even more recently, a randomized feasibility study showed that fecal microbiome transplant in patients with cirrhosis reduced *E. faecalis* in stool and was associated with a decrease in cytolysin and systemic inflammation.[27] Our findings of an OCA-associated reduction in intestinal *Enterococcus* would explain the significantly lower levels of AST and higher levels of serum albumin observed in OCA-treated compared with placebo-treated animals. Notably, there was a trend toward greater ascites resolution (at time of laparotomy) in OCA-treated animals (5 of 24 vs. 3 of 24).

A previous study used OCA in a rat model of cirrhosis with ascites and investigated its effect on the intestinal microbiome.[14] Although this study also reported a decrease in intestinal *Enterococcus* in OCA-treated animals, there was a more significant decrease in *E. coli* that was not observed in our study. Their model differed from ours in that CCl_4 was administered by gavage, and this route may induce modulation of gut microbial abundance, diversity, and function.[18,19] In fact, their BT rate of 78% in controls is higher than previously reported rates of about 50% in rats with CCl_4 cirrhosis induced by inhalation.[28,29]

When considering all isolated bacteria, we observed decreased BT in the OCA-treated group, which could indicate an overall effect of OCA in decreasing BT. However, when we excluded what we considered "nonpathogenic" bacteria (e.g., Lactobacillus and Bifidobacteria) because they have not been isolated in patients with cirrhosis and/or in other experimental studies of bacterial translocation, we could no longer find any differences in BT or in colonic inflammation between OCA and placebo-treated animals. In fact, the significant decrease in intestinal *Enterococcus* in OCA-treated animals was not associated with an obvious reduction in the translocation of this organism to the bloodstream, as it was isolated in blood cultures of two animals in the OCA group and one animal in the placebo group (Table 1). Nevertheless, the small number of animals studied does not permit making a firm conclusion in this respect. The proposed mechanism of *Enterococcus*-induced liver injury is through the activation of specific pattern-recognition receptors including toll-like receptors by the bacteria and pathogen-associated molecular pattern (PAMPs) promoting signaling cascades that lead to hepatocyte inflammation and damage.[6,30] One would therefore have to assume that, although the OCA-driven decrease in intestinal *Enterococcus* is sufficient in preventing PAMPs activation and thereby hepatocyte inflammation and damage, it is not sufficient to prevent its passage to extra-intestinal sites.

TABLE 1. MICROORGANISMS ISOLATED IN THE DIFFERENT EXTRA-INTESTINAL SITES IN ANIMALS RANDOMIZED TO PLACEBO OR OCA

Placebo (19 of 24 Had a Positive Culture)

MLNs	Blood	Ascites
—	*Lactococcus garvieae*	*Lactobacillus reuteri*
Lactobacillus vaginalis	—	—
Lactobacillus spp.	—	—
Bifidobacterium pseudolongum	—	—
Lactococcus murinus	—	—
Lactococcus garvieae	—	*No ascites*
—	*Lactococcus garvieae*	—
Lactobacillus murinus	—	—
Lactococcus garvieae	—	—
—	***Streptococcus agalactiae***	—
Lactobacillus spp	***Staphylococcus xylosus***	*Lactobacillus spp*
—	***Staphylococcus aureus***	—
Variable rods	—	—
Pseudomonas aeruginosa	*Lactobacillus reuteri*	***Escherichia coli***
Pseudomonas aeruginosa	***Enterococcus avium, Escherichia coli, Pseudomonas aeruginosa***	***Pseudomonas aeruginosa, Escherichia coli***
Staphylococcus warneri	—	*Staphylococcus epidermidis,* ***Bacteroides thetaiotaomicron***
—	—	***Escherichia coli***
Pseudomonas spp	***Escherichia coli***	—
Lactococcus garvieae, ***Escherichia coli***	***Staphylococcus aureus***	***Escherichia coli***

OCA (11 of 24 Had a Positive Culture)

