Research Article

EASL JOURNAL OF HEPATOLOGY

Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats

María Úbeda[1,2], Margaret Lario[1,2], Leticia Muñoz[1,2], María-José Borrero[1,2], Macarena Rodríguez-Serrano[3], Ana-María Sánchez-Díaz[4], Rosa del Campo[4], Lourdes Lledó[5], Óscar Pastor[6], Laura García-Bermejo[3], David Díaz[1,2,7], Melchor Álvarez-Mon[1,2,7,†], Agustín Albillos[1,2,8,*,†]

[1]*Laboratorio de Enfermedades del Sistema Inmune, Departamento de Medicina, Universidad de Alcalá, Alcalá de Henares, Spain;* [2]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain;* [3]*Unidad de Biomarcadores y Dianas Terapéuticas, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain;* [4]*Servicio de Microbiología, Hospital Universitario Ramón y Cajal, IRYCIS, Red Española de Investigación en Enfermedades Infecciosas (REIPI), Instituto de Salud Carlos III, Madrid, Spain;* [5]*Departamento de Microbiología, Universidad de Alcalá, Alcalá de Henares, Spain;* [6]*Servicio de Bioquímica Clínica, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain;* [7]*Servicio de Enfermedades del Sistema Inmune y Oncología, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Spain;* [8]*Servicio de Gastroenterología y Hepatología, Hospital Universitario Ramón y Cajal, IRYCIS, Madrid, Spain*

Background & Aims: In advanced cirrhosis, gut bacterial translocation is the consequence of intestinal barrier disruption and leads to bacterial infection. Bile acid abnormalities in cirrhosis could play a role in the integrity of the intestinal barrier and the control of microbiota, mainly through the farnesoid X receptor. We investigated the long-term effects of the farnesoid X receptor agonist, obeticholic acid, on gut bacterial translocation, intestinal microbiota composition, barrier integrity and inflammation in rats with CCl_4-induced cirrhosis with ascites.

Methods: Cirrhotic rats received a 2-week course of obeticholic acid or vehicle starting once ascites developed. We then determined: bacterial translocation by mesenteric lymph node culture, ileum expression of antimicrobial peptides and tight junction proteins by qPCR, fecal albumin loss, enteric bacterial load and microbiota composition by qPCR and pyrosequencing of ileum mucosa-attached contents, and intestinal inflammation by cytometry of the inflammatory infiltrate.

Results: Obeticholic acid reduced bacterial translocation from 78.3% to 33.3% (p <0.01) and upregulated the expression of the farnesoid X receptor-associated gene small heterodimer partner. Treatment improved ileum expression of antimicrobial peptides, angiogenin-1 and alpha-5-defensin, tight junction proteins zonulin-1 and occludin, and reduced fecal albumin loss and liver fibrosis. Enteric bacterial load normalized, and the distinctive mucosal microbiota of cirrhosis was reduced. Gut immune cell infiltration was reduced and inflammatory cytokine and Toll-like receptor 4 expression normalized.

Conclusions: In ascitic cirrhotic rats, obeticholic acid reduces gut bacterial translocation via several complementary mechanisms at the intestinal level. This agent could be used as an alternative to antibiotics to prevent bacterial infection in cirrhosis.

Introduction

The translocation of bacteria and bacterial products from the gut (GBT) is a hallmark of spontaneous bacterial infection, the systemic inflammatory response and remote organ injury in cirrhosis [1,2]. The high rate of GBT observed in patients and animal models of cirrhosis and ascites is the consequence of concurrent damage at several levels of intestinal barrier defence. Such abnormalities in patients and experimental models include: i) structural defects in the epithelial barrier, i.e. damaged tight junction (TJ) proteins, brush border membrane peroxidation, and an increased intestinal permeability to macromolecules [3,4]; ii) impaired innate defences, such as compromised Paneth cell synthesis of antimicrobial peptides, or impaired function of phagocytic cells, such as dendritic cells [5,6]; and iii) the overgrowth and dysbiosis of intestinal microflora due to gut hypomotility, innate defence system damage and reduced bile flow [3,7–9]. Intestinal barrier damage parallels with cirrhosis progression and is particularly severe when ascites and GBT have developed.

Keywords: Inflammation; Permeability; Dysbiosis; Ascites.
Received 18 June 2015; received in revised form 22 November 2015; accepted 18 December 2015; available online 23 December 2015

* Corresponding author. Address: Departamento de Medicina y Especialidades Médicas, Facultad de Medicina y Ciencias de la Salud-Campus Universitario, Universidad de Alcalá, Carretera Madrid-Barcelona km. 33.600, 28871 Alcalá de Henares, Madrid, Spain. Tel.: +34 918854870; fax: +34 918854526.
E-mail address: agustin.albillos@uah.es (A. Albillos).
† These authors share senior authorship.

Abbreviations: GBT, gut bacterial translocation; TJ, tight junction; ZO-1, zonula occludens-1; FXR, farnesoid X receptor; MLN, mesenteric lymph nodes; OCA, obeticholic acid; SHP, small heterodimer partner; OTU, operational taxonomic units; TNF, tumor necrosis factor; IL, interleukin.

Research Article

Bile acids play a pleitropic role in maintaining intestinal barrier homeostasis. The intestinal microbiota is directly affected by the bacteriostatic properties of bile acids, and indirectly via the synthesis of antimicrobial peptides and modulation of innate immunity through farnesoid X receptor (FXR) stimulation [10,11]. FXR, the nuclear receptor for bile acids, is mainly expressed in tissues frequently exposed to these molecules including the liver, intestine and kidneys. Accordingly, experimental models lacking bile acids in the intestinal lumen, such as models of cholestasis by bile duct ligation, feature intestinal bacterial overgrowth, inflammation, increased permeability and also GBT to mesenteric lymph nodes (MLN) [11–13]. This scenario is reproduced in *FXR* knockout mice and can be rescued by synthetic FXR agonists, conjugated bile acids or internal biliary drainage [11,13,14].

