Clinical, experimental and pathophysiological effects of Yaq- 001: a non-absorbable, gut- restricted adsorbent in models and patients with cirrhosis

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Abstract

Objective Targeting bacterial translocation in cirrhosis is limited to antibiotics with risk of antimicrobial resistance. This study explored the therapeutic potential of a non-absorbable, gut-restricted, engineered carbon bead adsorbent, Yaq-001 in models of cirrhosis and acute-on-chronic liver failure (ACLF) and, its safety and tolerability in a clinical trial in cirrhosis.

Design Performance of Yaq-001 was evaluated in vitro. Two-rat models of cirrhosis and ACLF, (4 weeks, bile duct ligation with or without lipopolysaccharide), receiving Yaq-001 for 2 weeks; and two-mouse models of cirrhosis (6-week and 12-week carbon tetrachloride (CCl_4)) receiving Yaq-001 for 6 weeks were studied. Organ and immune function, gut permeability, transcriptomics, microbiome composition and metabolomics were analysed. The effect of faecal water on gut permeability from animal models was evaluated on intestinal organoids. A multicentre, double-blind, randomised, placebo- controlled clinical trial in 28 patients with cirrhosis, administered 4 gr/day Yaq-001 for 3 months was performed.

Results Yaq-001 exhibited rapid adsorption kinetics for endotoxin. In vivo, Yaq-001 reduced liver injury, progression of fibrosis, portal hypertension, renal dysfunction and mortality of ACLF animals significantly. Significant impact on severity of endotoxaemia, hyperammonaemia, liver cell death, systemic inflammation and organ transcriptomics with variable modulation of inflammation, cell death and senescence in the liver, kidneys, brain and colon was observed.

Yaq-001 reduced gut permeability in the organoids and impacted positively on the microbiome composition and metabolism. Yaq-001 regulated as a device met its primary endpoint of safety and tolerability in the clinical trial.

Conclusions This study provides strong preclinical rationale and safety in patients with cirrhosis to allow clinical translation

Trial registration number NCT03202498

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Current strategies to target bacterial translocation in cirrhosis are limited to antibiotics with risk of resistance.

WHAT THIS STUDY ADDS

- Yaq-001 rapidly adsorbs endotoxin, ammonia and bile acids and impacts positively on markers of gut permeability, liver injury, fibrosis, portal pressure, brain and kidney dysfunction in animal models of cirrhosis and reduces mortality from acute-on-chronic liver failure.
- In models of cirrhosis, Yaq-001 restores microbiome composition and reduces endotoxaemia, ammonia, severity of inflammation, liver cell death, signalling pathways and lipopolysaccharide sensitivity.
- Enhanced permeability of intestinal organoids following incubation with faecal water from cirrhosis animals is prevented by Yaq-001.
- In a multicentre, double-blind, randomised, placebo-controlled clinical trial of Yaq-001 versus placebo in patients with cirrhosis, Yaq-001 was found to be safe and well tolerated.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The data provide the preclinical rationale and clinical safety to proceed to the next phase of clinical trials in patients with cirrhosis aiming to prevent the occurrence of complications.

Introduction

Gut dysbiosis and gut-derived bacterial ligands, in particular endotoxin, drive a dysregulated inflammatory response, which has been implicated in the development of cirrhosis and its complications such as sepsis, spontaneous bacterial peritonitis, renal dysfunction and hepatic encephalopathy.[1–3] This dysregulated inflammatory response is also central to the development of acute-on-chronic liver failure (ACLF).[4] Markers of bacterial translocation such as endotoxin and bacterial DNA have been shown to be associated with complications of cirrhosis and diminished survival highlighting their pathogenic importance.[5–7] The microbiome in cirrhosis is characterised by reduced diversity and abundance of autochthonous bacteria.[1] While antibiotics have been shown to impact positively on complications of cirrhosis, their use is associated with antibiotic resistance.[8 9] Furthermore, antibiotics reduce bacterial diversity rendering the microbiome less resilient.

One of the consequences of bacterial translocation in cirrhosis is that the endotoxin-sensing pathways in different organs are known to be primed resulting in heightened susceptibility to organ injury.[3 10] Adsorption of free endotoxin without exerting direct effects on bacterial growth kinetics, therefore, has the potential to attenuate susceptibility to organ injury without producing the deleterious effects on the microbiome. Considering this, we developed a synthetic non-absorbable, non-antibiotic, endotoxin sequestrant and generated the hypothesis that this may be a novel therapeutic strategy to restore the microbiome, prevent bacterial translocation, systemic inflammation progression of fibrosis and cirrhosis complications. Yaq-001 is a gut-restricted, non-absorbable, highly engineered, activated carbon of multiple porosities tailored to the micro (<2 nm) and meso-macroporous (30–200 nm) range and has a high surface area.[11–13] These properties confer a high adsorptive capacity for larger biologically relevant molecules such as bacterial toxins in addition to smaller intraluminal targets. The most closely associated experimental oral adsorbent is

AST-120, a microporous carbon bead, which has not been shown to be efficacious in patients with hepatic encephalopathy.[14]

In this study, we sought to determine the adsorptive capacity of Yaq-001 and its effect on bacterial growth kinetics in *in vitro* studies. We then evaluated the *in vivo* biological effects of Yaq-001 in four animal models representing characteristics of fibrosis, cirrhosis and ACLF. We studied the effects of Yaq-001 on measures of multiorgan function, systemic and portal haemodynamics, immune function, multiorgan transcriptomics and microbiome composition. Finally, we performed a phase 2 equivalent, multicentre, double-blind, randomised, placebo-controlled clinical trial to assess the safety and tolerability of Yaq-001 in patients with decompensated cirrhosis.

Methods

Methodological details are described in online supplemental section.

Functional and structural characteristics of Yaq-001

Adsorption of biomolecules of varying molecular weights (albumin, myoglobin and caffeine) was evaluated. Bacterial growth was studied for *Staphylococcus aureus* and *Escherichia coli*. Scanning electron microscopy was performed to characterise the beads and pore size distribution was assessed using mercury porosimetry.

Studies in animal models

Study design

These studies aimed to characterise the therapeutic potential of Yaq-001 in rats and mice models to define its role in prevention of occurrence of cirrhosis, progression of cirrhosis and occurrence of ACLF (online supplemental figures S1 and S2).

Animal models

Four-week bile-duct ligation model of advanced fibrosis

1. Cirrhosis: Sham (n=36); Sham-Yaq-001 (n=30); bile duct ligation (BDL) (n=37); BDL-Yaq-001 (n=44).
2. Prevention of ACLF: Sham-lipopolysaccharide (LPS) (n=9); Sham-LPS-Yaq-001 (n=10); BDL-LPS (n=16); BDL-LPS-Yaq-001 (n=12).

Yaq-001 (0.4 g/100 g body weight per day) was administered for 2 weeks prior to sacrifice. At the time of sacrifice, mean arterial pressure (MAP) and portal pressure were measured.

Carbon tetrachloride treated model of cirrhosis

1. Advanced fibrosis and early cirrhosis (CCl4 for 6 weeks): control (n=6); control-Yaq-001 (n=6); CCl4 (n=12); CCl4-Yaq-001 (n=12).
2. Advanced cirrhosis (CCl4 for 12 weeks): control (n=6); control-Yaq-001 (n=6); CCl4 (n=12); CCl4-Yaq-001 (n=12).

Yaq-001 (0.4 g/100 g body weight per day) was administered from 0 to 6 weeks in the 6-week model and from 6 to 12 weeks in the 12-week model.

Collection and analysis of biosamples

Blood, stool and tissue samples were collected for later analysis. Portal venous blood was collected where possible. Peripheral blood cells and Kupffer cell reactive oxidant species (ROS) were measured. H&E, picrosirius red (PSR) staining and TUNEL stains were performed in liver tissues. The mRNA in different organs was analysed by using nSolver V.4.0 software (NanoString Technologies). To define effect on the microbiome, 16s microbiome study was performed. To determine the effect of Yaq-001 on modulating metabolism, urinary ^{1}H-NMR analysis was performed.

Assessment of gut permeability in intestinal organoids

Permeability of mouse intestinal organoids was detected using established protocols.[15 16] Faecal water generated from the stools obtained from the four groups of 6-week CCl4 mice was incubated with the organoids.[15 16] Permeability of the organoids was assessed.

Clinical trial of Yaq-001 versus placebo, CARBALIVE-SAFETY study

Study design

The CARBALIVE-SAFETY clinical trial was a first in man, multicentre, double-blind, randomised, placebo-controlled clinical trial of oral Yaq-001 in decompensated cirrhosis. Details of the study protocol are available in online supplemental section (CONSORT, online supplemental figure S3). As Yaq-001 is regulated as a device, it followed both ISO standards and ICH-GCP guidance. Informed consent was obtained from each patient. The study was closely monitored and overseen by an independent data safety monitoring board (NCT03202498).

Study design is described as online supplemental figure S4. The primary endpoint was assessed at 12 weeks. Blood and stool samples were taken at the time of randomisation, 4 weeks and 12 weeks for assessment of some of the secondary and exploratory endpoints. Safety assessments were performed on weeks 1, 4, 8 and 12 and comprised a physical examination, clinical laboratory tests, urinalysis, 12-lead ECG and an assessment of reported and observed adverse events. ECGs were analysed independently. Nutritional status was assessed by the Royal Free Hospital Global Assessment tool at each safety assessment together with micronutrient analysis at baseline, weeks 4 and 12. Vitamin B_{12}, A, D, E, folate, and K1 and, trace elements copper, zinc and selenium were analysed.

Main inclusion and exclusion criteria

The main inclusion criteria were participants aged 18 years or above, clinical diagnosis of diuretic-responsive cirrhotic ascites (Child-Pugh score=7–11 inclusive), abstinence from alcohol for at least 4 weeks prior to screening and written informed consent. The main exclusions were lack of informed consent, use of oral antibiotics, immunosuppressants or antiviral medication within 4 weeks prior to recruitment, change in dose of proton pump inhibitor therapy within 4 weeks before the start of the study treatment, hospital admission for liver-related indication for at least 4 weeks (except paracentesis), body mass index (BMI) >35 or BMI<18 kg/m^2 and the presence of a transjugular intrahepatic portosystemic shunt (see protocol in online supplemental file for details).

Randomisation, dosing and compliance

Patients were randomised 1:1 to receive 4 g of oral Yaq-001 or equivalent placebo nocte after dinner for 12 weeks. The interval between Yaq-001 and concomitant medication administration was 4 hours treatment compliance was assessed by the number of used or unopened sachets returned to the clinical site at each visit. Patients taking ≥70% of study medication were considered compliant.

Endpoints and assessments

Primary endpoints

The main objective of this clinical investigation was to assess the safety and tolerability of Yaq-001 throughout the 3 months' treatment period.

