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Original Article

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Modulation of the Gut Microbiota-farnesoid X Receptor Axis Improves Deoxycholic Acid-induced Intestinal Inflammation in Mice

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Abstract

Background and Aims: Inflammatory bowel disease (IBD) is associated with gut dysbiosis and dysregulation of bile acid metabolism. A high luminal content of deoxycholic acid (DCA) with consumption of a Westernised diet is implicated in the pathogenesis of IBD. The aim of the study is to explore the role of intestinal microbiota and bile acid metabolism in mice with DCA-induced intestinal inflammation.

Methods: Wild-type C57BL mice, 4 weeks old, were fed with AIN-93G (control diet), AIN-93G+0.2% DCA, AIN-93G+0.2% DCA+6 weeks of fexaramine (FXR agonist), or AIN-93G+0.2% DCA+antibiotic cocktail, for 24 weeks. Histopathology, western blotting, and qPCR were performed on the intestinal tissue. Faecal microbiota was analysed by 16S rDNA sequencing. Faecal bile acid and short chain fatty acid (SCFA) levels were analysed by chromatography.

Results: Gut dysbiosis and enlarged bile acid pool were observed in DCA-treated mice, accompanied by a lower farnesoid X receptor (FXR) activity in the intestine. Administration of fexaramine mitigated DCA-induced intestinal injury, restored intestinal FXR activity, activated fibroblast growth factor 15, and normalised bile acid metabolism. Furthermore, fexaramine administration increased the abundance of SCFA-producing bacteria. Depletion of the commensal microbiota with antibiotics decreased the diversity of the intestinal microbiota, attenuated bile acid synthesis, and reduced intestinal inflammation induced by DCA.

Conclusions: DCA induced-intestinal inflammation is associated with alterations of gut microbiota and bile acid profiles. Interventions targeting the gut microbiota-FXR signalling pathway may reduce DCA-induced intestinal disease.

Key Words: Microbiota; farnesoid X receptor axis; inflammation; fexaramine

1. Introduction

Inflammatory bowel disease (IBD) is an immune-mediated chronic gastrointestinal disease driven by genetic and environmental factors.[1] Large-scale population-based studies demonstrated a rise in the incidence rates of IBD associated with Westernisation of dietary patterns and industrialisation.[2,3] The prevalence rate of IBD exceeds 0.3% in Western countries and has evolved into a global chronic disease associated with substantial economic impact and use of health care resources.[4]

Although genetic and environmental factors have been implicated, the pathogenesis of IBD is poorly defined. Increased consumption of a Westernised diet (dietary fat), associated with high luminal content of deoxycholic acid (DCA), is a risk factor for the development of IBD.[5] Emerging studies showed that DCA mediates gut dysbiosis which may lead to injury of the gut mucosal barrier, ultimately resulting in intestinal inflammation.[6–8] In addition, clinical studies demonstrated significant accumulation of DCA levels in the inflammatory lesions of patients with IBD.[9] All these show that DCA plays an important part in the development of intestinal inflammation. However, the mechanism of DCA in the development of IBD remains to be clarified, to provide potential therapeutic targets.

Intestinal micro-ecology is of great significance to the energy balance and metabolism of the human body.[10] Gut dysbiosis, defined as a decrease in the gut microbial diversity, is an important pathogenic mechanism in the development and disease progression in IBD. Clinical observations demonstrating the efficacy of antibiotics[11,12] and faecal microbiota transplantation for ulcerative colitis[13] support the role of intestinal bacteria contributing to the inflammatory response. Small molecular metabolites of gut bacteria, such as bile acids, amino acids, and short chain fatty acids (SCFAs), contribute to the pathophysiology of intestinal inflammation. Furthermore, gut bacteria are responsible for the biotransformation of bile acids which are important regulatory molecules involved in the gut mucosal defence.[14–16] For example, bile salt hydrolases (BSH) in the intestinal bacteria play a key part in bile acid modification. Dysbiosis may affaect farnesoid X receptor (FXR) signalling.[17] FXR, a bile acid-activated nuclear receptor, widely participates in the pathophysiology of gastrointestinal diseases and liver metabolism, as well as in the prevention of bacterial translocation.[18,19] Specifically, farnesoid X receptor (FXR) has recently been shown to participate in mucosal barrier function by regulating intestinal antibacterial growth and mucosal immune response.[20] FXR plays a pivotal role in regulating enterohepatic bile acid circulation, and agonists or antagonists of FXR are promising therapies for diseases associated with metabolic syndrome and inflammation.[18,21,22]

In the previous study, we demonstrated that excessive DCA-induced gut dysbiosis plays an important role in the development of intestinal inflammation and disruption of bile acid metabolism by downregulation of the FXR-FGF15 axis.[23] In the current study, we explored whether FXR activation can prevent pathological development of DCA-induced intestinal inflammation, using FXR agonist, fexaramine, and antibiotics. Furthermore, we aimed to evaluate whether FXR activation can restore gut microbiota and bile acid homeostasis in DCA-fed mice.

2. Methods

2.1. Mice and treatment

Wild-type female C57BL/6J mice, 4 weeks old, from the Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China) were placed in a pathogen-free (SPF) laboratory. Mice were fed a control diet (standard AIN-93G diet with water *ad libitum*) or a 0.2% DCA supplemented diet (C97% titration, Sigma-Aldrich, St Louis, MO) for 24 weeks, as previously described.[6] A proportion of the mice on the 0.2% DCA-supplemented diet were administered 50 mg/kg of fexaramine in corn oil by oral gavage daily (DCA+Fex group) for the last 6 of 24 weeks.[24] Another group of mice on the 0.2% DCA-supplemented diet were administered a cocktail of antibiotics for 24 weeks (DCA+Abx group) for depletion of gut microbiota. A mixture of 500 mg ampicillin, 500 mg neomycin, 250 mg vancomycin, and 250 mg metronidazole (Sigma-Aldrich, St Louis, MO) was administered as previously described.[25] All treatments of the experimental animals were approved by the care commission at Zhejiang Chinese Medical University, Zhejiang, China.

2.2. Intestinal tissue collection

Mice were sacrificed at 28 weeks, and intestinal tissue was collected as previously described.[6,26] Tissue sections were prepared for haematoxylin and eosin (H&E), immunohistochemical (IHC), and immunofluorescence (IF) staining. The ileum, colon, and liver tissues were harvested, immediately frozen in liquid nitrogen, and stored at -80° C for analysis of mRNA and protein expression.

2.3. Histopathology and immunohistochemical staining

Paraffin-embedded blocks were generated from ileal and colonic tissue samples fixed in 10% formalin. Samples were sectioned into 5-µm slices, stained with H&E, and examined under light microscope (Nikon Eclipse TE2000-U, NIKON, Japan) at 20x magnification for the histological evaluation.

Intestinal histology grade, ranging 0 to 6, was determined by assessing the degree of inflammatory cell infiltration (0–3) and tissue damage (0–3) by examiners blinded to group assignments as previously described.[27] A sodium citrate buffer (PH = 6.0) was used for antigen retrieval with high-pressure boiling for 10 min. Paraffin slices were incubated with primary antibodies, anti-FXR (1:50, Invitrogen), anti-FGF-15 (1:150, Santa Cruz), and anti-8OHdG (1:1500, GeneTex), respectively, for 2 h at room temperature, followed by incubation with a horseradish peroxidase secondary antibody kit (Elabscience, China) for 1 h. Afterwards, the sections were prepared with DAB stain and haematoxylin counterstain. The immunostained samples of paraffin slices were analysed with an optical microscope (Nikon Eclipse TE2000-U, NIKON, Japan) at 20x magnification. An immunostain score (0, absent target location staining; 1, weak staining; 2, moderate staining; 3, strong staining) was assigned after examining five randomly selected fields for each sample by examiners blinded to group assignments.

2.4. Imunofluorescence

After de-waxing and antigen retrieval, the paraffin sections of the colonic tissue were blocked with 5% blocker BSA for 1 h at room temperature. The sections were stained with anti-ZO-1 (1:30, Invitrogen) overnight at 4°C, and then incubated with a goat anti-rabbit IgG (H+L) secondary antibody (Alexa Fluor® 488 conjugate, 1:500, Beyotime) for 2 h at room temperature to obtain green fluorescence. The cell nuclei were stained with DAPI to obtain blue fluorescence. Confocal images were collected using an Olympus Bx53 confocal fluorescence microscope system at 20x magnification.

2.5. Pyrosequencing analysis

16S rDNA pyrosequencing was performed by the Realbio Genomics Institute (Shanghai, China). The deoxyribonucleic acid (DNA) isolated from faeces was used as an amplified template of the 16S rDNA V3-V4 hypervariable area, and Illumina MiSeq PE250 multiplex sequencing was performed for the16S analysis. Optimising sequences were selected for 97% similarity and were clustered into operational taxonomic units (OTUs). The similarity based on shared OTU count between samples was shown by Venn diagram. The resulting matrix of distances between groups was shown by principal component analysis (PCA). Chao 1 diversity index, the Shannon and

Simpson concentration index were carried out for community diversity analysis. The taxonomic analysis was performed by clustering of reads based on the OTUs using the QIIME platform.