MLNs	Blood	Ascites
Bacillus spp.	—	—
Lactobacillus murinus	—	*No ascites*
Lactobacillus murinus	—	*No ascites*
Lactococcus garvieae	—	—
Lactobacillus murinus	—	—
Lactococcus garvieae, Lactobacillus johnsonii, ***Enterococcus gallinarum***	*Lactococcus spp., Lactobacillus spp.*	*Lactococcus garvieae*
Enterococcus faecalis	***Enterococcus faecalis***	***Enterococcus faecalis***
Staphylococcus aureus	***Pseudomonas aeruginosa,*** *Streptococcus agalactiae*	*No ascites*
Proteus mirabilis, Escherichia coli	—	—
Proteus mirabilis	*Lactobacillus spp,* ***Proteus mirabilis, Pseudomonas aureus***	*Lactobacillus spp,* ***Proteus mirabilis, Pseudomonas aureus***
Klebsiella penumoniae	***Klebsiella pneumoniae***	***Klebsiella pneumoniae***

Note: Each row represents one rat with a positive bacteriological culture at any site. Pathogens in bold represent potentially "pathogenic" organisms (as opposed to possible contaminants). Shaded areas represent animals in whom intestinal inflammation was evident in cecum.

On this basis, we could postulate that OCA would probably not have an effect in preventing bacterial infections or the inflammatory state that leads to further decompensation or organ failures in decompensated cirrhosis; however, it could be associated with a beneficial intrahepatic effect that could lead to less hepatocyte injury and an improved liver function that could be more effective at an earlier, compensated, stage of cirrhosis and could lead to prevention of decompensation.

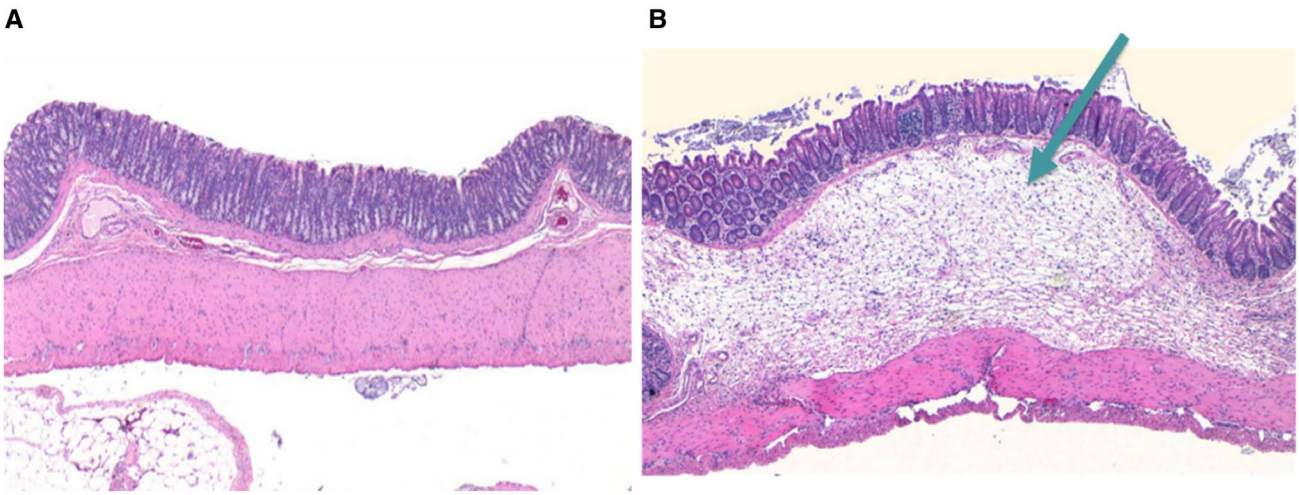

FIG. 4. Cecum histology (magnification ×100). (A) Normal cecum, seen in 15 of 24 (63%) of OCA-treated rats, versus 14 of 24 (58%) of placebo-treated rats. (B) Submucosal edema/inflammation (green arrow) seen in 9 of 24 (38%) of OCA-treated rats, versus 10 of 24 (42%) of placebo-treated rats.

In conclusion, although we could not find any significant changes in the rate of BT to MLNs or other extra-intestinal sites in rats with cirrhosis and ascites treated with OCA, we found that during OCA treatment there was a significant reduction in *Enterococcus* species in the intestine. There was a concomitant improvement in AST and serum albumin, and a trend toward reduction in ascites formation. We postulate that OCA would probably be more beneficial in patients with compensated cirrhosis by improving the intrahepatic milieu and thereby preventing decompensation. Further studies investigating the relationships among OCA, intestinal *Enterococcus*, its adherence to the intestinal mucosa, inflammatory markers, and liver injury would be of great interest.

Acknowledgment: The authors thank Dr. Michael Nathanson for his critical review of our paper.

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