Secondary and exploratory endpoints

Blood and stool samples were collected for later analysis for markers of endotoxemia, systemic inflammation, bile acids, short-chain fatty acids, gut permeability and the microbiome (results not reported in this paper).

Statistical analysis

Animal studies

Based on the *in vitro* studies, we anticipated a 50% decrease in circulating endotoxin in the treatment groups with an alpha error of 0.05 and power of 80%, resulting in a minimum sample size of 5 animals/group. As this study included several pathophysiological endpoints,

multiple experimental groups were included. All the data accrued from these studies are described in this paper. All the rats in eight groups from three independent batches were included in the analysis as shown in online supplemental figure S1. All the mice studied in eight groups were included in online supplemental figure S2.

Group comparisons for continuous variables were performed using Man-Whitney U test (no-normal distribution) or unpaired t-test (normal distribution) and for categorical variables by using χ^2 test. The data were analysed using R package (R V.4.4.4). 16s microbiome study and circos correlation were analysed by using Wilcoxon rank sum test and Spearman correlation. Software used Graphpad Prism V.9.0 (GraphPad software, San Diego, California, USA).

CARBALIVE-SAFETY clinical trial

This first-in-man clinical investigation was not powered to demonstrate statistical significance for any endpoint. All statistical analyses of study data were carried out using SAS V.9.3 or a later version. For categorical variables, summary tabulations of the number and percentage of patients within each category (with a category for missing data) of the parameter are presented. Percentage calculations are based on non-missing data unless otherwise specified. Please also see protocol (online supplemental file).

Results

Functional and structural characteristics of Yaq-001

Yaq-001 beads exhibited a consistent predefined structure with a bead diameter within the 250–500 µm range and the prescribed porosity (online supplemental figure S5A). Yaq-001 rapidly adsorbed albumin (66.5 kDa), myoglobin (16.7 kDa) and caffeine (0.194 kDa) representing different sized biomolecules (online supplemental figure S5B). Yaq-001 adsorbed LPS (18 kDa) reducing the concentrations from 2.5 to 1.5 EU/mL (60%) within 30 min. No endotoxin was detected in the control solution (0 EU/mL) (online supplemental figure S5B). Yaq-001 also adsorbed a range of bile acids (online supplemental figure S5C). Direct coincubation of Yaq-001 with bacterial suspensions of either *E. coli* or *S. aureus* indicated that Yaq-001 did not affect bacterial growth kinetics for either species following direct contact in comparison to the antibiotic controls (online supplemental figure S5D). Mercury porosimetry showed that Yaq-001 used in the clinical trial had a consistent pore size distribution plot in the meso-macroporous range from 30 to 200 nm (online supplemental figure S5E).

Yaq-001 exhibited better performance in adsorptive capacity and effect on endotoxin kinetics than AST-120 (Kremezin, Kureha, Japan) (online supplemental figure S5).

Studies in animal models

Studies in BDL rat model of advanced fibrosis

Effect of Yaq-001 on liver injury and portal pressure

BDL rat model was used to assess the effect of Yaq-001 in cirrhosis (figure 1A). Significant reduction in 4-week body weight was observed in BDL rats (p<0.0001), which was prevented by administration of Yaq-001 (p=0.045) (figure 1A). Yaq-001 was associated with a significantly lower plasma ALT (p=0.007). ALP, TBIL and albumin were not impacted by Yaq-001 (online supplemental figure S6A–C). Total bile acid concentrations were not different between the BDL and Sham groups and there was no significant impact of Yaq-001 (online supplemental figure S6E). Mean arterial pressure (MAP) was lower in BDL animals and no effect of Yaq-001 was observed (online supplemental figure S6F). Yaq-001 resulted in a significant reduction in portal pressure compared with untreated BDL rats ((median (IQR) 11.1 mm Hg (10.3–11.7) vs 12.4 mm Hg (10.8–13.3), (p=0.025)) (figure 1A). TUNEL assay showed significantly more intense staining in the liver tissue of BDL compared with Sham rats (figure 1A) (p<0.0001), which were significantly reduced in Yaq-001-treated BDL rats compared with untreated-BDL rats (p=0.025). Collagen proportionate area (CPA) was

significantly higher in BDL rats (p=0.0007) compared with Sham rats, which was unchanged with Yaq-001 (p=0.122) (online supplemental figure S6D).

Effect of Yaq-001 on ammonia, organ dysfunction, endotoxaemia and bacterial translocation
Ammonia: Arterial and portal venous ammonia concentrations were significantly increased in BDL rats (p<0.0001), which were significantly reduced by Yaq-001 ((p=0.003) and (p=0.004), respectively) (figure 1A). None of the animals showed signs of overt hepatic encephalopathy.

Kidneys: BDL animals had significantly higher plasma creatinine (p=0.049), which was significantly reduced with Yaq-001 (p=0.025) (figure 1A). Urea was higher in BDL group (p=0.092), which was reduced with Yaq-001 treatment (p=0.095) (figure 1A).

Gut permeability, endotoxaemia, bacterial DNA and cytokines: The microbial metabolite, D-lactate, a marker of gut-specific intestinal barrier damage and translocation[16] was significantly increased in BDL rats (p=0.032) and was significantly reduced by Yaq-001 (p=0.035) (figure 1A). BDL rats exhibited marked endotoxaemia in the portal vein and the artery (p<0.0001 for each), which was significantly reduced with Yaq-001 ((p<0.0001) (p=0.003), respectively) (figure 1A). Portal venous bacterial DNA was detectable in significantly higher number of BDL rats (p<0.05), which was markedly reduced in Yaq-001 administered BDL rats (p=0.08) (figure 1A). Plasma IL- β concentration was higher in the BDL rats but no significant differences were observed in TNF-a, IL-6 and IL-10. No significant changes were seen with Yaq-001 (online supplemental table S1).

Studies in the BDL model of ACLF
This experiment was performed to determine whether Yaq-001 treatment for 2 weeks prevents the occurrence of ACLF when BDL animals are administered LPS (online supplemental figure S1, figure 1B).

Survival: Animals were sacrificed either at coma stages (considered as a surrogate for mortality) or at 6 hours post-LPS. Yaq-001 significantly impacted on time to coma of BDL-LPS rats compared with untreated controls (p<0.01) (figure 1B). All animals in the two Sham groups were alive at 6 hours following LPS (data are not shown).

Liver: Yaq-001 was associated with significantly lower ALT in BDL-LPS rats compared with untreated rats (p=0.004) (figure 1B). No significant effect of Yaq-001 was observed on ALP, TBIL and albumin (online supplemental figure S7A–C). The severity of fibrosis measured using CPA and the body weight was unchanged (online supplemental figure S7D,E).

Systemic and portal haemodynamics: No significant difference in MAP was observed between the groups treated with or without Yaq-001 (online supplemental figure S7F) but Yaq-001 produced a significant reduction in portal pressure in BDL-LPS animals compared with the untreated group (p=0.003), (figure 1B).

Brain: Yaq-001 significantly reduced brain water in BDL-LPS compared with the untreated group (p=0.017) (figure 1B). Arterial and portal venous ammonia concentrations were significantly increased in BDL-LPS rats, which were significantly reduced in Yaq-001-treated animals ((p=0.007) and (p=0.017) respectively) (figure 1B).

Kidneys: Creatinine concentration was significantly higher in BDL-LPS animals (p=0.004), which was significantly reduced by Yaq-001 (p=0.03) (figure 1B).

Cytokines: BDL-LPS group had a significantly higher plasma IL-1β, which was significantly reduced with Yaq-001 (p=0.003). Plasma IL-10 was higher in BDL-LPS and was significantly reduced with Yaq-001 (p=0.028) (figure 1B). No significant differences were observed in IL-6 or TNF-α concentrations between any of the groups (online supplemental table S1).

Effect of Yaq-001 on peripheral blood cells and Kupffer cells

Significant increase in total leucocyte, neutrophil and monocyte counts in the artery and portal vein was observed with BDL rats (online supplemental figure S8A,B) (p=0.008 and p=0.016, respectively), which were significantly reduced with Yaq-001 in the arterial blood and insignificantly reduced in the portal vein (online supplemental figure S8B). To determine whether Yaq-001 impacts on the response of peripheral inflammatory cells and Kupffer cells to generate reactive oxygen species (ROS) to LPS ex vivo, studies using isolated cells incubated with LPS, were performed. Yaq-001 was associated with significantly lower LPS-induced ROS production in CD163− Kupffer cells in BDL rats (p=0.036) and portal venous $CD43^{hi}$ monocyte populations of BDL rats (p=0.029) (online supplemental figure S8C).

Transcriptomic analysis of gene expression profiles from the liver, colon, brain and kidneys

Multiorgan transcriptomic analysis was performed to determine the possible molecular mechanisms underlying the clinical effects of Yaq-001. The four groups studied were as follows: Sham (n=3), Sham-Yaq-001 (n=3), BDL (n=3) and BDL-Yaq-001 (n=4) (figure 2A). All differentially expressed genes (DEGs) and related pathways in the liver, colon, kidney and brain are listed in online supplemental table S2. The top 20 and significant DEGs are listed in online supplemental table S3.

Effect of Yaq-001 on gene expression profiles in the liver and gut in BDL rats

Liver: Analysis of liver tissue showed 82 DEGs at the threshold of 1.2-fold change and p=0.1 in the four groups (figure 2B). Compared with the Sham group, expression of 62 genes was upregulated, and 15 genes were downregulated in BDL. These significantly changed genes were associated with inflammation, cell death and senescence. Compared with the untreated BDL group, the expression of 7 genes was upregulated and 12 genes were downregulated in the Yaq-001-treated BDL group, indicating the potential role of Yaq-001 in reducing inflammation, cell death and cellular senescence. Furthermore, two genes were upregulated, and four genes were downregulated in Sham-Yaq-001 group in comparison to Sham group (figure 2C). Functional analysis demonstrated that BDL rats had enriched pathways related to inflammation, cellular senescence, cell death, TLR signalling and other related signalling pathways in comparison with Sham (online supplemental figure S9A). Yaq-001 treatment targeted the altered pathways compared with untreated BDL group. Additionally, Yaq-001 treatment also changed the pathways in the liver when compared with Sham group, demonstrating its effect in rats even without cirrhosis (online supplemental figure S9A).

Colon: 43 DEGs were identified from the colonic tissue (figure 2D). Five genes that correlated with inflammation and cell death were upregulated and 15 genes were downregulated in BDL compared with the Sham group. Moreover, the expression of 10 genes were upregulated, and 13 genes were downregulated with Yaq-001 treatment. Only 1 gene was upregulated in the Sham-Yaq-001 group, and 16 genes were downregulated with Yaq-001 compared with the untreated Sham group (figure 2E). Functional analysis indicated that inflammation, cellular senescence, cell death, TLR signalling and intracellular signalling were associated with BDL in comparison with the Sham group (online supplemental figure S9B). Yaq-001 targeted the altered pathways, indicating the potential mechanisms in the prevention of gut dysfunction and permeability (online supplemental figure S9B).