2.6. Faecal bile acids measurement

Mice faeces was collected at 28 weeks and stored in isopropanol at -20°C. Faecal bile acid levels were quantitated by an ultra-performance

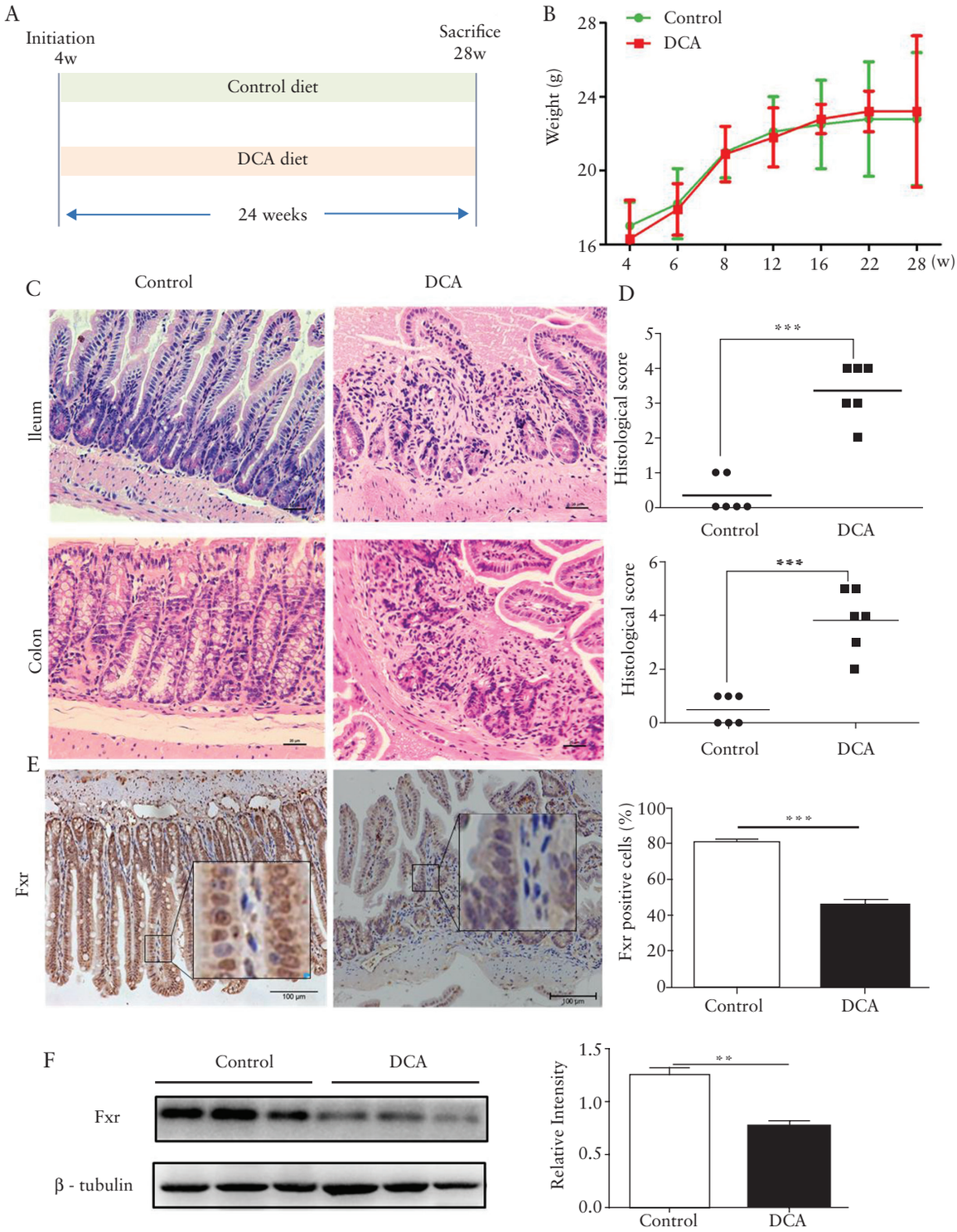

Figure 1. DCA-induced intestinal inflammation and down-regulated the level of FXR expression. (a) Experimental flow chart. Mice were administered a 0.2% DCA diet or control diet and were sacrificed 24 weeks after treatment. (b) Changes in body weight in each group throughout the treatment period ($p > 0.05$). (c and d) The intestinal histological appearances and pathological scores in mice, scale bar = 20 μm. (e) Immunohistochemistry results indicated that the level of FXR expression in the ileum was decreased in the DCA group, scale bar = 100 μm. (f) The expression of FXR in the ileum was assessed by western blot analysis. DCA, deoxycholic acid; FXR, farnesoid X receptor. ***$p < 0.001$. $n = 6$ for each group.

liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Briefly, the faecal samples were thoroughly blended, centrifuged, and freeze-dried. The frozen samples were subsequently dissolved with BAP Ultra reagent, acetonitrile/methanol, and ultrapure water, and were centrifuged at 13 500*g* and 4°C for 20 min. Supernatant was transferred to the plates for LC-MS analysis. Bile acid concentration was quantified using the UPLC-MS/MS system (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA).

2.7. Faecal short chain fatty acid quantification

The SCFA concentrations were determined by UPLC-MS/MS. Briefly, the faecal samples were thawed on ice and dissolved in water with sirconia bead homogenized for 3 min. Afterwards, the samples were mixed with acetonitrile/methanol (8:2 ratio) for extraction. The extracts were further centrifuged at 18 000*g* for 20 min. Supernatant was transferred to plates and mixed with derivatisation reagents for derivatisation at the Biomek 4000 workstation for 60 min at 30°C.

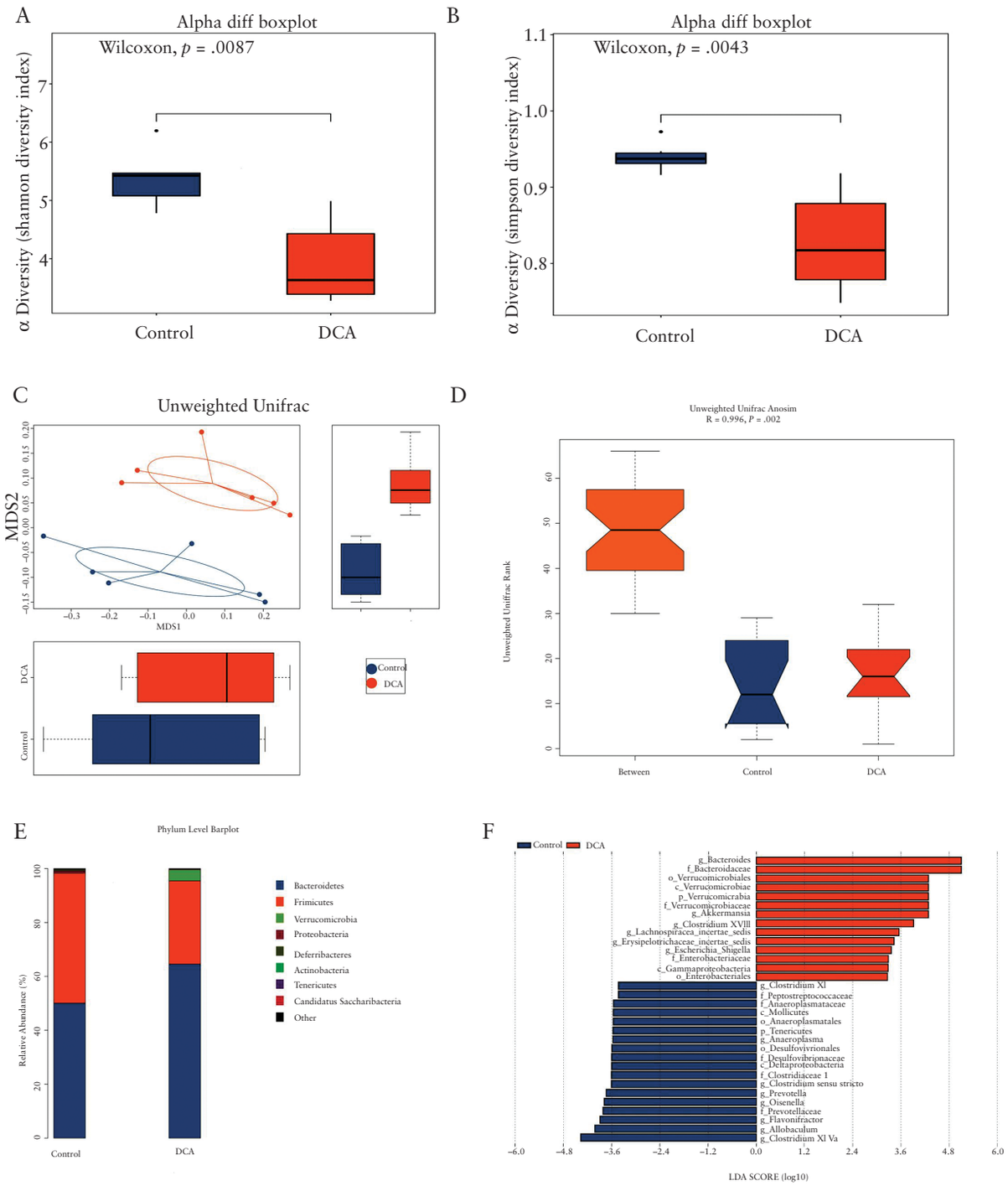

Figure 2. DCA altered the faecal microbiota composition significantly. (a and b) α diversities (Shannon and Simpson) of the DCA group and the control group. (c and d) β diversities between the two groups by the MDS2 and ANOSIM analysis. (e)The microbiota community structure between the two groups. (f)The LEfSe analysis of the microbial compositions between the two groups at the genus level. DCA, deoxycholic acid; $^{**}p$ <0.01, and $^{***}p$ <0.001. n = 6 for each group.

