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Lactobacillus rhamnosus GG supernatant promotes intestinal barrier function, balances T_{reg} and T_H17 cells and ameliorates hepatic injury in a mouse model of chronic-binge alcohol feeding

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HIGHLIGHTS

- LGG supernatant ameliorates experimental ALD in a chronic-binge alcohol exposure model.
- LGG supernatant normalizes the balance of T_{reg} and T_H17 in peripheral blood of mice exposed to chronic-binge alcohol.
- LGG supernatant decreases the serum level of IL-17 in chronic-binge alcohol mice model.

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ABSTRACT

Impaired intestinal barrier function plays a critical role in alcohol-induced hepatic injury, and the subsequent excessive absorbed endotoxin and bacterial translocation activate the immune response that aggravates the liver injury. *Lactobacillus rhamnosus* GG supernatant (LGG-s) has been suggested to improve intestinal barrier function and alleviate the liver injury induced by chronic and binge alcohol consumption, but the underlying mechanisms are still not clear. In this study, chronic-binge alcohol fed model was used to determine the effects of LGG-s on the prevention of alcoholic liver disease in C57BL/6 mice and investigate underlying mechanisms. Mice were fed Lieber–DeCarli diet containing 5% alcohol for 10 days, and one dose of alcohol was gavaged on Day 11. In one group, LGG-s was supplemented along with alcohol. Control mice were fed isocaloric diet. Nine hours later the mice were sacrificed for analysis. Chronic-binge alcohol exposure induced an elevation in liver enzymes, steatosis and morphology changes, while LGG-s supplementation attenuated these changes. Treatment with LGG-s significantly improved intestinal barrier function reflected by increased mRNA expression of tight junction (TJ) proteins and villus-crypt histology in ileum, and decreased *Escherichia coli* (E. coli) protein level in liver. Importantly, flow cytometry analysis showed that alcohol reduced T_{reg} cell population while increased T_H17 cell population as well as IL-17 secretion, which was reversed by LGG-s administration. In conclusion, our findings indicate that LGG-s is effective in preventing chronic-binge alcohol exposure-induced liver injury and shed a light on the importance of the balance of T_{reg} and T_H17 cells in the role of LGG-s application.

1. Introduction

Alcoholic liver disease (ALD) encompasses a variety of disorders ranging from simple steatosis to steatohepatitis, cirrhosis and hepatocelluar carcinoma (Tsukamoto and Xi, 1989; Stewart et al., 2001). ALD is an important cause of morbidity and mortality but no targeted therapy is available. Besides the direct toxic effects on the

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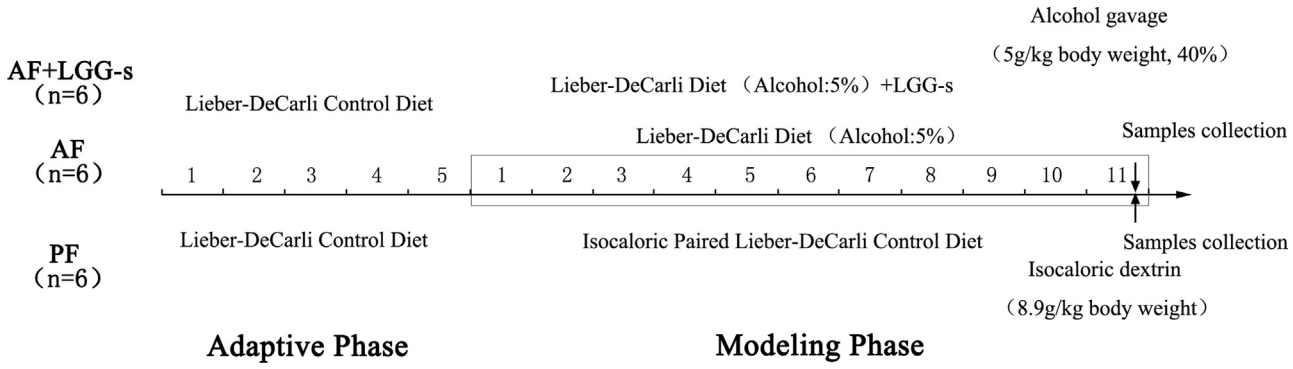

Fig. 1. Animal feeding schedule. All C57BL/6 mice were fed Lieber-DeCarli diet for 5 days, and then the mice were divided in three groups: (1) AF group: the mice were fed Liber-DeCarli diet containing 5% EtOH (w/v) for 10 days, and a bolus of EtOH (5 g/kg) was gavaged; (2) PF group: the mice were isocaloric fed a isocaloric diet replacing EtOH with maltose dextrin; (3) AF + LGG-s group: the mice were fed LGG-s at a dose of equivalent to 10^9 CFU/day along with alcohol as indicated in AF group. Nine hours later, the mice killed and the blood and tissue samples were collected for analysis.

liver, alcohol increases gut permeability and endotoxemia (Purohit et al., 2008; Elamin et al., 2014), and induces immune deregulation during alcohol-induced liver injury (Lefkowitch, 2005).