Effect of Yaq-001 on gene expression profiles in the brain and kidney in BDL rats

Brain: 17 DEGs were identified from the brain tissue (figure 2F). Compared with Sham group, expression of 2 genes were upregulated and 13 genes were downregulated in BDL animals. These significantly changed genes were associated with inflammation, cell death and cellular senescence. Compared with the untreated-BDL group, the expression of five genes were upregulated and two genes were downregulated in the Yaq-001-treated BDL group (figure 2G). Functional analysis demonstrated that BDL rats had enriched pathways related to inflammation, cellular senescence, cell death, TLR signalling and intracellular

signalling (online supplemental figure S9C). Yaq-001 targeted cytokine–cytokine receptor interaction, cytosolic DNA-sensing pathway, TLR signalling pathway, NOD-like receptor signalling pathway, neutrophil extracellular trap formation, TGF-beta signalling pathway and cytokine-cytokine receptor interaction pathways compared with untreated-BDL group (online supplemental figure S9C).

Kidneys: 30 DEGs were identified from kidney tissue (figure 2H). Nine genes that correlated with inflammation were downregulated in BDL. The expression of five genes was upregulated and four genes were downregulated with Yaq-001 treatment compared with untreated-BDL group. Five genes were upregulated in Sham-Yaq-001 group, and three genes were downregulated with Yaq-001 compared with untreated-Sham group (figure 2I). Functional analysis indicated that inflammation and TLR signalling were associated with BDL in comparison with Sham (online supplemental figure S9D). Compared with the untreated-BDL group, Yaq-001 targeted the altered pathways, indicating the potential mechanisms in the prevention of renal dysfunction (online supplemental figure S9D).

Effect of Yaq-001 on the gut microbiome profile

The effects of Yaq-001 on the microbiome bacterial composition were assessed by metataxonomics. At the family level, an abundance of six bacteria was significantly changed at the threshold of twofold change and *Porphyomonadaceae* was significantly changed ($p<0.05$) comparing BDL with Sham (figure 3A). At genus level, 19 bacteria were significantly changed in abundance. *Barnesiella* was significantly changed ($p<0.05$) comparing BDL with Sham group (figure 3B). These changes were reversed with Yaq-001 treatment compared with untreated-BDL rats (online supplemental figure S10A,B, online supplemental table S4 and online supplemental figure S10C,D). For between-groups sample diversity, PERMANOVA analysis revealed a significant difference in beta diversity between groups ($R^2=0.32$, $p=0.001$). Yaq-001 appeared to moderately restore the beta diversity in the BDL group especially in PCoA2 axis (online supplemental figure S10E,F).

To further investigate the potential importance of the changes in the microbiome induced by Yaq-001, we correlated these with all significantly changed DEGs and the top 20 DEGs in the four organs. Circos plots indicated a significant correlation between them (figure 3C,D and online supplemental figure S11A–C). *Porphyromonadaceae* was observed to positively correlate with three DEGs—TGFB2 and CASP1 in liver tissue, and FOS in colonic tissue. Also, it correlated negatively with five DEGs—TGFB2, IL-18 and CCR5 in brain tissue, CXCL10 in colon tissue and CCL24 in kidney tissue.

Effect of Yaq-001 on metabolomic profile

Significant difference in acetate/creatinine, glycine/creatinine, lactate/creatinine, betaine/creatinine, trimethylamine oxide/creatinine and bile acid/creatinine ratio were observed in BDL compared with Sham. Treatment of BDL rats with Yaq-001 resulted in significant resolution of acetate/creatinine, glycine/creatinine and lactate/creatinine compared with the untreated BDL animals (online supplemental figure S12).

Studies in CCl4 mice

Effect of Yaq-001 on liver injury and fibrosis

CCl4 mice models (6 weeks and 12 weeks) were used to further confirm the effect of Yaq-001 on liver injury and fibrosis in models of cirrhosis (figure 4A,B). Yaq-001 was associated with a significantly lower plasma ALT ($p<0.0001$, $p<0.0001$) in both 6-week and 12-week CCl4 models. ALP and TBIL were reduced by Yaq-001 in 6-week CCl4 mice ($p=0.040$, $p=0.001$) (figure 4A,B). CPA was significantly higher in both CCl4 mice compared with control animals ($p=0.0001$, $p=0.0001$), which were significantly reduced with Yaq-001 ($p=0.024$, $p=0.012$) (figure 4A,B). TUNEL assay showed significantly more intense staining in the liver tissue of CCl4 compared with control mice ($p<0.001$, $p<0.001$), which were

significantly reduced in Yaq-001-treated CCl4 mice compared with untreated-CCl4 mice (p=0.021, p=0.017) (figure 4A,B).

Effect of Yaq-001 on ammonia, organ dysfunction and endotoxaemia

Ammonia: Ammonia concentrations were significantly increased in the 6-week and 12-week CCl4 mice compared with controls (p=0.002, p=0.001), which were significantly reduced by Yaq-001 (p=0.025, p=0.035) (figure 4A,B). None of the animals showed signs of overt hepatic encephalopathy.

Kidneys: Higher plasma creatinine was significantly reduced by Yaq-001 treatment (p=0.005, p=0.003) in 6-week and 12-week CCl4 animals (figure 4A,B).

Endotoxaemia: Both 6-week and 12-week CCl4 mice exhibited marked endotoxaemia compared with controls (p=0.007, p=0.007), which were significantly reduced with Yaq-001(p=0.007, p=0.043) (figure 4A,B).

In vitro studies in intestinal organoids to assess gut permeability

Intestinal organoids were successfully derived and cultured from small intestine of C57BL/6 mice. Intestinal organoids underwent eversion into apical-out polarity in the first 12 hours of suspension culture and collected for identification and subsequent experiments (figure 5A). Immunostaining of the microvilli (mv; F-actin) demonstrated that intestinal organoids in suspension had reversed polarity such that the apical surface faced outward (figure 5A). Apical-out intestinal organoids possessed goblet cells, which were identified with MUC2 staining (figure 5B). Gut permeability of apical-out intestinal organoids was significantly increased by coculturing with faecal water from CCl4 group compared with the control group (p=0.003) (figure 5C,D). The gut permeability was significantly decreased with faecal water from Yaq-001 treated CCl4 animals compared with the CCl4 group (p=0.001) (figure 5C,D).

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CARBALIVE-SAFETY clinical trial

The data regarding safety and tolerability are reported here. Other secondary and exploratory endpoints will be described elsewhere.

Patient characteristics

34 patients were screened for this study at 8-European centres. 28 patients met the study entry criteria and were randomised to either active or placebo groups. Six patients screened did not meet the study entry criteria. Dosing was not initiated in two patients randomised to placebo due to withdrawal of consent (online supplemental figure S3, CONSORT). Three patients were included for the second dosing cohort of 8 g. This part of the study was terminated prematurely due to the coronavirus pandemic with none of the patients completing the study duration (data are not included).

In accordance with study entry criteria, all patients had cirrhosis with diuretic-responsive ascites and Child-Pugh score of 7–8. The baseline demographics were similar across treatment groups. The ratio of male to female patients was reflective of the disease state. Compliance in the active and placebo groups was 92.9% and 66.7%, respectively (table 1).

Safety and tolerability

Of the 14 patients enrolled in the Yaq-001 treatment group, 13 (93%) completed 12 weeks of therapy. The median duration of exposure was 83 (6–94) days. 10 of the 12 (83%) patients who received placebo completed the treatment. The median duration of exposure was 83 (14–86) days. No deaths or serious adverse events were reported in the study. The difference in treatment-emergent adverse events (TEAEs) in patients treated with Yaq-001 and those treated with placebo is presented in table 2. The most frequently reported TEAEs were gastrointestinal in nature in both the active and placebo groups. Of these, only constipation and diarrhoea were evaluated by the clinical investigator as possibly related to the investigational product. One placebo-treated patient withdrew from the study due to diarrhoea.

Across both treatment groups, 40/51 (78%) of the reported TEAEs were evaluated by the clinical investigator as not related or unlikely related (32/38; 84% for the active treatment group; 8/13; 62% for the placebo group). The incidence of adverse events reported was reflective of the targeted subject population for this clinical investigation. The majority of the TEAEs reported were not considered by the clinical investigator to be related to treatment and were mild in intensity. Systemic antibiotics were administrated for the following TEAEs in the active arm: amoxicillin—acute bronchitis; clarithromycin—acute bronchitis; phosphomycin—urinary tract infection. None of these infections were related to the administration of the investigational product. Drugs received by the patients at the time of randomisation and during follow-up are listed in online supplemental tables S5 and S6. Treatment-emergent, clinically significant laboratory abnormalities are listed in table 3. None were deemed treatment related by the investigator.

Clinical, haematological and biochemical variables

The data are summarised in table 3. No significant changes in any of the clinical parameters were observed in any of the groups. Although there was a trend towards a reduction in the white cell count and C reactive protein in the Yaq-001 group, the differences were not statistically significant.

Nutritional status

The data are summarised in table 3. No significant differences were observed in either treatment group with regard to global nutritional status, vitamin B_{12} and folate, vitamin A or E, or copper zinc, and selenium. Median vitamin A, zinc and baseline vitamin D concentrations were below the limit of normal range but no differences between treatment groups were observed. No changes were observed in any of the micronutrient parameters with treatment and these were evenly matched between groups. Any baseline abnormalities were attributable to the underlying cirrhosis.

Discussion

The results of the study showed that Yaq-001 prevented progression of liver injury and fibrosis in animal models of cirrhosis and significantly reduced the mortality of ACLF animals. This effect of Yaq-001 on ACLF mortality will need to be confirmed in patients. This was

associated with positive impact on markers of gut permeability, liver injury, portal pressure, brain and kidney dysfunction. These pleiotropic effects of Yaq-001 were associated with partial restoration of the composition of the microbiome bacterial community, reduction in the severity of endotoxaemia, ammonia, inflammation, cell death, signalling pathways and LPS sensitivity. A phase 2 equivalent, multicentre, double-blind, randomised, placebo-controlled clinical trial in patients with cirrhosis confirmed regulatory compliance and, safety and tolerability of Yaq-001, thereby, providing evidence of clinical translatability. The data provide the rationale to proceed to further clinical trials.