Subsequently, 50% methanol solution was added for dilution and centrifuged at 4000*g* for 30 min. Finally, the supernatant was transferred to the plates for LC-MS analysis (ACQUITY UPLC-Xevo TQ-S, Waters Corp., Milford, MA, USA). SCFA standards were purchased from Sigma-Aldrich (St Louis, MO, USA).

2.8. Quantitative reverse transcriptase PCR

Tissue RNA sample was extracted with RNAiso Plus reagent (Kangwei Biotechnology, China) following the manufacturer's protocol. Each RNA sample was reverse transcribed into cDNA using the TIANScript RT reagent Kit (Kangwei Biotechnology, China). Forward (F) and reverse (R) primers were used (Supplementary Table S1, available as Supplementary data at *ECCO-JCC* online). Values were normalised to GAPDH. Relative mRNA expression in our study was calculated using the ΔΔCt method.

2.9. Western blot

Proteins were extracted from a RIPA lysis buffer with protease inhibitor cocktail to fragment whole harvested tissue from the distal ileum, colon, and liver. Protein concentrations of the samples were determined using a BCA Protein Assay kit (Kangwei Biotechnology, China). A total of 50 mg protein sample was denatured with sodium dodecyl sulphate (SDS) sample buffer, separated on 10% SDS-PAGE, and transferred to PVDF membranes. After blocking with 5% skimmed milk for 2 h at room temperature, the membranes were incubated with antibodies (anti-FXR, anti-FGF-15) overnight at 4°C, followed by horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology, Inc.) for 2 h at room temperature. Protein expression was visualised with an enhanced chemiluminescence system. The intensity of the bands value was adjusted according to the expression of the internal protein (β-actin). Image J software was used to quantify the protein densitometry.

2.10. Statistical analysis

The results are presented as the mean values (± standard error of the mean [SEM)]) for continuous variables and Student's ttest was used to compare the groups. Analysis of variance [ANOVA] with the Bonferroni method was performed for posthoc analysis for

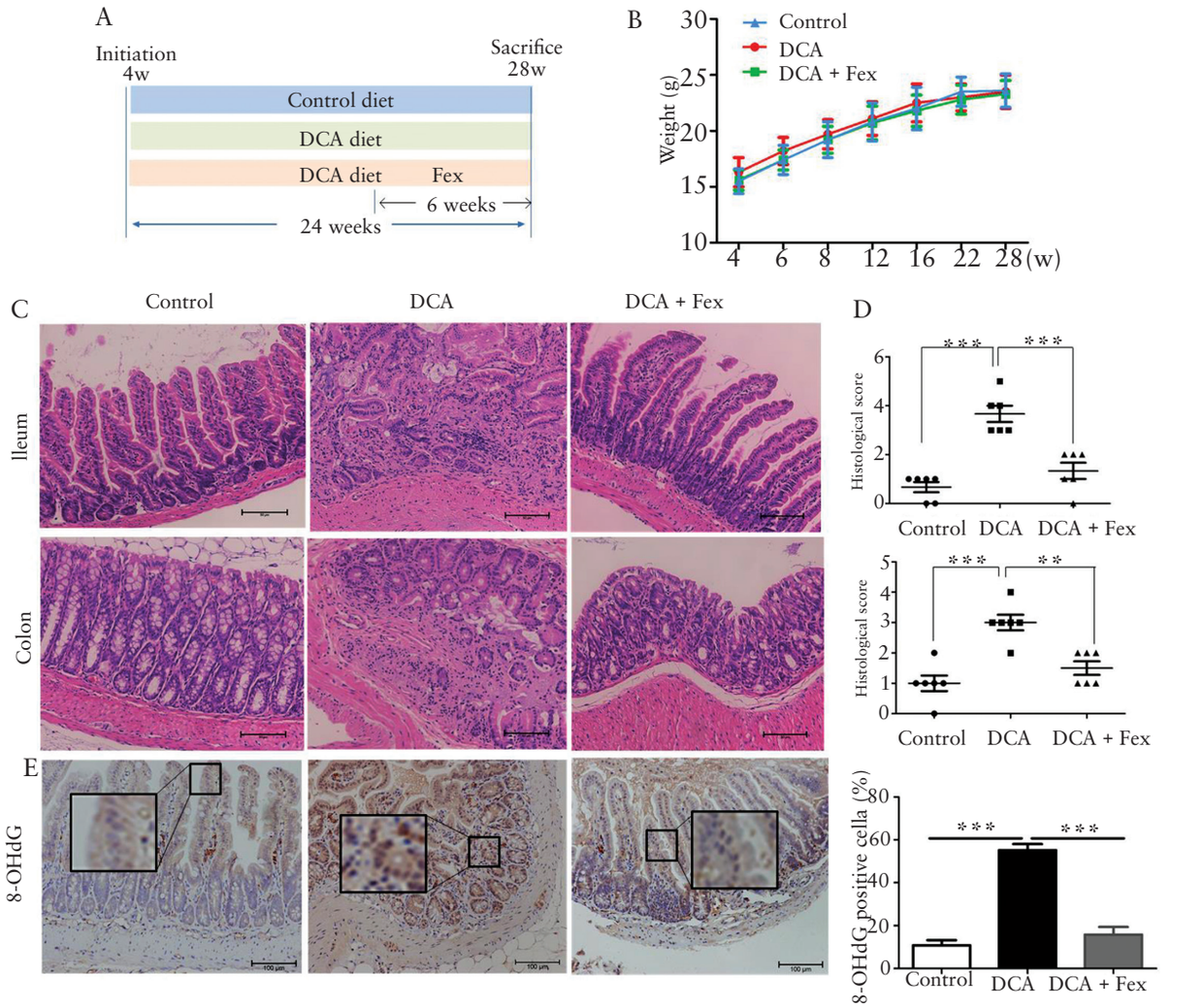

Figure 3. Fexaramine administration abrogated the progression of DCA-induced intestinal inflammation. (a) Experimental flow chart. The mice of the DCA+Fex group were fed with fexaramine for 6 weeks before sacrifice. (b)The weekly growth trends of body weight between the three diet groups. (c and d) H&E staining indicated both mucosal inflammatory cell infiltration decreased and less severe epithelial layer destruction in the DCA+Fex group, scale bar = 50 μm. (e) An immunohistochemical analysis of intestinal sections revealed that the oxide stress protein 8-OHdG was less expressed in the mice treated with fexaramine. Scale bar = 100 μm. The percentage of positive cells was calculated from five randomly selected tissues from each section. Fex, fexaramine; DCA, deoxycholic acid; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; $^{**}p < 0.01$, and $^{***}p < 0.001$. $n = 6$ for each group.

comparing more than two groups. Two-sided p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS 22 (Chicago, IL, USA).

3. Results

3.1. DCA induces intestinal inflammation, gut dysbiosis, and intestinal FXR deactivation

All mice thrived, and were sacrificed at 24 weeks. The body weight of mice in the control and DCA groups increased gradually without significant difference (Figure 1b). Chronic DCA-induced murine intestinal inflammation model was characterised by mucosal oedema and thickening, crypt distortion, and high levels of lymphocyte and neutrophil infiltration (Figure 1c). By 24 weeks, the DCA group had significantly higher histological scores compared with the control group (ileum 0.3 ± 0.2 vs 3.3 ± 0.3, p <0.0001; colon 0.5 ± 0.2 vs 3.8 ± 0.5, p <0.0001) (Figure 1d). Furthermore, IHC staining and western blot showed lower FXR expression in the ileum of the DCA group (Figure 1e and f).