Lemmers et al. (2009) found that patients with ALD displayed elevated level of IL-17 in plasma compared with healthy counterparts, and higher proportion of IL-17 secreting cells among $CD4^+$ lymphocytes (T_H17) was observed in patients with alcoholic cirrhosis. In the presence of cytokines such as TGF-β, IL-6 and IL-21, naïve CD4 T cells differentiate to T_H17 subset, which is the main source of IL-17. IL-17 is recently reported to play a pivotal role in the stimulation and mobilization of neutrophils (Kolls and Linden, 2004), which exacerbates innate immune responses with the combined effect of IL-6 and TNF-α in mice (Hammerich et al., 2011). T_{reg} cells are characterized by its suppressive function on the proliferation and activation of effector T cells *in vitro* and *in vivo* (Guo et al., 2015; Sarris et al., 2008). The T_{reg} subset depleted mice displayed higher levels of proinflammatory cytokines and biochemical indicators related to hepatotoxicity in some models of acute liver injury (Kim et al., 2014; Wang et al., 2015). The balance of different subsets including T_{reg} and T_H17 cells contributes to the immune homeostasis in gut mucosa. However, the details of derangement of T_{reg} and T_H17 cells and its contribution to the course in the early stage of ALD remain to be elucidated.

Although remarkable achievement has been made in the field of ALD in recent years, there is still no effective therapy for ALD. Therefore, there is an urgent need to develop efficient strategies for patients with ALD. Recent studies have revealed that changes in intestinal microbiome play a critical role in ALD development in patients (Mutlu et al., 2012) and mouse model of ALD (Yan et al., 2011). *Lactobacillus rhamnosus* GG (LGG), a widely studied probiotic (Vandenplas et al., 2014), has been shown to restore gut microbiota and the expression of TJ proteins in mice with non-alcoholic fatty liver disease (NAFLD) (Ritze et al., 2014) and alcoholic steatohepatitis (Forsyth et al., 2009). However, it could be difficult for live probiotics to colonize intestine while there are lesions on intestinal mucosa. Moreover, LGG could also cause adverse outcomes like bacteremia (Land et al., 2005). Recently, LGG culture supernatant (LGG-s) generated from LGG culture has been used in ALD. Several studies have indicated that LGG-s is a safe and stable protector against acute and chronic alcohol induced liver injury (Wang et al., 2013, 2012). However, whether LGG-s is effective in chronic-binge alcohol exposure model is unknown. Chronic-binge alcohol exposure mimics the common drinking pattern in patients experiencing an excessive drinking with a history of chronic alcohol consumption (Bertola et al., 2013; Ki et al., 2010).

Recently, probiotic is found to generate T_H17 cells response, direct the differentiation of T_{reg} cells and even facilitate cytokine expressions (Lecuyer et al., 2014), but mechanisms of LGG protection of ileum permeability and regulation of T_H cells differentiation and cytokine expression in alcohol associated liver diseases have not been understood. Accordingly, we employed the chronic-binge model to investigate the effects of LGG-s on gut permeability and regulation of T cells differentiation in chronic-binge alcohol exposure-induced hepatic injury.

2. Material and methods

2.1. Culture of LGG and preparation of LGG-s

LGG was purchased from American Type Culture Collection (ATCC 53103, Rockville, MD) and cultured in MRS broth according to ATCC guidelines. The LGG supernatant (LGG-s) was harvested after filtered through 0.22 μm filters when the bacterial density reached 10^9 colony-forming units/ml (CFU/ml). The supernatant was stored at 0–4 °C for use in a week.

2.2. Animal experiments and sample collection

As shown in the pattern below (Fig. 1), male C57BL/6 mice (10 weeks of age) were supplied with a Lieber-DeCali liquid diet containing alcohol (5% w/v, AF, $n = 6$) or isocaloric maltodextrin (PF, $n = 6$) for 10 days, as previously described (Bertola et al., 2013). An additional group of mice (AF + LGG-s, $n = 6$) were fed the same diet as AF group supplemented with LGG-s at a dose of equivalent to 10^9 CFU/ml per mouse per day. On Day 11, a bolus of ethanol (5 g/kg body weight) was gavaged. Nine hours later, blood, liver and ileum tissues were collected (Fig. 1). All mice were treated according to the protocols reviewed and approved by the institution ethics committee of Wenzhou Medical University.

2.3. Biochemical analysis

Serum was collected and stored at −80 °C until use. Serum levels of ALT and AST were determined by the clinical laboratory of the First Affiliated Hospital of Wenzhou Medical University using automatic biochemistry analyser (AU5800, Beckman Coulter, USA).

2.4. Liver and ileum histology hematoxylin/eosin staining and liver Oil Red O staining

Tissues were harvested after mice were sacrificed. The paraffin-embedded tissue sections after fixation with 4% formalin were processed for staining with hematoxylin/eosin (HE). The frozen