Translocation of bacteria, its products and metabolites are critically important in the progression of hepatic fibrosis and pathogenesis of complications of cirrhosis.[1 17–20] Indeed, selective gut decontamination using norfloxacin or rifaximin is the current standard of care for secondary prophylaxis of patients with spontaneous bacterial peritonitis and hepatic encephalopathy, respectively.[21 22] However, the use of these antibiotic strategies is limited to patients with advanced cirrhosis and induces the risk of antibiotic resistance.[23] The data presented here provide a safe, gut-restricted, non-antibiotic strategy, Yaq-001, which has the potential to diminish translocation and prevent the progression of hepatic injury, fibrosis and prevent extrahepatic organ injury in models of cirrhosis. The *in vitro* studies demonstrate that Yaq-001 has the optimal pore size distribution to bind intraluminal factors such as free endotoxin. We also tested *in vitro* bacterial growth kinetics of two species, which were not affected by Yaq-001, an observation that was subsequently confirmed in the studies in the BDL animal model where no change diversity was observed.

Endotoxaemia has been implicated in immune dysfunction resulting in a dysregulated systemic inflammatory response, which is strongly associated with the progression of fibrosis, cirrhosis and occurrence of ACLF.[24 25] Yaq-001 reduced the severity of endotoxaemia and bacterial DNA positivity, which was associated with attenuated systemic inflammation. Significant improvements in LPS-induced ROS production were observed in trafficking portal venous monocytes suggesting that Yaq-001 had attenuated the primed state of monocyte/macrophage populations within the gut–liver axis. This observed reduction in LPS-induced ROS production may be important in explaining the reduction in plasma IL-1β in LPS-treated BDL rats.

Plasma D-lactate, a marker of increased gut permeability was reduced in the Yaq-001 treated BDL rats.[26] Elevated plasma D-lactate levels in cirrhosis are associated with decompensation.[22] Transcriptomic analysis of colonic tissue demonstrated upregulation of genes associated with necroptosis, apoptosis and inflammation in BDL animals. Functional analyses pointed to the modulation of colonic inflammation by Yaq-001, IL-17 signalling, which is known to have diverse biological functions, promoting protective immunity against many pathogens, neutrophil recruitment, antimicrobial peptide production and enhanced barrier function.[27 28] To further validate the potential effect of Yaq-001 in modulating gut permeability, we performed experiments in intestinal organoids that were incubated with faecal water.[29] The data confirmed that even in *in vitro* settings, faecal water obtained from the faeces of CCl4-induced cirrhosis animals enhanced permeability of the organoids, which was prevented in the faecal water obtained from animals that were treated with Yaq-001. The data support the hypothesis that Yaq-001 impacts on the factors in the gut responsible for increasing gut permeability in cirrhosis.

Yaq-001 significantly reduced the severity of liver injury and portal hypertension in both the BDL and BDL-LPS models of cirrhosis and ACLF. The lack of significant differences in CPA between untreated and Yaq-001-treated BDL groups suggests that the reduction in portal pressure is possibly due to modulation of the dynamic component of portal hypertension, in which inflammation is known to play a role and proposes Yaq-001 as a potential treatment for portal hypertension.[30 31] Reduction in ALT levels and TUNEL staining confirmed a reduction in liver injury in the Yaq-001 treated animals. The reduction in liver injury in the LPS treated BDL animals suggests that Yaq-001 has a particular effect on endotoxin

sensitivity *in vivo*. This hypothesis was tested in isolated Kupffer cells, which confirmed that LPS-induced ROS production was significantly impacted by Yaq-001 treatment.

Transcriptomic analysis of liver tissue demonstrated that the upregulated genes, CXCL16, CASP1 and TGFB2 in BDL rats was prevented by Yaq-001 administration. Silencing of CXCL16 alleviates hepatic ischaemia reperfusion injury and CXCL16 variant is also associated with hepatitis B virus-related acute liver failure.[32] CASP1 mediates proinflammatory cytokine release and pyroptotic cell death in cirrhosis and its inhibition has been shown to prevent ACLF.[33] TGFB2 is an important mediator of cellular senescence.[34 35] Of note, Yaq-001 also modified necroptosis and cytosolic DNA-sensing pathways representing cell death, which are known to be activated by LPS and can trigger systemic inflammation.[36] These effects of Yaq-001 potentially explain the effect of Yaq-001 in reducing liver injury.[33 37]

Yaq-001 administration had a significant impact on time to coma of ACLF rats, which is considered a surrogate for mortality compared with untreated controls. Whether it impacts truly on mortality of ACLF animals will require more confirmation. Yaq-001 also significantly lowered portal venous and arterial ammonia levels, which was associated with reduced brain water. Transcriptomic analysis of brain tissue showed that IL-18, TGFB2, CCR5 and IL-23A were dysregulated in BDL rats and these were corrected by Yaq-001. IL-18 is released during pyroptosis by activation of the inflammasome complex in neuroinflammatory and neurodegenerative diseases.[38] The effect of Yaq-001 on TGFB2 may mean that it has an impact on cellular senescence, which is known to be associated with hepatic encephalopathy. CCR5 has been implicated in neuroprotection and is a novel therapeutic target in stroke.[39] The impact of Yaq-001 on IL-23A indicates possible reduction in neuroinflammation.

In both cirrhosis and ACLF models, Yaq-001 reduced renal dysfunction. Transcriptomic analysis of kidney tissue showed that CCL24 was downregulated in BDL rats, which was prevented in the Yaq-001-treated animals. CCl4 protects renal function in the development of early diabetic nephropathy by exerting an anti-inflammatory effect.[40] Yaq-001 impacted, on the cytokine-cytokine receptor interactions and chemokine and toll-like signalling pathways, which were abnormal in the BDL rats.

BDL animals become sarcopenic and lose weight, which were significantly abrogated by Yaq-001.[41] The possible mechanisms underlying this effect are likely multifactorial.[42] Yaq-001 reduced ammonia significantly, which has been shown to induce sarcopenia.[43] Weight loss in cirrhosis is also attributed to increased catabolic state in the context of systemic inflammatory response and thus the observed improvement in body weight may reflect the diminished catabolic state with reduced inflammation.[42]

The clinical effects of Yaq-001 observed in the BDL models were validated in the CCl4-induced liver injury animal models. Two models were studied. In the first (6-week model), Yaq-001 was administered in a preventative mode starting its administration with the onset of liver injury during administration of CCl4. The results showed significant reduction in the severity of liver injury, fibrosis and progression to cirrhosis, endotoxaemia, creatinine and ammonia levels. In the second (12-week model), Yaq-001 was administered starting at 6 weeks when the animal already had advanced fibrosis/cirrhosis. Again, significant reduction in markers of liver injury, fibrosis, endotoxaemia, creatinine and ammonia were observed. Extrapolating these observations to humans, the results from the 6-week model suggest that Yaq-001 may be useful to prevent the progression of fibrosis in patients without cirrhosis and, from the 12-week model, the possibility of prevention of progression of liver disease in those with well-compensated cirrhosis.

Gut microbiota are important in modulating intestinal health, permeability, bacterial translocation, systemic inflammation and complications of cirrhosis.[44–46] BDL was associated with marked changes in the abundance of microbiota, which were reversed by Yaq-001. In

particular, the abundance of *Porphyromonadaceae* and *Barnesiella* were significantly elevated in BDL rats and significantly decreased with Yaq-001. This change is potentially important as *Porphyromonadaceae* is a proinflammatory bacterium that has been positively correlated with hepatic encephalopathy and, *Barnesiella* and *Porphyromonadaceae* have been associated with liver cancer.[47–49] Urinary nuclear magnetic resonance (NMR) analysis reflects the combined metabolic status of both host and microbiota. Yaq-001 was associated with a distinct shift of acetate, glycine and lactate in metabolomic profile in BDL rats. These metabolites are generated by mixed acid fermentation (MAF), typically by bacteria such as Enterobacter. MAF is not the preferred metabolic pathway for facultative anaerobes and may be indicative that Enterobacter populations are under conditions of metabolic stress in Yaq-001 treated BDL animals. As these species are often pathogenic in cirrhosis, this may represent a beneficial change. However, the exact mechanisms by which the change in the microbiome results in improvement in distant organ function and gene expression cannot be directly inferred from the data derived from this study. One possibility is that alongside LPS adsorption and modulation of other unmeasured toxins, the milieu of the gut is changed allowing proliferation of more autochthonous bacteria, which impacts on gut inflammation and reduces gut permeability.[46] This hypothesis is supported by the organoid experiments. Reduction in permeability would result in a reduction in endotoxaemia, systemic inflammation, improvement of organ function and LPS-sensitivity. In this study, most of these changes have been described individually but whether this is happening in sequence has not been investigated.

As Yaq-001 is completely excreted unchanged in the stool, it is regulated in Europe as a device. Therefore, the clinical trial was performed both according to ISO standards and ICH-GCP guidance. The results of this first-in-man, multicentre, double-blind, randomised, placebo-controlled clinical trial suggested that oral Yaq-001 at a dose of 4 g nocte was well tolerated with a favourable safety profile. Despite the rapid adsorption kinetics for bacterial toxins and metabolites, Yaq-001 treatment had no negative impact on micronutrient levels or on nutritional profile as assessed by the gold standard Royal Free Global Assessment tool. These data must be interpreted keeping in mind that Yaq-001 was administered postprandially, separated from drugs by 4 hours as necessitated by the protocol. It is important to note that the studies were performed in stable cirrhosis patients, many of whom had minimal evidence of systemic inflammation and therefore, any clinical effect of this intervention was difficult to gauge. However, future analysis of the available samples from the blood and stool will provide answers as to whether Yaq-001 modulates the gut microbiome, inflammation and endotoxaemia.

These results must be considered in view of some limitations. First, the rodent microbiome is not directly analogous to the human and further clinical studies will be required to verify the effects on the gut microbiome's bacterial composition. Second, although Yaq-001 was effective in adsorbing a variety of bile acids *in vitro* and reduced bile acids significantly in Sham animals, no impact on bile acids was seen in BDL animals. This possibly reflects the effect of the BDL model, where no increase in bile acids was observed. Also, no changes in bile acids were observed in CCl4 animals but these animals did not have elevated bile acid either. Further studies on effect of Yaq-001 bile acids both in the circulation and stool need to be performed to confirm safety. Third, although, Yaq-001 was observed to impact positively on the gene expression profiles of multiple pathways, their exact relevance at the protein or cellular level has not been explored. Additionally, the limited genes in the different organs assessed in this study are a potential source of bias as they focused on pathways likely to be impacted by Yaq-001. Thus, further studies using unbiased approaches should be performed to define the true effect of Yaq-001. Fourth, although survival benefit was observed with prior Yaq-001 administration in the ACLF model, further studies are needed to confirm this finding. Fifth, as only one dose of Yaq-001 was tested in the clinical trial, further dose-ranging studies will be needed to define optimal dosing for safety and efficacy. However, the animal toxicity studies that were performed by an independent laboratory for

regulatory purposes, showed evidence of safety in much larger doses than that administered in the present studies (summary in online supplemental file). Finally, although Yaq-001 was safe and tolerable, further analyses are needed to clarify its clinical effects in patients.