On faecal microbiota analysis, DCA supplementation significantly decreased α-diversity (the Shannon and Simpson index [Shannon is an index that quantifies the uncertainty in a population. Simpson is an index described as the probability that two consecutive samples of a community result in the number of individuals belonging to the same species]) (Figure 2a and b), suggesting that DCA altered faecal microbiota diversity. The MDS2

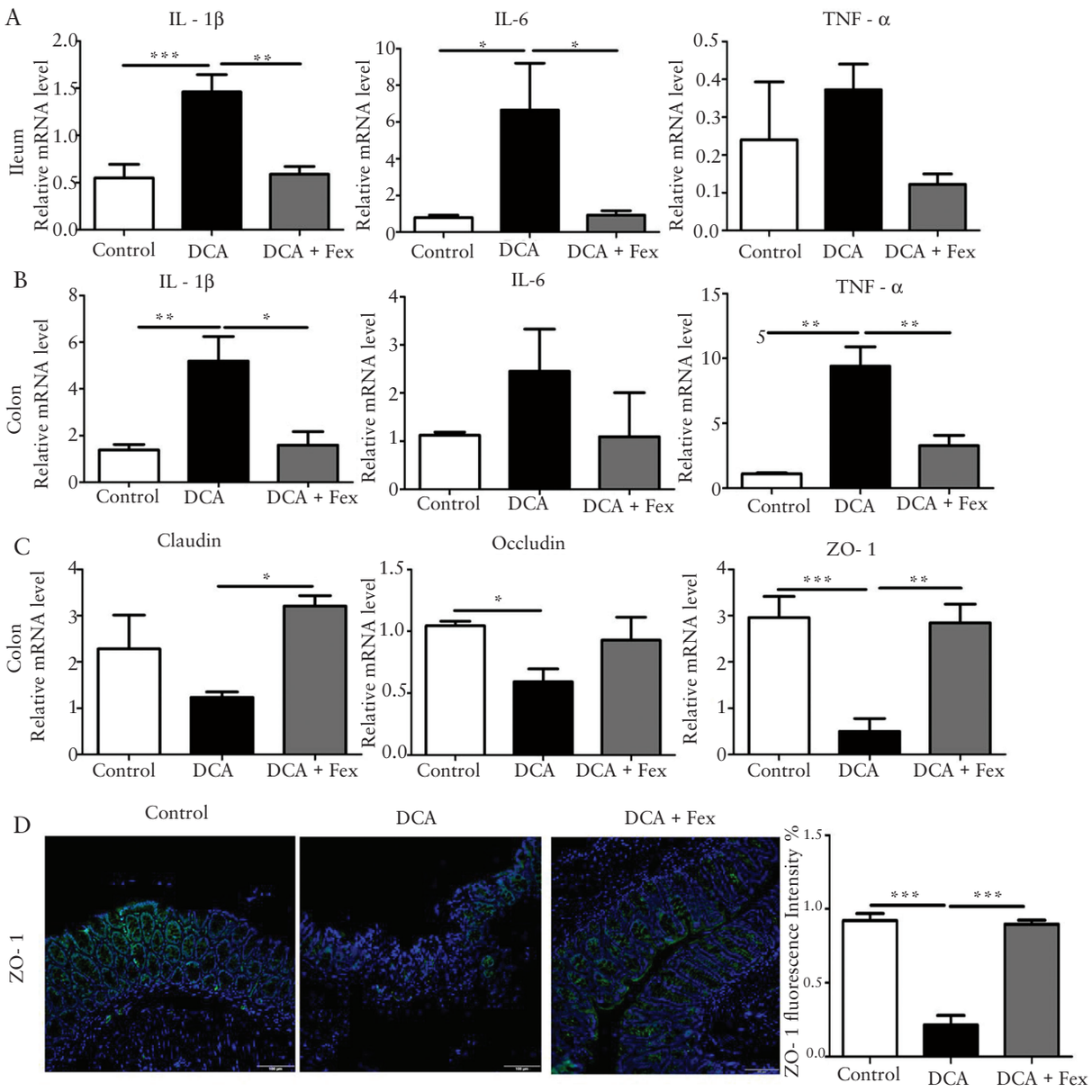

Figure 4. Fexaramine treatment alleviated low-grade intestinal inflammation and stabilised the impairment of the intestinal mucosal barrier induced by DCA. (a and b) Levels of proinflammatory cytokines IL-1β, IL-6, TNF-α in the ileum and colon in each diet group were determined by real-time qPCR. (c) The mRNA level of claudin, occludin, and ZO-1 in the colon in mice. (d) Immunofluorescence analysis revealed the expression of ZO-1 was increased in the colon sections after administration with fexaramine, and nuclei were stained with DAPI (blue). The presence of ZO-1 (green) was analysed with fluorescence microscopy. Fex, fexaramine; DCA, deoxycholic acid; ZO-1, zonula occludins-1. *p <0.05, **p <0.001, and ***p <0.0001. n = 6 for each group.

and ANOSIM analysis also showed significant differences in β-diversity between the two groups (Figure 2c and d). The microbiota community structure in the control group was dominated by Firmicutes (48.3%) and Bacteroidetes (50.1%), whereas the proportions of Firmicutes and Bacteroidetes were 30.9% and 64.6%, respectively, in the DCA group (Figure 2e). At the genus level, the LEfSe analysis of the microbial compositions showed that the DCA-treated mice had higher levels of opportunistic pathogens (Bacteroides and Escherichia-Shigella), whereas bile salt hydrolase (BSH)-producing bacteria, including Clostridium XI, and Clostridium XIVa, were reduced compared with the control group (Figure 2f).

3.2. Fexaramine administration abrogates the progression of DCA-induced intestinal inflammation

To investigate the protective effects against the development of intestinal inflammation, daily gavage of fexaramine (Fex; 50 mg/kg body weight) were administered to DCA-treated mice for the last 6 weeks. Intestinal inflammation was decreased with the administration of fexaramine, evidenced by a significant decrease in mucosal inflammatory cell infiltration and reduction in epithelial destruction (Figure 3c and d). Furthermore, lower expressions of sensitive oxidative stress marker (8-hydroxy-2'-deoxyguanosine, 8-OHdG) were observed in the intestine of mice in the DCA+Fex and control groups compared with the DCA group (Figure 3e).