Cirrhosis reduces the amount of bile produced and modifies its composition. This results in decreased bile acids in the gut, as observed in patients, and in models of cirrhosis such as those induced by CCl_4 [14–16]. Interestingly, low intestinal bile acid levels have been linked to dysbiosis in patients with cirrhosis [7]. Hence, it could be that the cirrhosis-induced reduction in bile acid pool size could impair intestinal FXR axis activation, leading to intestinal dysbiosis, inflammation and barrier damage, thus contributing to GBT. Accordingly, FXR agonists, such as obeticholic acid (OCA), could restore intestinal barrier function and inhibit GBT to the MLN in rats with CCl_4-induced cirrhosis with ascites, a non-cholestatic model of cirrhosis featuring a high rate of GBT. OCA, also known as INT-747, is a first-in-class bile acid analogue, derived from chenodeoxycholic acid – the natural FXR agonist, and shows a 100-fold greater FXR agonistic activity than the natural compound [17]. The aim of this study was to investigate the long-term effects of the FXR agonist OCA on GBT, and intestinal microbiota composition, barrier integrity and inflammation in rats with CCl_4-induced cirrhosis with ascites.

Materials and methods

Experimental model of cirrhosis

Male Sprague-Dawley rats (Harlan, Horst, Netherlands) were used for all experiments. Cirrhosis was induced by weekly gavage with CCl_4 and phenobarbital in the drinking water, as described in Supplementary material. Phenobarbital-treated age- and sex-matched rats were used as the control group. All experiments were approved by and performed in accordance with the ethics committee of the University of Alcalá regulations, the Guide for the Care and Use of Laboratory Animals.

Study design

OCA, a synthetic FXR ligand, was prepared weekly by dissolving in methylcellulose, according to manufacturer's protocol (*Intercept Italia S.r.l.*, Perugia, Italy). Once ascites developed, rats were randomized to OCA (5 mg/kg·day) or vehicle (methylcellulose 0.5%) by oral gavage for two weeks [18]. Control animals were randomized into the same groups.

Instrumentation

Experiments were performed 7 days after the last CCl_4 dose. Animals underwent laparotomy under anesthesia with sevofluorane (Abbott Laboratories, Madrid, Spain) in strict aseptic conditions. Rats were shaved, the skin disinfected and the following tissues and fluids were consecutively removed: ascitic fluid using calibrated sterile syringes, blood by aortic puncture, MLN, liver and small intestine. Samples of MLN, ascites and stool of the terminal ileum were used for bacteriological study. Samples of small intestine and liver were snap-frozen and/or formalin-fixed. Mononuclear cells from blood, MLN and intestinal lamina propria were obtained.

Methods

All methods are described in the Supplementary material. In brief, GBT to MLN was assessed through conventional bacteriological culture. The FXR signaling pathway was examined by analyzing the genomic ileum expression of FXR and the *FXR* target gene, small heterodimer partner (SHP). To address intestinal epithelial integrity, we determined the genomic ileum expression of the antimicrobial peptides, angiogenin-1 and alpha-5-defensin and examined the epithelial TJ proteins, zonula occludens-1 (ZO-1) and occludin, along with fecal albumin loss. Bacterial load in ileum feces was quantified by conventional culture and qPCR, and the composition of the microbiota attached to the mucosa by latest generation sequencing. The effect of OCA on intestinal and systemic inflammation was assessed by the distribution and activation of intestinal and peripheral blood immune cells by flow cytometry, and the ileum expression of inflammation-related cytokine genes. Hepatic fibrogenesis was examined by quantifying collagen fibres by Sirius Red staining, and hydroxyproline, and the expression of pro-fibrogenic genes.

Results

Characteristics of the rats

Forty-eight out of 85 rats (56%) subjected to the CCl_4 cirrhosis induction protocol survived and developed ascites. On average, rats developed ascites 13 weeks (range, 8–22) after the initial CCl_4 dose. Once ascites developed, the 48 rats were randomized to receive the 2-week course of OCA or vehicle. Four (2 allocated to the OCA and 2 to the placebo group) of the 48 cirrhotic rats with ascites died during treatment such that the final study population was comprised of 44 animals, 21 treated with OCA and 23 with vehicle. On the day of the experiment, ascites was non-significantly reduced in the OCA-treated compared to vehicle-treated rats (Supplementary Table 1). Three rats in the OCA group and no rats in the vehicle group showed ascites reabsorption. The OCA-treated animals also showed reduced liver necro-inflammatory activity, as shown by their lower aminotransferases and bilirubin, and greater plasma albumin levels (Supplementary Table 1).

OCA reduces GBT to MLN

As shown in Fig. 1A and Table 1, OCA reduced GBT to MLN from 78.3% in vehicle-treated cirrhotic rats to 33.3% (p <0.01). Notably, OCA also reduced the frequency of *Escherichia coli* detected in the MLN of rats with GBT from 100% in vehicle-treated cirrhotic rats to 28.5% (Table 1). None of the 21 OCA-treated rats had ascites infection, which developed in 5 of the vehicle-treated animals (21.7%) and was caused by the same bacterial species identified in the MLN. None of 18 control rats treated with OCA or vehicle developed GBT.

To elucidate the mechanisms of GBT reduction by OCA, we explored its effects on the different intestinal barrier levels of defence.

OCA upregulates the FXR pathway improving epithelial integrity and intestinal permeability

We first examined the ileum FXR signaling pathway in the cirrhotic animals and the changes produced by OCA. *FXR* expression