In conclusion, the data provide compelling evidence for the potential of Yaq-001 as a novel gut-restricted adsorbent targeting endotoxin and ammonia that impacts on the gut microbiome, gut permeability, systemic inflammation, liver injury and fibrosis and, organ function in models of cirrhosis and improves survival in ACLF. The placebo-controlled clinical trial of Yaq-001 in cirrhosis patients provides evidence of safety and tolerability allowing translation to the next phase of clinical studies to define its potential as a novel therapeutic for patients with cirrhosis.

Data availability statement

Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as online supplemental information.

Ethics statements

Patient consent for publication

Not applicable.

Ethics approval

This study involves human participants and was approved by UK: South West—Exeter Research Ethics Committee; Ref No. 17/SW/0144France: Le Comité de Protection des Personnes Sud Méditerranée II, agréé par arrêté: Ref No. 17/SW/0144Portugal: Assunto: Estudo Clinico com Intervenao de Dispositivo Medico com o Protocolo n2 Yaq001-S- 001 e Codigo CEIC 1712GK893. Participants gave informed consent to participate in the study before taking part.

Acknowledgments

We would like to thank Fraser Simpson (Department of Genetics, Evolution and Environment, University College London) for his support to perform the Nanostring. The urinary NMR studies were facilitated by a Medical Research Council research grant under the High-throughput 'omic' Science and Imaging funding scheme (MC_PC_13045). University of Brighton coauthors acknowledge Prof Sergey Mikhalovsky and Dr Carol Howell in discussion and research leading to the CARBALIVE grant. The authors would like to thank all the patients and public who provided input into designing the study and those that took part in the study.

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Figure 1

Effect of Yaq-001 on organ dysfunction, endotoxaemia and bacterial translocation in BDL and ACLF rats. (A) Rats underwent bile duct ligation for 4 weeks as a model of cirrhosis (n=23–37/group). Treatment groups received Yaq-001 for 2 weeks before sacrifice. (A) 4-week body weight in four groups: Sham (n=31), Sham-Yaq-001 (n=24), BDL (n=31) and BDL-Yaq-001 (n=38). Significantly lower final body weights were observed in BDL compared with Sham controls ($p<0.001$). Yaq-001-treated BDL rats had a significantly higher body weights compared with untreated-BDL rats ($p<0.05$). Plasma alanine transaminase (ALT) concentrations in Sham (n=17), Sham-Yaq-001 (n=14), BDL (n=17) and BDL-Yaq-001 (n=26) groups and Portal pressure (PP) measurements in Sham (n=17), Sham-Yaq-001 (n=19), BDL (n=14) and BDL-Yaq-001 (n=26) groups. Significantly higher ALT and PP were observed in BDL compared with Sham controls ($p<0.0001$). Yaq-001-treated BDL rats had a significantly lower ALT and PP compared with untreated-BDL rats ($p<0.01$, $p<0.05$). TUNEL assay of liver tissue with quantification of staining by digital image analysis. Significantly higher TUNEL staining was observed in BDL compared with Sham controls ($p<0.0001$). Yaq-001-treated BDL rats had a significantly lower TUNEL staining compared with untreated-BDL rats ($p<0.05$) indicative of a reduction in liver cell death with Yaq-001 treatment. Arterial ammonia concentrations in Sham (n=7), Sham-Yaq-001 (n=5), BDL (n=19), BDL-Yaq-001 (n=21) groups and Portal venous ammonia concentrations in Sham (n=6), Sham-Yaq-001 (n=5), BDL (n=13), BDL-Yaq-001 (n=18) groups. Significantly increased arterial ammonia concentrations and portal venous ammonia concentrations were observed in BDL compared with Sham controls ($p<0.0001$, $p=0.0001$). Yaq-001 significantly decreased arterial and portal venous ammonia concentrations in BDL rats ($p<0.01$ for both). Serum creatinine in Sham (n=19), Sham-Yaq-001 (n=17), BDL (n=20), BDL-Yaq-001 (n=17) and serum urea in Sham (n=28), Sham-Yaq-001 (n=23), BDL (n=30), BDL-Yaq-001 (n=34) groups. Yaq-001 markedly decreased serum creatinine levels in BDL rats ($p<0.05$). Plasma D-lactate in Sham (n=7), Sham-Yaq-001 (n=8), BDL (n=6), BDL-Yaq-001 (n=7). Plasma D-lactate was significantly increased in the BDL group compared with Sham animals ($p<0.05$). Yaq-001 resulted in a significant reduction in plasma D-lactate in BDL rats ($p<0.05$). Portal venous endotoxin (Sham (n=6), Sham-Yaq-001 (n=5), BDL (n=12) and BDL-Yaq-001 (n=7)) and arterial endotoxin (Sham (n=6), Sham-Yaq-001 (n=5), BDL (n=12) and BDL-Yaq-001 (n=7)). Portal venous bacterial DNA positivity (Sham (n=6), Sham-Yaq-001 (n=5), BDL (n=12) and BDL-Yaq-001 (n=13)) and arterial plasma bacterial DNA positivity (Sham (n=6), Sham-Yaq-001 (n=6), BDL (n=12) and BDL-Yaq-001 (n=7)). Significantly higher portal venous endotoxin and arterial endotoxin were observed in BDL rats compared with Sham rats ($p<0.0001$). Significantly higher portal venous plasma bacterial DNA positivity was observed in BDL rats compared with Sham rats ($p<0.05$). Yaq-001 administration was associated with a significant reduction of portal venous and arterial endotoxin compared with untreated-BDL rats ($p<0.0001$, $p<0.01$). Yaq-001 administration reduced bacterial DNA positivity, which was not statistically different ($p>0.05$). (B) Rats underwent sham biliary surgery or BDL for 4 weeks. The treated group received Yaq-001 for 2 weeks prior to LPS injection. Animals were sacrificed either at coma stages or 6 hours after LPS injection (n=9–16/group). Kaplan-Meier analysis of BDL-LPS rats with (n=16) or without (n=12) Yaq-001 treatment. Yaq-001 treatment significantly improved the survival of BDL-LPS rats compared with untreated-BDL-LPS rats (log rank test, $p=0.003$). Plasma ALT concentrations in Sham-LPS (n=7), Sham-LPS-Yaq-

001 (n=5), BDL-LPS (n=10) and BDL-LPS-Yaq-001 (n=9) groups and portal pressure measurements in Sham-LPS (n=8), Sham-LPS-Yaq-001 (n=10), BDL-LPS (n=9) and BDL-LPS-Yaq-001 (n=9) groups. Yaq-001-treated BDL-LPS rats had a significantly lower ALT and potal pressure compared with untreated-BDL-LPS rats ($p<0.005$). Brain water percentage in Sham-LPS (n=4), Sham-LPS-Yaq-001 (n=4), BDL-LPS (n=7), BDL-LPS-Yaq-001 (n=13) groups. Arterial ammonia concentrations in Sham-LPS (n=5), Sham-LPS-Yaq-001 (n=5), BDL-LPS (n=7), BDL-LPS-Yaq-001 (n=7) groups. Portal venous ammonia concentrations in Sham-LPS (n=5), Sham-LPS-Yaq-001 (n=5), BDL-LPS (n=6), BDL-LPS-Yaq-001 (n=5) groups. Yaq-001 decreased brain water percentage and arterial/portal venous ammonia concentrations in BDL-LPS rats compared with untreated rats ($p<0.05$, $p<0.01$, $p<0.05$). Serum creatinine in Sham-LPS (n=4), Sham-LPS-Yaq-001 (n=3), BDL-LPS (n=12) and BDL-LPS-Yaq-001 (n=6) groups. Serum urea in Sham-LPS (n=8), Sham-LPS-Yaq-001 (n=4), BDL-LPS (n=12) and BDL-LPS-Yaq-001 (n=8) groups. Yaq-001 significantly decreased creatinine levels in BDL-LPS rats ($p<0.05$). Plasma cytokines in Sham-LPS (n=6), Sham-LPS-Yaq-001 (n=9), BDL-LPS (n=8) and BDL-LPS-Yaq-001 (n=8) groups. Yaq-001 significantly decreased plasma IL-1β and IL-10 concentrations in BDL-LPS groups ($p<0.01$, $p<0.05$). *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$. ACLF, acute-on-chronic liver failure; BDL, bile duct ligation; LPS, lipopolysaccharide.

Figure 2

Effect of Yaq-001 on gene expression profiles in the liver and extrahepatic organs in BDL rats. (A) Rats underwent BDL for 4 weeks as a model of cirrhosis (n=3–4/group) and the treatment groups received Yaq-001 for 2 weeks before sacrifice. Liver, colon, brain and kidney were collected for transcriptomic analysis. (B, D, F, H) Heatmap of differentially expressed genes (DEGs) in different organs between Sham (n=3), Sham-Yaq-001 (n=3), BDL (n=3) and BDL-Yaq-001 (n=4) groups. DEGs were identified at 1.2-fold change and p=0.1 threshold in three pairwise groups (BDL vs Sham, BDL-Yaq-001 vs BDL, Sham-Yaq-001 vs Sham). (C, E, G, I) Volcano plot of pairwise DEGs in four organs among Sham (n=3), Sham-Yaq-001 (n=3), BDL (n=3) and BDL-Yaq-001 (n=4) groups. The vertical dashed lines indicated the threshold for 1.2-fold change. The horizontal dashed line indicated the adjusted p=0.05 and p=0.1 threshold. The right part indicates upregulation of gene expression, and the left part indicates downregulation of gene expression. For top 20 genes indicated by gene names, please see online supplement Table 3. BDL, bile duct ligation.

Figure 3

Effect of Yaq-001 treatment on the microbiome composition. (A) Heatmap of gut microbiome associated with the effect of Yaq-001 as determined by 16S PCR at the family level. The family *Porphyromonadaceae* with asterisk was statistically differently abundant between BDL (n=7) vs Sham (n=6), and between BDL-Yaq-001 (n=7) vs BDL groups (n=7) (Wilcoxon rank sum test, $p<0.05$). The abundance of this family was statistically higher in BDL group than in Sham group, and its abundance statistically decreased in the BDL-Yaq-001 group than in the BDL group. The other six families in the heatmap were with marked fold changes between BDL vs Sham, and between BDL-Yaq-001 vs BDL groups ($|log2FC|>2$). Of these, five were more abundant in the BDL group than in the Sham group. The abundance largely decreased in the Yaq-001-treated group. In addition, to these, one family was less abundant in the BDL group than in the Sham group. The abundance increased in

the Yaq-001-treated group. (B) Heatmap of gut microbiome at the Genus level. The Genus *Barnesiella* with asterisk was statistically differently abundant between BDL vs Sham, and between BDL-Yaq-001 vs BDL groups (Wilcoxon rank sum test, $p<0.05$). The abundance of this genus was statistically higher in BDL group than in the Sham group, and its abundance statistically decreased in the BDL-Yaq-001 group. The other 19 genera in the heatmap represent those with significant fold change values between BDL vs Sham, and between BDL-Yaq-001 vs BDL groups ($|log2FC|>2$). Of these, 14 were more abundant in the BDL group compared with the Sham group. The abundance decreased in the Yaq-001-reated BDL group. In addition, five genera were less abundant in the BDL group than in the Sham group. Their abundance increased in the Yaq-001-treated BDL animals. (C, D) Correlation plots between markedly changed genes and gut microbiome at family/genus. The genes were from among the top 20 changed genes in BDL animals with Yaq-001 treatment. Nodes represent either genes (lower semi-circular part) or bacteria (upper semicircular part) at the family and genus level. The nodes are coloured based on the log-fold change for the differential gene expression and differences in the bacterial abundance. The red nodes indicate an increase and blue nodes indicate a decrease. Edges represent the correlation coefficient calculated between genes and microbial genus or family with red indicating a positive correlation and blue a negative correlation. Correlation coefficients greater or equal to 0.4 were plotted in plot C (Spearman's coefficient≥0.4), and D shows all correlations. *$p<0.05$. BDL, bile duct ligation.