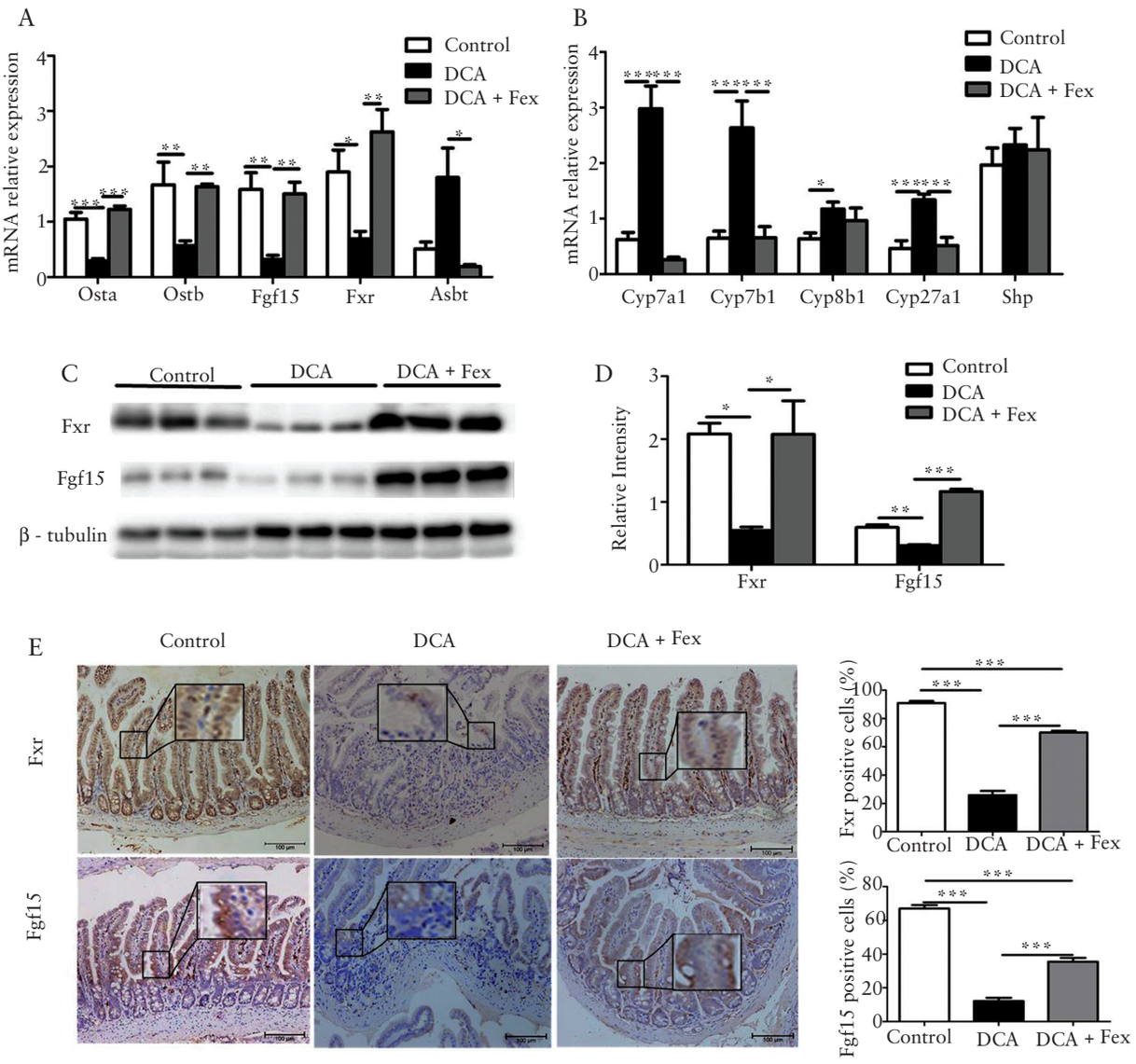

Figure 5. Fexaramine activated intestinal FXR-fgf15 and modulated the bile acid synthesis. (a) Changes in Ostα, Ostβ, Fgf15, Fxr, and Asbt in the ileum of mice with fexaramine administration. (b) The mRNA expression of hepatic bile acid synthesis enzymes in each diet group. (c) The autoradiography image of western blot with the expression of FXR, Fgf15, and β-tubulin antibodies in the ileum. (d) The relative expression in FXR and Fgf15 in the ileum in each diet group. The proteins were quantified using Image J software. (e) Immunohistochemical results of FXR and Fgf15 in the intestinal sections. Ostα, organic solute transporter alpha; Ostβ, organic solute transporter Ostβ; Asbt, apical sodium bile acid transporter; Fgf15, fibroblast growth factor 15; FXR, farnesoid X receptor; Cyp7a1, cholesterol 7α-hydroxylase; Cyp7b1, cholesterol 7β-hydroxylase; Cyp8b1, sterol-12alpha-hydroxylase; Cyp27a1, cholesterol 27α-hydroxylase; Shp, small heterodimer partner. Scale bars = 100 μm. *p <0.05, **p <0.01, and ***p <0.001. n = 6 for each group.

The mRNA levels of proinflammatory cytokines, including IL-1β and IL-6 in the ileum and IL-1β and TNF-α in the colon, were notably lower in both the control and DCA+Fex groups (Figure 4a and b) compared with the DCA group. Real-time PCR demonstrated upregulation of colonic claudin and zonula occludin-1 (ZO-1) after the fexaramine treatment (Figure 4c). Furthermore, immunofluorescence showed that the expression of ZO-1 increased in the colonic tissue in the DCA+Fex group, and distribution was relatively continuous (Figure 4d). The results indicated that fexaramine repaired the intestinal permeability and reversed chronic intestinal low-grade inflammation induced by DCA.

3.3. Fexaramine induces intestinal Fgf15 and modulates bile acid synthesis

Fexaramine administration increased the expression of organic solute transporter alpha (Ostα), beta (Ostβ), and decreased the expression of intestinal bile acid transporter of apical sodium bile acid transporter (Asbt) in the ileum of mice in the DCA group (Figure 5a). Furthermore, mice in the DCA+Fex group demonstrated sustained increase of intestinal Fxr and Fgf15 production (Figure 5c–e). Fgf15 is known to inhibit hepatic bile acid synthesis through suppressing related enzymes. Consistent with the increase in intestinal Fgf15 expression, hepatic bile acid synthesis enzymes, including Cyp7a1, Cyp7b1, and Cyp27a1, were significantly suppressed at the mRNA level after chronic fexaramine treatment, whereas the expression of Cyp8b1 was slightly affected (Figure 5b).

Fexaramine administration also produced marked changes in the composition of the faecal bile acid pool in the DCA group. In addition to reducing the size of the total bile acid pool, fexaramine notably decreased the levels of primary, secondary, and unconjugated bile acids (Figure 6a–c). With the exception of faecal secondary bile acids, no significant differences between the DCA+Fex and the control groups was observed. For individual faecal bile acid, DCA, tauro-β-murine cholic acid (Tβ-MCA), and Tα-MCA levels were decreased with administration of fexaramine (Figure 6d). Finally, fexaramine administration increased the concentration of faecal ursodeoxycholic acid (UDCA) described as a scrubber of bile acid.[28]

3.4. Fexaramine alters DCA-induced gut microbiota dysbiosis

On pyrosequencing analysis, 411 OTUs were identified in the DCA group, 429 OTUs in the DCA+Fex group, and 363 OTUs were shared by the two groups (Figure 7a). PCA results demonstrated that the faecal microbial community in the DCA+Fex group was significantly different from the DCA group (Figure 7b). Chao 1 diversity index and Shannon index, representing the α-diversity (Figure 7c and d), were not significant different between DCA+Fex and DCA groups. However, β-diversity, including MDS2 and ANOSIM analysis, showed significant differences between the two groups (Figure 7e and f).

Intestinal microbiota was dominated by the four major phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. However, compared with the DCA group, fexaramine treatment did not statistically affaect the phylum community or Firmicutes/Bacteroidetes (F/B) ratio (Figure 8a). More importantly, at the genus level, a heatmap of the microbial compositions showed that the DCA+Fex group had higher levels of beneficial bacteria, including Bifidobacterium, Lactobacillus, Akkermansia, and SCFA-producing bacteria, such as Clostridium sensu stricto and Clostridiaceae compared with the DCA group. In contrast, the abundance of Helicobacteria, an opportunistic pathogen, was lower in the DCA+Fex compared with the DCA group (Figure 8b, d and e).

We further explored the interactions between the microbiota communities. Inverse relationship was observed between Clostridium IV and Oscillbacter, as well as, Clostridium XIVa and Intestinimonas. Interestingly, a positive relationship between Clostridium sensu stricto and Anaerostipes, Akkermansia and Rikenella, Allobaculum and Butyricicoccus, Parasutterella, and Intestinimonas were observed (Figure 8c).

3.5. Fexaramine increases faecal concentrations of SCFAs

Gut microbiome-derived SCFAs are immunomodulatory molecules that contribute to the maintenance of the organic immune homeostasis.[29] Results of the 16S DNA sequencing analysis showed an

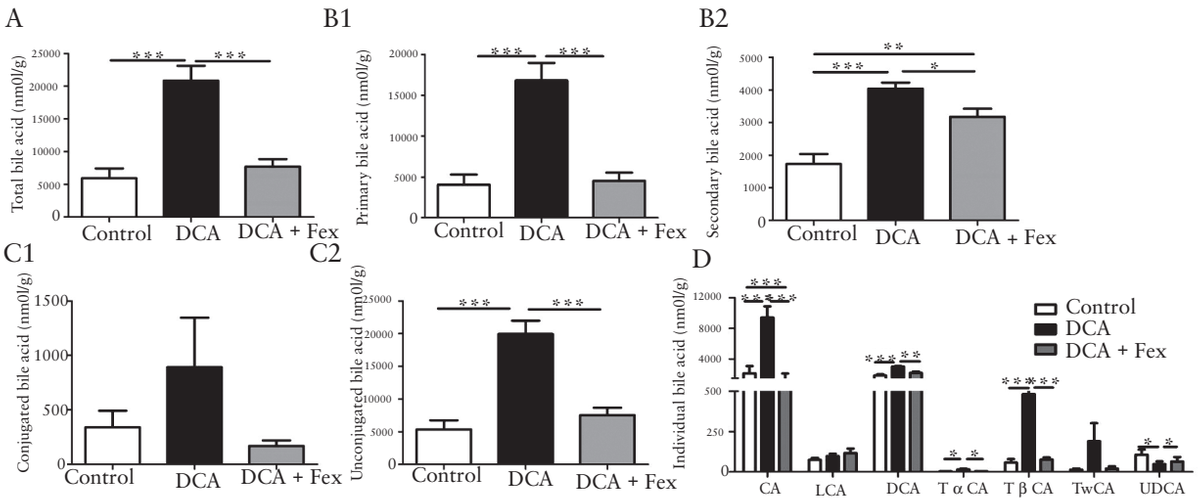

Figure 6. Fexaramine affected bile acid metabolism. (a) The concentrations of the total faecal bile acids. (b) The concentrations of the faecal primary bile acid (b1) and secondary bile acid (b2). (c) The concentrations of the faecal conjugated (c1) and unconjugated bile acid (c2). (d) Individual bile acid concentrations in faeces between the three groups. Fex, fexaramine; DCA, deoxycholic acid; CA, cholic acid; LCA, lithocholic acid; Tβ-MCA, tauro-β-murine cholic acid; Tα-MCA, tauro-α-murine cholic acid; Tω-MCA, tauro-ω-murine cholic acid; UDCA, ursodeoxycholic acid. *p <0.05, **p <0.001, and ***p <0.0001. n = 6 for each group.