Figure 4

Effect of Yaq-001 on organ dysfunction, ammonia and endotoxaemia in CCl4 mice. (A) Mice underwent CCl4 injection for 6 weeks as a model of cirrhosis (n=6–12/group) and the treatment groups received Yaq-001 for 6 weeks before sacrifice. Plasma ALT, ALP and TBIL concentrations in control (n=6), control-Yaq-001 (n=6), 6-week CCl4 (n=12) and 6-week CCl4-Yaq-001 (n=12) groups. Significantly higher ALT, ALP and TBIL were observed in CCl4 compared with controls (p=0.0001, p=0.0007, p=0.012). Yaq-001-treated CCl4 mice had a significantly lower ALT compared with untreated-CCl4 mice (p<0.0001, p=0.040, p=0.001). H&E and PSR staining of liver tissue. CCl4 mice were associated with a significant increase in collagen proportionate area (CPA) compared with controls (p=0.0001). Yaq-001 had significant effect on CPA in CCl4-Yaq-001 compared with CCl4 mice(p=0.024). TUNEL staining liver tissues. Significantly greater staining was observed in CCl4 compared with controls (p=0.0001). Yaq-001-treated CCl4 mice had a significantly lower TUNEL staining compared with untreated-CCl4 (p=0.021) with Yaq-001 treatment. Venous ammonia concentrations and serum creatinine levels in control (n=6), control-Yaq-001 (n=6), 6-week CCl4 (n=12) and 6-week CCl4-Yaq-001 groups (n=12). Significantly increased ammonia concentrations were observed in CCl4 compared with controls (p=0.0020). Yaq-001 significantly decreased venous ammonia concentrations and serum creatinine levels in CCl4 mice (p=0.025, p=0.005). Endotoxin concentrations in control (n=3), control-Yaq-001 (n=3), 6-week CCl4 (n=10) and 6-week CCl4-Yaq-001 groups(n=10). Significantly higher endotoxin was observed in CCl4 mice compared with control mice (p=0.007). Yaq-001 administration was associated with a significant reduction of venous endotoxin compared with untreated-CCl4 mice (p=0.007). (B) Mice underwent CCl4 injection for 12 weeks as a model of cirrhosis (n=6–12/group) and the treatment groups received Yaq-001 for 6

weeks before sacrifice. Plasma ALT, ALP and TBIL concentrations in control (n=6), control-Yaq-001 (n=6), 12-week CCl4 (n=12) and 12-week CCl4-Yaq-001 (n=12) groups. Significantly higher ALT, ALP and TBIL were observed in CCl4 compared with controls (p=0.0001, p=0.0008, p=0.007). Yaq-001-treated CCl4 mice had a significantly lower ALT compared with untreated-CCl4 mice (p<0.0001). H&E and PSR staining of liver tissue in CCl4 mice. CCl4 mice were associated with a significant increase in collagen proportionate area (CPA) compared with controls (p=0.0001). Yaq-001 had significant effect on CPA in CCl4-Yaq-001 compared with CCl4 mice(p=0.012). TUNEL staining of liver tissues. Significantly higher TUNEL staining was observed in CCl4 compared with controls (p=0.0001). Yaq-001-treated CCl4 mice had a significantly lower TUNEL staining compared with untreated-CCl4 (p=0.017) indicative of a reduction in liver cell death with Yaq-001 treatment. Venous ammonia. Significantly increased ammonia concentrations were observed in CCl4 compared with controls (p=0.001). Yaq-001 significantly decreased venous ammonia concentrations in CCl4 mice (p=0.035). Serum creatinine: Yaq-001 significantly decreased serum creatinine levels in CCl4 mice (p=0.003). Endotoxin concentrations in control (n=3), Control-Yaq-001 (n=3), 12-week CCl4 (n=10) and 12-week CCl4-Yaq-001 groups(n=10). Significantly higher venous endotoxin was observed in CCl4 mice compared with control mice (p=0.007). Yaq-001 administration was associated with a significant reduction of venous endotoxin compared with untreated-CCl4 mice (p=0.043). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. ALT, alanine aminotransferase; ALP, alkaline phosphatase; PSR, picrosirius red; TBIL, total bilirubin.

Figure 5

Effect of Yaq-001 on gut permeability in intestinal organoids. (A) Intestinal organoids derived and cultured from small intestine of C57BL/6 mice underwent eversion into apical-out polarity in the first 12 hours of suspension culture. Immunostaining of the microvilli (mv; F-actin) demonstrated that intestinal organoids in suspension have reversed polarity from basolateral-out to apical-out. (B) Apical-out intestinal organoids in suspension culture generate goblet cells (MUC2). (C) Gut permeability of apical-out intestinal organoids was significantly increased by coculturing with faecal water from CCl4 group than control group (p=0.003). Gut permeability was notably decreased in faecal water from CCl4-Yaq-001 group compared with CCl4 group (p=0.001). (D) Quantification of the integrated density/area of each group. **p<0.01.

Figure 1

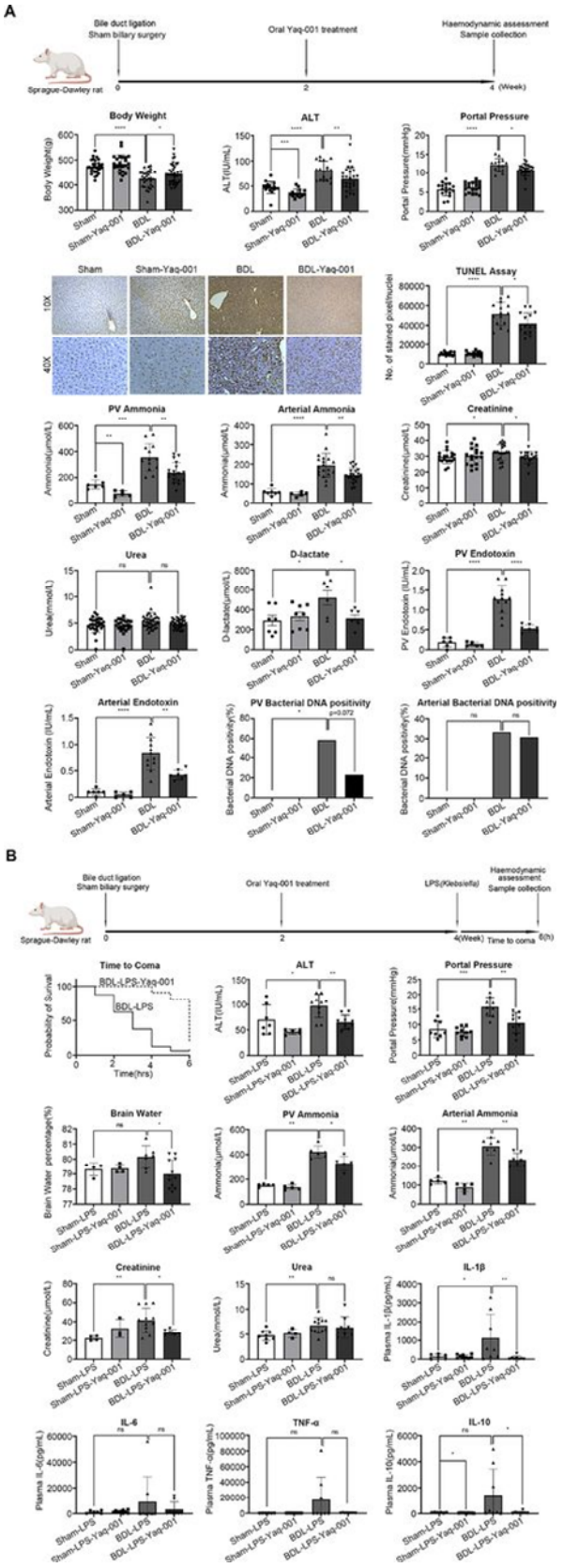

A
Bile duct ligation
Sham bilary surgery
Oral Yaq-001 treatment
Haemodynamic assessment
Sample collection
Sprague-Dawley rat
0
2
4 (Week)
Body Weight
ALT
Portal Pressure
Sham
Sham-Yaq-001
BDL
BDL-Yaq-001
TUNEL Assay
PV Ammonia
Arterial Ammonia
Creatinine
Urea
D-lactate
PV Endotoxin
Arterial Endotoxin
PV Bacterial DNA positivity
Arterial Bacterial DNA positivity
B
Bile duct ligation
Sham bilary surgery
Oral Yaq-001 treatment
LPS(Klebsiella)
Haemodynamic assessment
Sample collection
Sprague-Dawley rat
4(Week)
Time to coma
6(h)
Time to Coma
BDL-LPS-Yaq-001
BDL-LPS
Probability of Survival
Time(hrs)
ALT
Portal Pressure
Brain Water
PV Ammonia
Arterial Ammonia
Creatinine
Urea
IL-1β
IL-6
TNF-α
IL-10
Sham-LPS
Sham-LPS-Yaq-001
BDL-LPS
BDL-LPS-Yaq-001