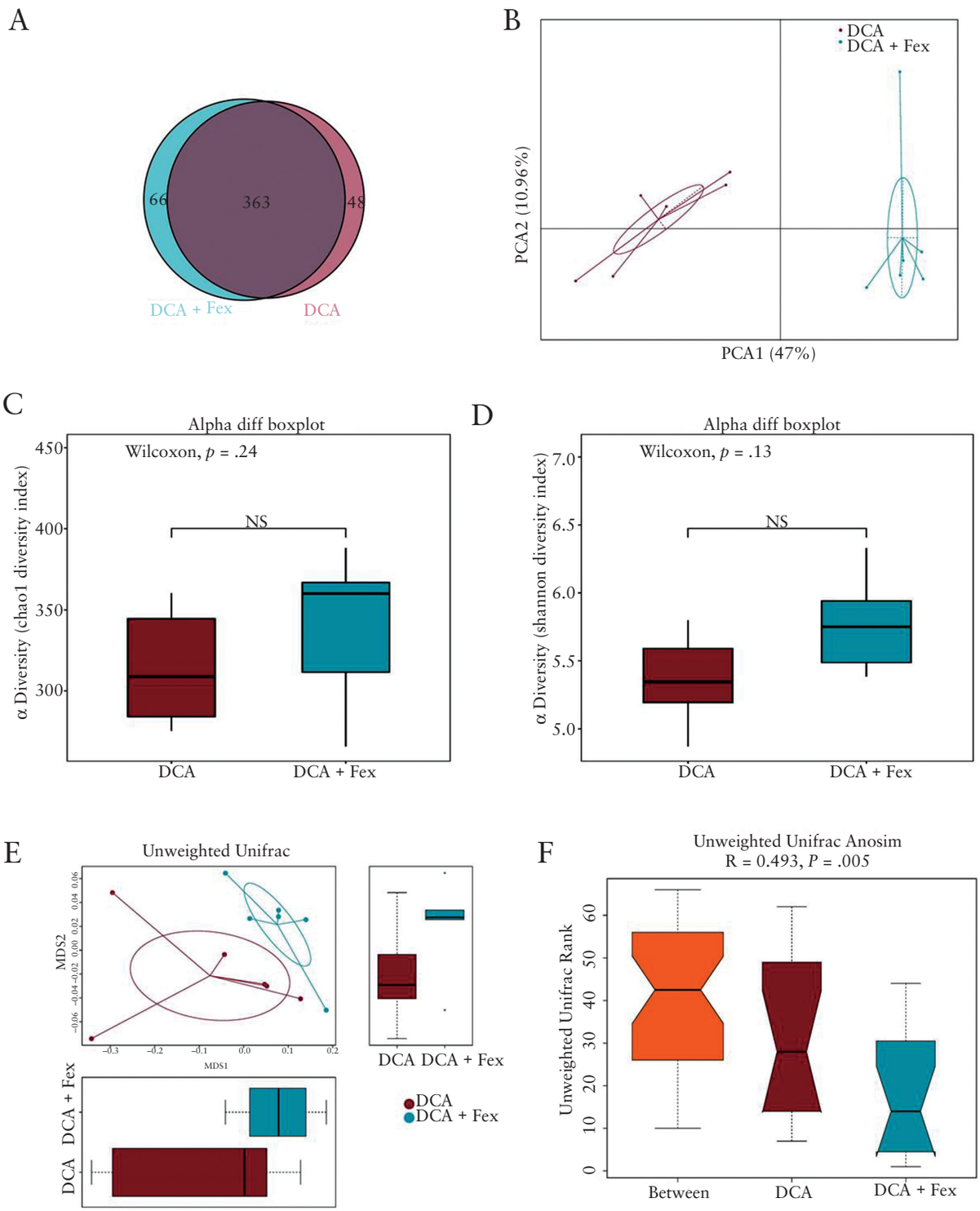

Figure 7. Fexaramine modulated the intestinal microbiota in mice. (a) Venn diagrams of bacterial OTUs between the DCA group and the DCA+Fex group. (b) The PCA focused on faecal bacterial communities in distinct groups with respect to DCA diet or DCA and fexaramine treatment. (c and d) α diversities of gut microbiota in each diet group. (e and f) β diversities of gut microbiota in each diet group. Fex, fexaramine; DCA, deoxycholic acid; OTU, operational taxonomic unit; PCA, principal components analysis. *n* = 6 for each group.

increased abundance of SCFA-producing bacteria in the fexaramine-treated mice including acetate, propionate, and butyrate. Higher level of faecal SCFAs, especially butyrate, were measured in the DCA+Fex compared with the DCA group (Supplementary Figure S1, available as Supplementary data at *ECCO-JCC* online).

3.5. Antibiotics prevent DCA-induced intestinal inflammation by reducing the bile acid pool

To investigate the role of gut microbiota in the development of intestinal inflammation, an antibiotic cocktail was administered to mice fed on DCA. Administration of broad-spectrum antibiotics

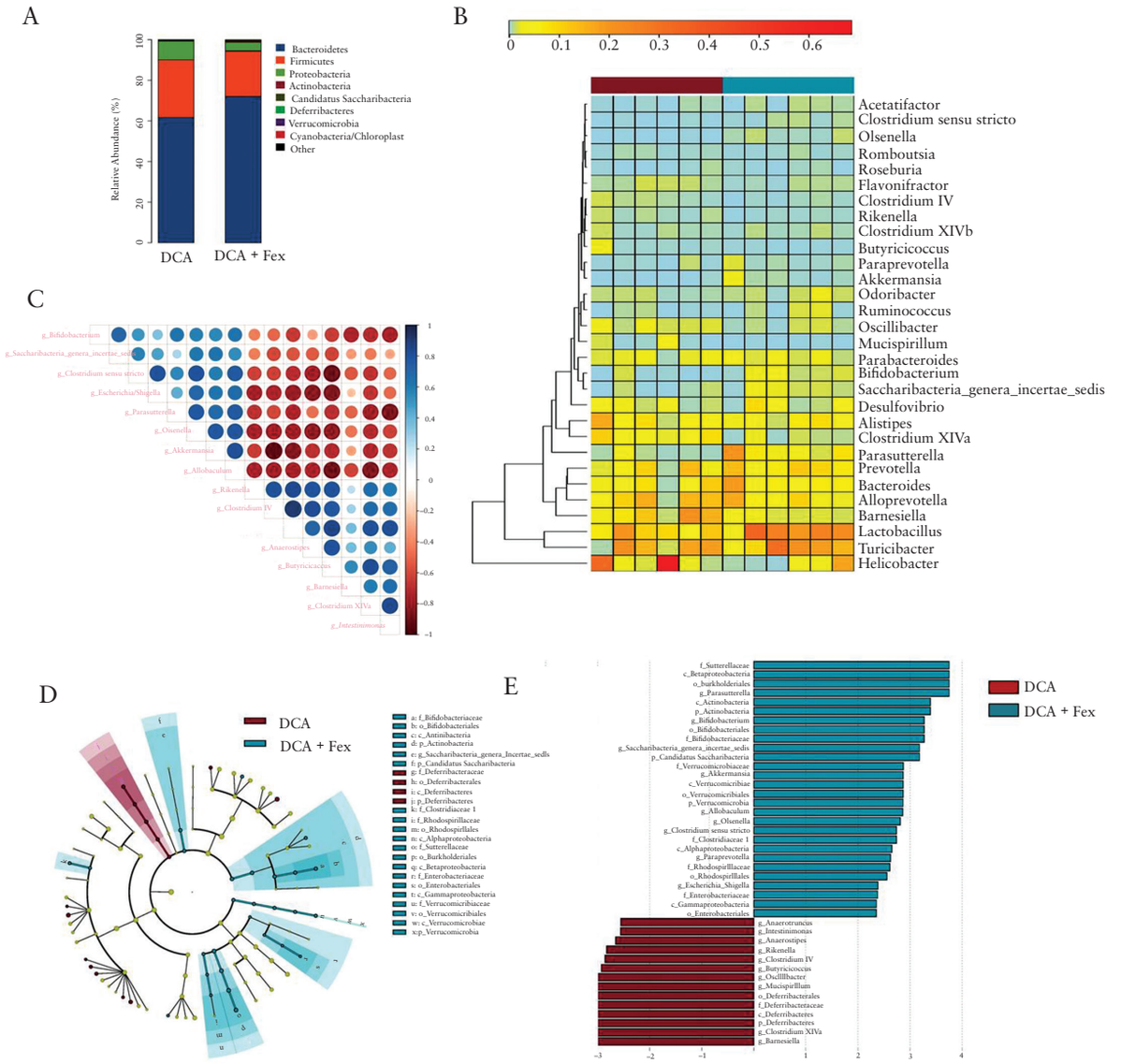

Figure 8. Population analysis of intestinal microbiota for each diet group by the 16S DNA sequencing analysis. (a)The relative abundance of intestinal microbiota at the phyla level between the two groups. (b) A heatmap of the microbial compositions at the genus level. (c)The interactions between different bacteria were shown. (d-e)The significantly different bacteria between the DCA group and the DCA+Fex group. Fex, fexaramine; DCA, deoxycholic acid. $n = 6$ for each group.

significantly decreased both the diversity and the abundance of the intestinal microbiota community. On pyrosequencing analysis, 326 OTUs were identified in the control group, 240 OTUs in the DCA group, 70 OTUs in the DCA+Abx group, and 35 OTUs were shared by the three groups (Figure 9a). Administration of antibiotics drastically decreased the proportion of both Gram-negative Bacteroidetes and Gram-positive Firmicutes (Figure 9b).

We further examined the faecal bile acid composition to study how the altered microbiota affects the microbial modification of the host compounds. Notably, the DCA+Abx group demonstrated decreased total bile acid pool including conjugated bile acids, whereas primary bile acids dominated the total bile acid pool (Figure 9f) compared with the DCA group. Finally, we examined the expression of the genes that play a role in the regulation of enterohepatic circulation of bile acids. Antibiotic administration inhibited the expression of Cyp7a1 and Cyp7b1 without an effect on other bile acid target genes, in both ilieal and hepatic tissues in mice from the DCA group (Figure 9c and d). Western blot also showed a slight change of Fxr and Fgf15 expression (Figure 9e). Taken together, antibiotics appear to inhibit microbial bile acid deconjugation and dehydroxylation.

Finally, we investigated whether an antibiotic cocktail therapy can prevent intestinal inflammation induced by DCA in mice. No differences in body weight were observed between the DCA+Abx and DCA groups during the experiment (Figure 10b). Histopathology examination of H&E-stained ileum and colon tissues showed less inflammatory cell infiltration in the epithelial layer and lower histological score in the DCA+Abx compared with the DCA group (Figure 10c). Furthermore, inflammatory cytokines including IL-Iβ and IL-6 in both the ileal and colonic tissues were dramatically decreased in the DCA+Abx compared with the DCA group.

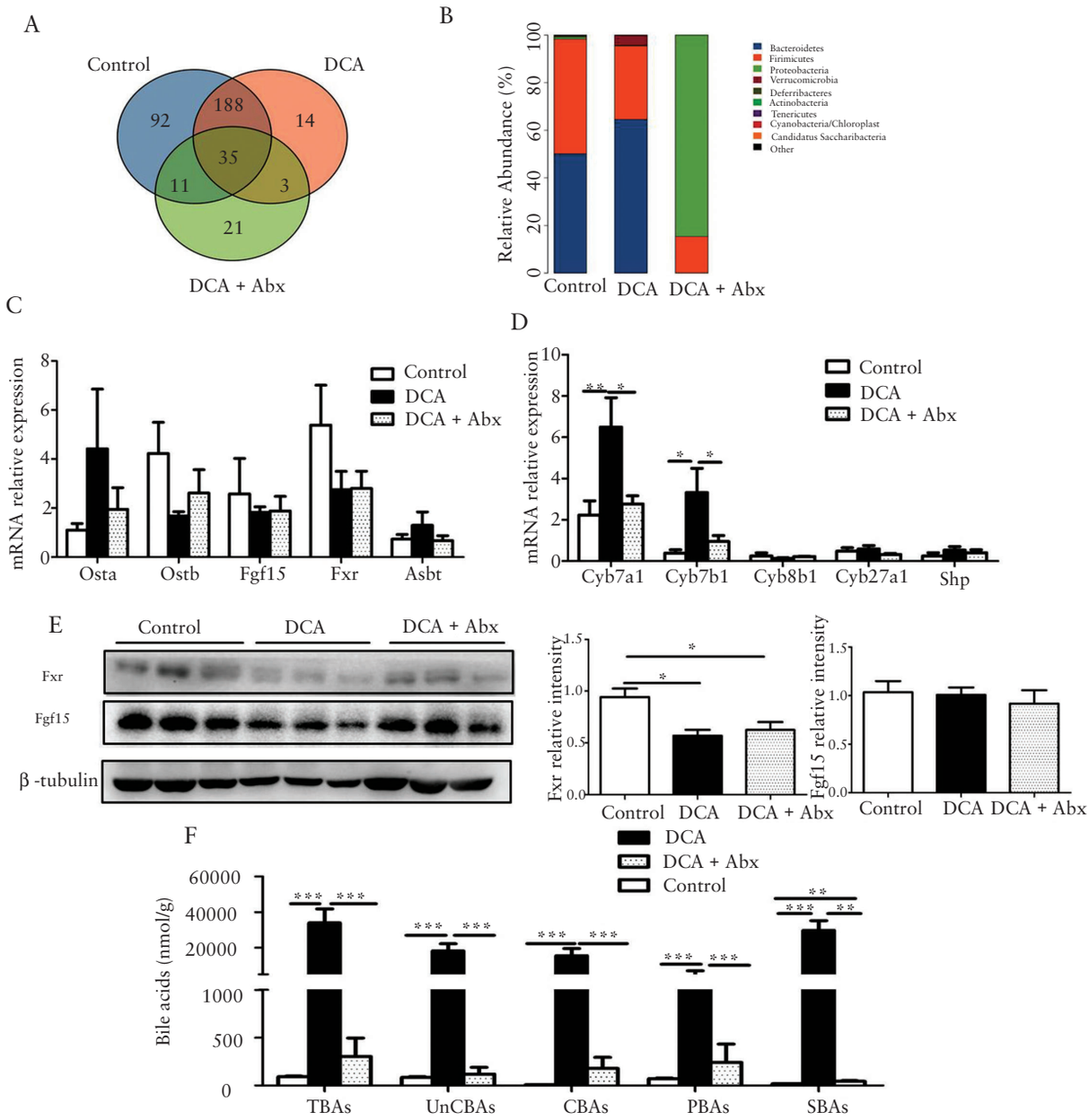

Figure 9. Effects of antibiotics cocktail (Abx) treatment on intestinal microbiota community and bile acid metabolism in mice. (a) The comparison of the OTUs among the three diet groups. (b) The relative abundance of bacteria at the phyla level among the three diet groups was presented. (c) Changes in Ostα, Ostβ, Fgf15, Fxr, and Asbt in the ileum of mice with Abx administration. (d) The mRNA expression of hepatic bile acid synthesis enzymes with Abx treatment. (e) The expression of FXR and Fgf15 in the ileum among the three diet groups was assessed by western blot analysis. Using β-tubulin as the internal reference, Image J software was used to quantity the proteins. (f) Types of bile acid detected in the faeces. Ostα, organic solute transporter alpha; Ostβ, organic solute transporter Ostβ; Asbt, apical sodium bile acid transporter; Fgf15, fibroblast growth factor 15; FXR, farnesoid X receptor; OTU, operational taxonomic unit. **p* <0.05, ***p* <0.01, and ****p* <0.001. *n* = 6 for each group.

4. Discussion

IBD is a chronic inflammatory disease characterised by dysregulation of the mucosal immune response.[30] Increased consumption of dietary fat intake and interaction with gut microbiota play a key role during the initiation process of inflammation.[31] In our study, we explored whether dietary DCA can affect gut microbiota and suppress intestinal FXR expression, in a mouse model of intestinal inflammation. The effects of DCA promoting epithelial injury were clearly observed, consistent with earlier findings.[23,32,33] Interestingly, DCA-induced intestinal inflammation was not accompanied by weight loss in our animal model. Although unclear, modest levels of intestinal inflammation in our animal model, supported by a lack of gastrointestinal symptoms or intestinal ulcers and possibly pro-obesity effects of DCA as a metabolite of high-fat diet, may have mitigated the impact of inflammation on weight loss.[5] Furthermore, administration of intestine-specific FXR agonist, fexaramine, was protective against DCA-induced intestinal inflammation by re-storing gut microbiota and bile acid metabolism. In addition, the beneficial effects of antibiotics on the gut microbiota were replicated,