Figure 2

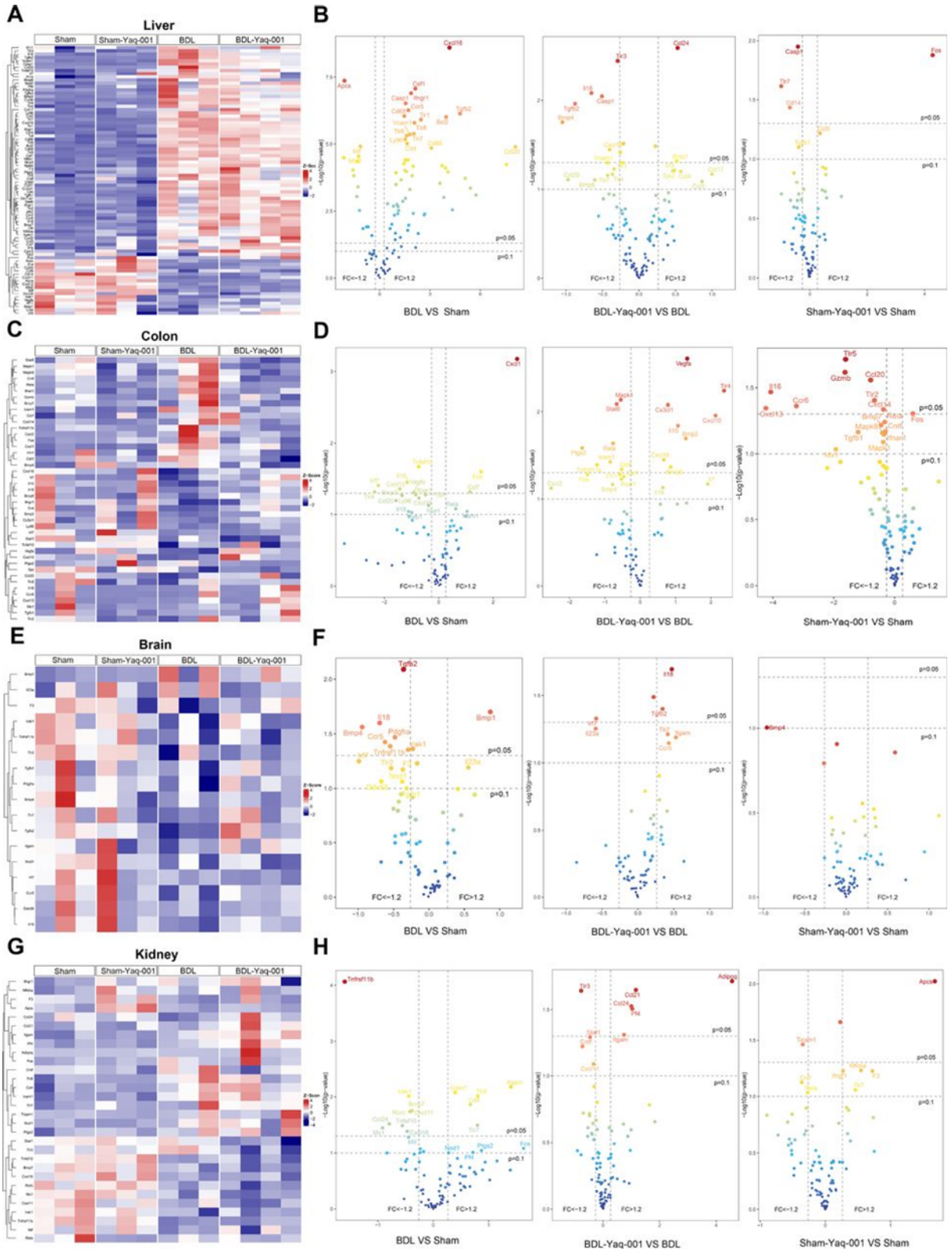

A
Liver
Sham
Sham-Yaq-001
BDL
BDL-Yaq-001
B
BDL VS Sham
BDL-Yaq-001 VS BDL
Sham-Yaq-001 VS Sham
C
Colon
D
E
Brain
F
G
Kidney
H
p=0.05
p=0.1
FC<=1.2
FC>1.2

Figure 3

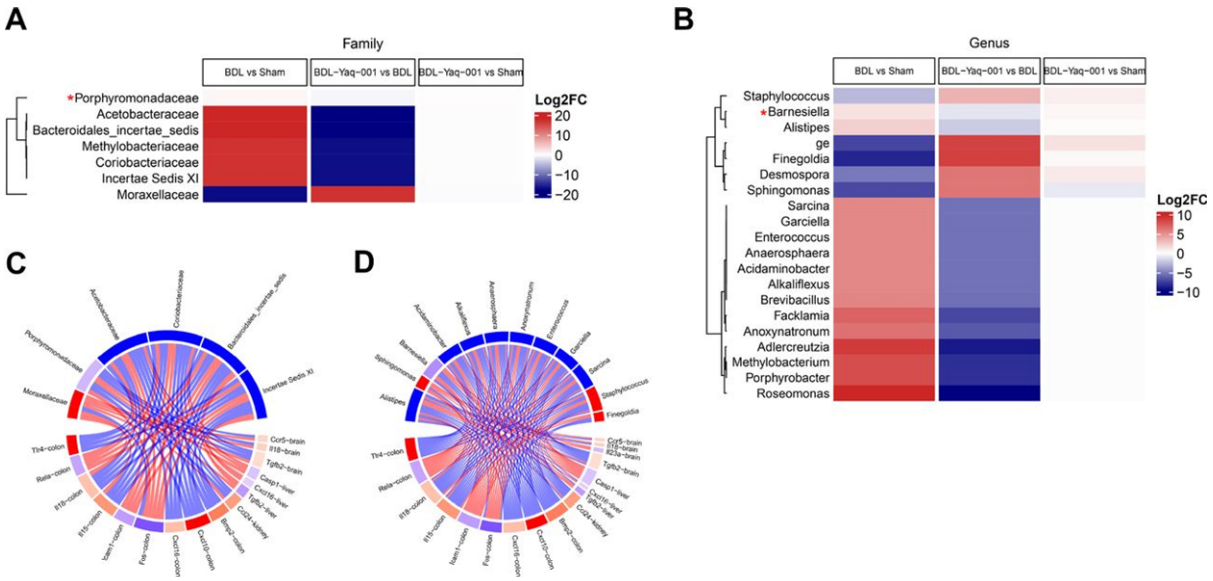

A
Family
BDL vs Sham
BDL−Yaq−001 vs BDL
BDL−Yaq−001 vs Sham
*Porphyromonadaceae
Acetobacteraceae
Bacteroidales_incertae_sedis
Methylobacteriaceae
Coriobacteriaceae
Incertae Sedis XI
Moraxellaceae
Log2FC
20
10
0
−10
−20
B
Genus
BDL vs Sham
BDL−Yaq−001 vs BDL
BDL−Yaq−001 vs Sham
Staphylococcus
*Barnesiella
Alistipes
ge
Finegoldia
Desmospora
Sphingomonas
Sarcina
Garciella
Enterococcus
Anaerosphaera
Acidaminobacter
Alkaliflexus
Brevibacillus
Facklamia
Anoxynatronum
Adlercreutzia
Methylobacterium
Porphyrobacter
Roseomonas
Log2FC
10
5
0
−5
−10
C
D

Figure 4

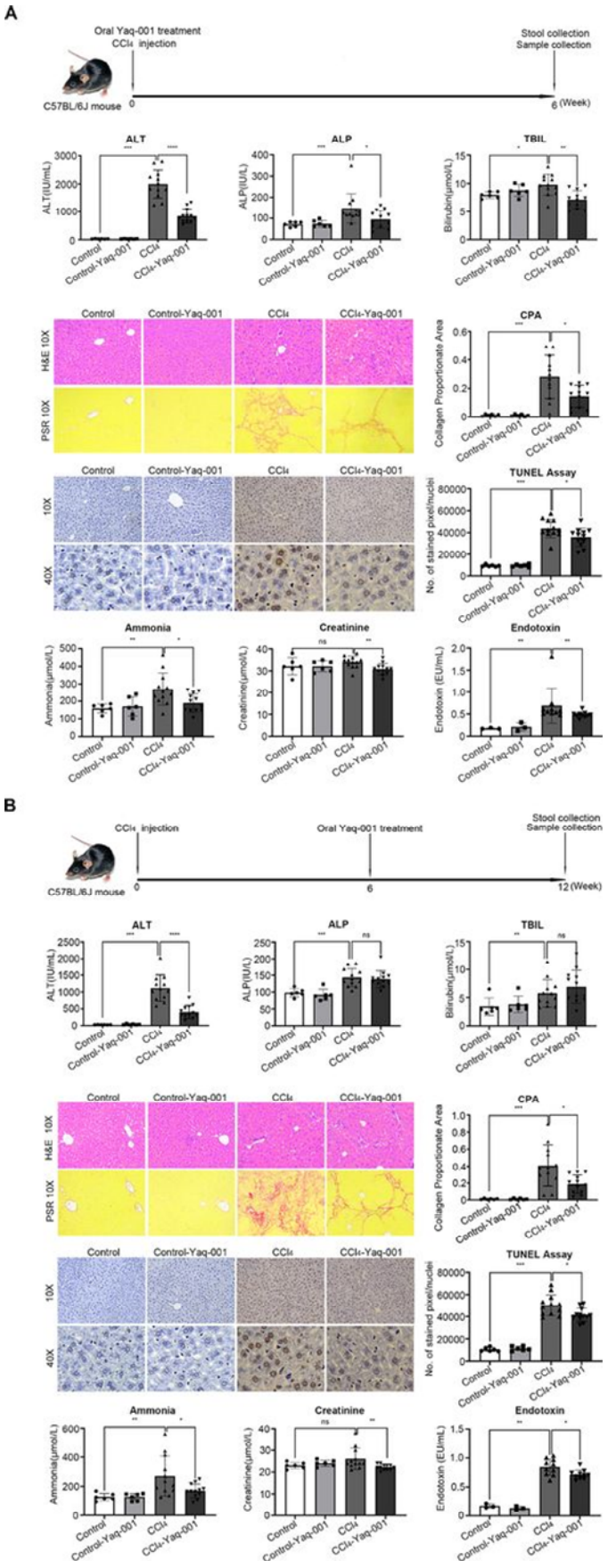
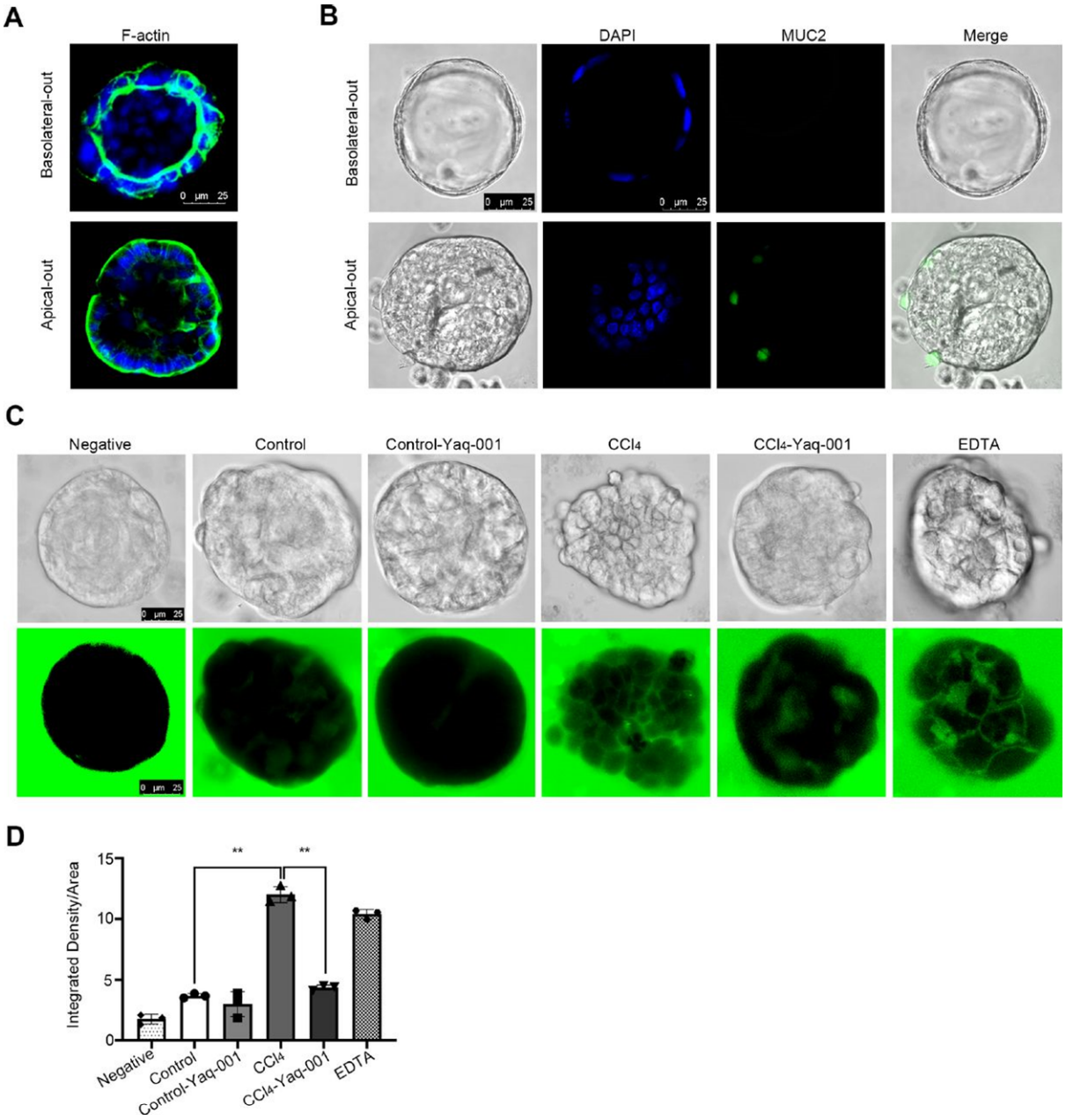