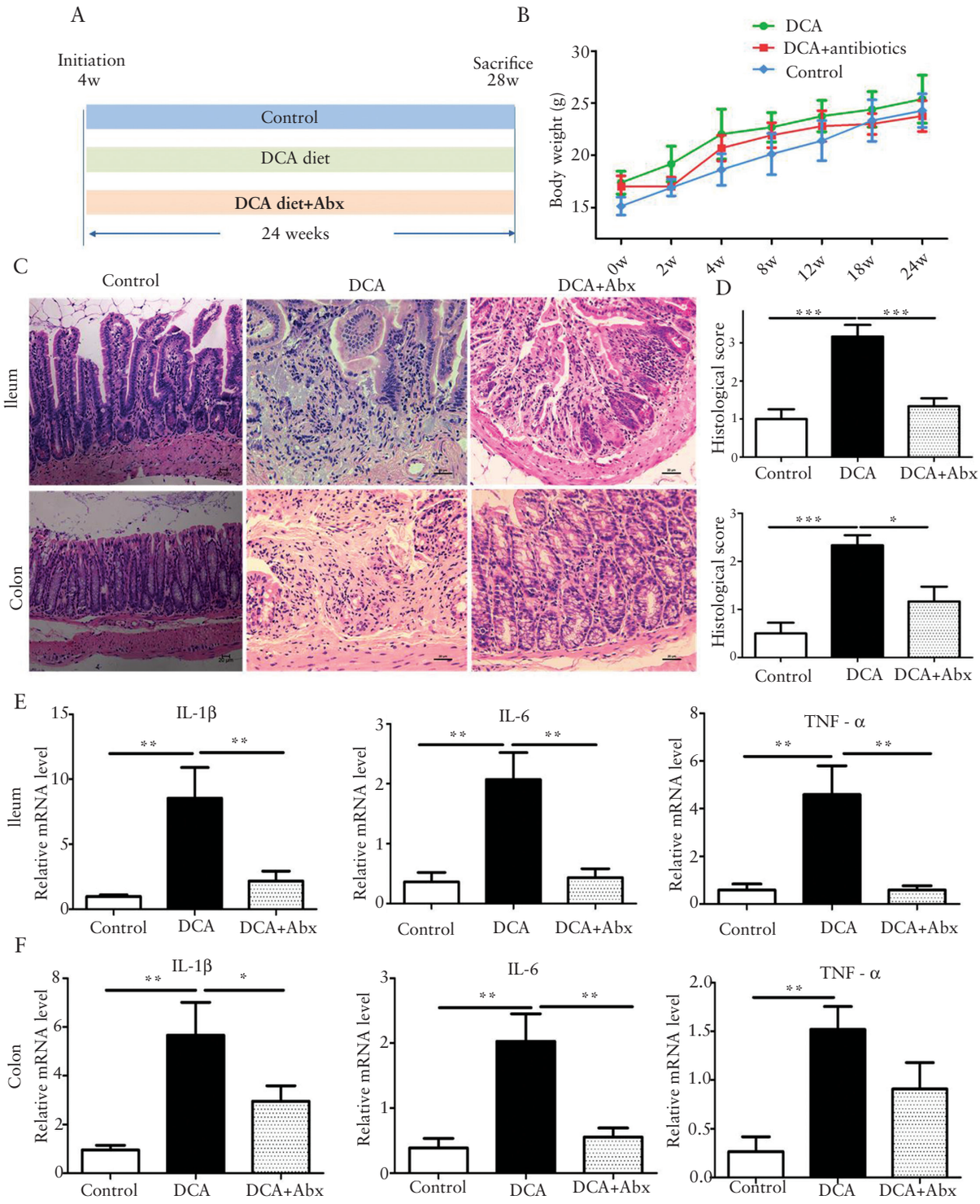

Figure 10. Antibiotics cocktail prevented the intestinal inflammation induced by DCA in mice. (a) Experimental flow chart. Mice were administered a DCA diet or DCA combined with antibiotics diet and killed 24 weeks after treatment. (b) The body weight of mice was noted among the three groups throughout the treatment period ($p > 0.05$). (c and d) Morphology analysis of H&E-stained gut tissues in mice between the three groups. (e and f) Inflammatory cytokines in the ileum and colonic tissues were detected by qPCR, respectively. DCA, deoxycholic acid. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. $n = 6$ for each group.

consistent with the other studies.[34,35] Our results indicate that the gut microbiota plays a crucial role in promoting inflammation, and the reduction of microbial abundance by antibiotics is protective against DCA-induced intestinal inflammation.

Recently, the gut microbiota has been recognised as an 'endocrine organ' modulating host physiology via production of metabolites, such as bile acids.[36,37] The main sites of bile acid metabolism include hepatocytes and microbes within the intestinal tract.[37] In our study,

we found that DCA mainly decreased the abundance of Clostridium that possess BSH. Subsequently, lower levels of tauro-muric cholic acid (TMCA), an intestinal FXR agonist, were observed, consistent with our previous findings.[23] Nuclear FXR regulates bile acid metabolism through the enterohepatic signalling pathway via fibroblast growth factor-15/19 (FGF-15/19),[38] which can bind to the receptors located on the hepatocytes and further dampen the expression of rate-limiting enzymes, like cholesterol 7α-hydroxylase (Cyp7a1), ultimately inhibiting bile acid synthesis.[39] Disruption of enterohepatic circulation of host bile acids promotes inflammation in the gastrointestinal tract by enterocyte DNA damage.[40]

Deficiency or inhibition of intestinal FXR increases the bile acid pool in mice.[40] In IBD, deficiency of FXR worsens gut inflammation,[41] and the activation of FXR can alleviate intestinal inflammation.[18] Fexaramine is a gut-specific FXR agonist that mitigated DCA-induced intestinal inflammation in our study. The protective effects of FXR activation were partly mediated by the suppression of hepatic Cyp7a1 and Cyp7b1 expression which inhibited the de novo synthesis of bile acids. Furthermore, fexaramine increased the production of beneficial bile acid, including UDCA, and increased the hydrophilic bile acid pool by diluting the concentration of toxic bile acids, including DCA.[36,37] Reduced concentration of luminal DCA can stabilise the intestinal barrier after DCA treatment.[42]

Apart from direct effects on bile acid metabolism, fexaramine likely has additional beneficial effects of restoring intestinal dysbiosis. Pathak *et al.* previously showed that activation of intestinal FXR altered intestinal microbiota by increasing the abundance of LCA (lithocholic acid)-producing bacteria, Acetatifactor and Bacteroides, in the leptin receptor-deficient rodent model.[43] Our study also showed that fexaramine administration altered the composition of intestinal microbiota by increasing the abundance of beneficial, SCFA-producing bacteria. However, fexaramine administration failed to increase the abundance of LCA-producing bacteria, Acetatifactor and Bacteroides, in the DCA-induced intestinal inflammation model. Although unclear, mechanistic differences of FXR activation between metabolic syndrome and IBD may account for the inconsistency and will require validation in future studies. Taken together, fexaramine modulated bile acid signalling and mitigated DCA-induced intestinal inflammation in our animal model.

The importance of interaction between the intestinal microbiome and bile acid homeostasis has been demonstrated in the gnotobiotic mouse model.[44] Bile acid pools of germ-free mice were less chemically diverse compared with the conventional mouse model.[37] In addition, administration of broad-spectrum antibiotics alters the community structure and function of the gut microbiota.[45,46] Consistent with previous studies, our results also demonstrated that antibiotic treatment significantly decreased bacterial density including both Gram-negative Bacteroidetes and Gram-positive Firmicutes.[35] As previously shown, antibiotic therapy led to dominance of host-derived primary bile acids over microbial-derived secondary bile acids in the faeces[47] as antibiotics depleted the faecal microbiota responsible for 7α-dehydroxylation.[48]

Our study demonstrated that mice treated with antibiotics had higher ratios of primary to secondary and conjugated to unconjugated bile acids, indicating that antibiotics almost completely suppressed microbial dehydroxylation and deconjugation. Therefore, the protective effects of antibiotics in DCA-induced intestinal inflammation are driven by the suppression of deconjugation and dehydroxylation of bile acids in the intestine and reducing enterotoxin-promoting bile acid composition. However, the choice of antibiotics is also likely important. For example, a 12-week trial of rifaximin, a non-absorbable antibiotic, demonstrated a trend towards higher remission rates in patients with active Crohn's disease.[49] Given that different antibiotics have variable effects on bile acid concentration and composition, as well as on IBD, antibiotic and patient selection will be important in future studies evaluating the efficacy of antibiotics in patients with IBD.[50]

In conclusion, chronic DCA administration resulted in intestinal dysbiosis associated with low intestinal FXR expression, and accelerated de novo synthesis of bile acid. As an FXR agonist, fexaramine is a promising therapeutic agent to treat bile acid-related intestinal inflammation by modulating dysregulated bile acid signalling. Adverse events associated with FXR agonist include pruritus.[51] Although the development of intestine-specific FXR agonist may potentially minimise the side effects, the long-term toxicity data are not currently available.[51] Furthermore, rather than targeting individual components, therapeutic interventions that modulate both gut microbiota and FXR may lead to a more robust effect in treating intestinal inflammation. Future research evaluating specific commensal microbiota and key metabolites which modulate intestinal inflammation will be invaluable.

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Conflict of Interest

The authors declare no conflict of interest.

Ethics Approval

This study was conducted with the approval of Institutional Animal Care and Use Committee at Zhejiang Chinese Medical University, Zhejiang, P. R. China.

Data Availability Statements

The data of this article will be shared on reasonable request to the corresponding author.

Author Contributions

Conceptualisation and funding acquisition: MQX; data curation: YQS and YBZ; formal analysis: MQX, YQS, and MSC; investigation: YQS, MSC, FLC, and XZ; methodology: YQS and YBZ, FLC and LLT; project administration: MQX, WLH, and ND; original draft: MQX and YQS; review and editing: MQX, JJK, WLH, and ND.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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