A
Oral Yaq-001 treatment
CCl4 injection
Stool collection
Sample collection
C57BL/6J mouse
0
6 (Week)
ALT
ALP
TBIL
ALT(IU/mL)
ALP(IU/L)
Bilirubin(μmol/L)
Control
Control-Yaq-001
CCl4
CCl4-Yaq-001
H&E 10X
PSR 10X
CPA
Collagen Proportionate Area
10X
40X
TUNEL Assay
No. of stained pixel/nuclei
Ammonia
Creatinine
Endotoxin
Ammonia(μmol/L)
Creatinine(μmol/L)
Endotoxin (EU/mL)
B
CCl4 injection
Oral Yaq-001 treatment
Stool collection
Sample collection
C57BL/6J mouse
0
6
12 (Week)
ALT
ALP
TBIL
CPA
TUNEL Assay
Ammonia
Creatinine
Endotoxin

Figure 5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F-actin
Basolateral-out
Apical-out
B
DAPI
MUC2
Merge
Basolateral-out
Apical-out
C
Negative
Control
Control-Yaq-001
CCl4
CCl4-Yaq-001
EDTA
D
Integrated Density/Area
15
10
5
0
**
**
Negative
Control
Control-Yaq-001
CCl4
CCl4-Yaq-001
EDTA

Table 1

Patient characteristics

	Placebo	Active
Number	12	14
Age (years)	58.5 (35–75)	58.5 (47–68)
Male sex	9 (75%)	10 (71.4%)
Race/ethnicity		
Black	0 (0%)	0 (0%)
Other	12 (100%)	14 (100%)
BMI (kg/m^2) (SD)	27.8 (22.9–32.8)	26.3 (20.4–32.8)
Child Pugh Score	7 (7–8)	7 (7–8)
MELD score	13.2 (10.2–16.1)	12.6 (9.7–13.5)
Decompensation history		
Alcoholic hepatitis	2 (16.7%)	0 (0.0%)
Ascites	8 (66.7%)	11 (78.6%)
Hepatic encephalopathy	5 (41.6%)	2 (14.3%)
Varices	2 (16.7%)	6 (42.9%)
Laboratory values		
Bilirubin (µmol/L)	31 (19–40)	41 (17–68)
Albumin (g/L)	34 (30–38)	34 (30–39)
Creatinine (µmol/L)	65 (53–83)	70 (60–81)
Sodium (mmol/L)	136.5 (134–137)	137.2 (134–140)

- **BMI, body mass index; MELD, model for end stage liver disease.**

Table 2

Adverse and serious events

Adverse event	Placebo n (%)	Active n (%)
Constipation	3 (25)	2 (14)
Epigastric pain	0 (0)	1 (7)
Nausea	0 (0)	1 (7)
Meteorism	0 (0)	1 (7)
Osophageal reflux	0 (0)	1 (7)
Diarrhoea	2 (17)	0 (0)
Diuresis	0 (0)	1 (7)
Serious event		
Death	0 (0)	0 (0)
50% increase in MELD	0 (0)	0 (0)
100% increase in creatinine	0 (0)	0 (0)
50% reduction in BMI	0 (0)	0 (0)
Acute decompensation	0 (0)	0 (0)
Episode of ACLF	0 (0)	0 (0)

- **ACLF, acute-on-chronic liver failure; BMI, body mass index; MELD, model for end stage liver disease.**

Table 3

Safety parameters: clinical laboratory assessments, royal free global assessment and micronutrient concentrations

	Placebo			Active		
Parameters	Baseline	4 weeks	12 weeks	Baseline	4 weeks	12 weeks
Laboratory parameters	Median (range)			Median (range)		
Haemoglobin (g/L)	122 (108–143)	119 (107–145)	119 (105–139)	122 (113–134)	123 (113–133)	121 (110–137)
Leucocyte count ($\times 10^9$/L)	4.22 (3.47–4.97)	4.05 (3.43–4.84)	3.94 (3.20–4.90)	5.22 (4.20–6.99)	5.12 (4.21–6.09)	4.70 (4.26–5.52)
Platelets ($\times 10^9$/L)	81 (75–113)	93 (83–104)	93 (85–100)	95 (81–127)	84 (75–107)	93 (75–105)
Bilirubin (μmol/L)	41 (17–68)	31 (16–79)	47 (20–71)	31 (19–40)	42 (21–52)	31 (17–47)
ALT (IU/L)	25.9 (19.88–8.55)	22.89 (18.07–31.33)	24.1 (18.07–31.33)	25.9 (24.1–34.94)	27.11 (24.1–33.13)	28.31 (19.88–34.94)
ALP (IU/L)	49.2 (37.8–52.8)	37.2 (24–54)	40.8 (27–56.4)	46.8 (31.2–51)	46.8 (30–69)	45 (30–61.2)
Albumin (g/L)	34 (30–39)	35 (31–40)	32 (31–38)	34 (30–38)	35 (30–38)	32 (30–38)
Sodium (mmol/L)	137 (135–140)	138 (132–140)	138 (135–140)	137 (134–137)	136 (132–137)	137 (136–138)
Creatinine (μmol/L)	70 (60–81)	77 (63–83)	65 (63–90)	65 (53–83)	64 (53–75)	70 (57–72)
INR	1.4 (1.3–1.6)	1.3 (1.2–1.5)	1.4 (1.3–1.5)	1.3 (1.2–1.4)	1.4 (1.2–1.4)	1.3 (1.2–1.4)
Child Pugh Score	7 (7–8)	7 (6–8)	8 (6–8)	7 (7–8)	8 (6.7–9)	8 (7–9)
MELD score	12 (10–17)	12 (10–17)	13 (12–16)	13 (11–14)	14 (11–14)	13 (9–15)
MELD Na score	15 (10–21)	13 (9–19)	16 (12–21)	15 (13–17)	16 (15–17)	14 (13–19)

	Placebo			Active		
Parameters	Baseline	4 weeks	12 weeks	Baseline	4 weeks	12 weeks
Nutritional status	n (%)			n (%)		
Adequate	10 (83)	8 (73)	10 (91)	13 (93)	13 (100)	10 (77)
Moderate malnourishment	2 (17)	3 (27)	1 (9)	1 (7)	0 (0)	3 (23)
Severe malnourishment	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Micronutrient Concentrations	Median (range)			Median (range)		
Vitamin B_{12} (ng/L)	533 (290–765)	530 (384–970)	524 (275–815)	503 (337–790)	483 (365–694)	462 (326–749)
Folate (µg/L)	16 (9–22)	17 (13–19)	23 (21–26)	22 (20–30)	20 (18–24)	21 (17–27)
Vitamin D (nmol/L)	28 (20–51)	45 (25–57)	43 (26–61)	32 (25–72)	57 (22–72)	70 (37–72)
Vitamin A (µmol/L)	0.48 (0.13–2.18)	0.47 (0.12–1.90)	0.39 (0.09–1.80)	0.46 (0.15–1.84)	0.39 (0.16–1.86)	0.62 (0.17–1.82)
Vitamin E (µmol/L)	28.4 (17.7–42.0)	32.5 (17.6–36.5)	29.9 (20.8–36.1)	30.0 (19.5–45.6)	30.3 (18.3–46.2)	29.4 (17.7–37.9)
Vitamin K (µg/L)	0.58 (0.16–3.28)	0.33 (0.12–2.5)	0.35 (0.17–4.2)	0.58 (0.14–4.3)	0.41 (0.12–5.7)	0.60 (0.14–2.8)
Copper (µmol/L)	13.6 (8.30–22.6)	13.6 (7.10–23.7)	13.5 (11.2–24.1)	13.7 (10.0–30.1)	15.5 (9.10–26.7)	15.1 (7.80–37.6)
Zinc (µmol/L)	9.10 (5.60–15.3)	8.85 (5.00–13.7)	8.40 (5.70–14.3)	8.20 (5,60–12.9)	8.60 (4.70–15.0)	9.00 (5.00–15.8)
Selenium (µmol/L)	0.78 (0.56–1.10)	0.86 (0.47–1.12)	0.90 (0.65–1.04)	0.77 (0.55–1.14)	0.93 (0.48–1.23)	0.91 (0.49–1.46)

- **ALP, alkaline phosphatase; ALT, alanine aminotransferase; INR, international normalised ratio; MELD, model for end stage liver disease; MELD-Na, model for end stage liver disease - sodium.**

Table 4