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Preventive effect of α-linolenic acid-rich flaxseed oil against ethanol-induced liver injury is associated with ameliorating gut-derived endotoxin-mediated inflammation in mice

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ABSTRACT

The effects of α-linolenic acid (ALA)-rich flaxseed oil (FDO) against ethanol-induced liver injury and the probable molecular mechanisms in a mouse model of chronic-plus-single-binge ethanol feeding were evaluated. Mice were fed Lieber-DeCarli ethanol or control liquid diets with corn oil (CNO) or flaxseed oil for 10 days. On day 11, mice are gavaged with a single dose of ethanol or maltose dextrin. Ethanol exposure with CNO caused severe liver injury, inflammation and oxidative stress in liver, which were remarkably ameliorated by FDO. FDO supplementation decreased the elevation of plasma endotoxin level, which might be attributed to ameliorating ethanol-induced intestinal barrier dysfunction via upregulating the expressions of tight junction proteins. Additionally, FDO supplementation suppressed endotoxin-triggered inflammation via blocking TLR4/MyD88/NF-κB cascades in liver. These findings suggest that ALA-rich flaxseed oil may have potential to be developed as an effective agent for ethanol-induced liver injury.

1. Introduction

Light-to-moderate alcohol consumption is thought to be associated with a lower risk of cardiovascular diseases (Thakker, 1998). However, chronic heavy drinking has been widely considered to be a predominant cause of death and disability among young people with alcohol abuse, and approximately 25% of total deaths in the age group 20–39 years are alcohol-related worldwide (World Health Organization, 2014). Ethanol

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Abbreviations: ALA, α-linolenic acid; ALD, alcoholic liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; CNO, corn oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EtOH, ethanol; FDO, flaxseed oil; GSH, glutathione; IVC, , individually ventilated cage; LBP, LPS-binding protein; LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor κB; PUFAs, polyunsaturated fatty acids; PVDF, polyvinylidene fluoride; ROS, reactive oxygen species; SDS-PAGE, sulphate-polyacrylamide; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TG, triacylglycerol; TJ, tight junction; TLR4, toll-like receptor-4; ZO-1, zonula occludens-1

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and its metabolites exert deleterious effects on multiple organs, especially in the liver where ethanol is primarily metabolized (Yeh & Brunt, 2014).

Aside from the well-established causes of alcoholic liver injury, e.g., oxidative stress and direct toxicity of alcohol and its metabolites, an increasing number of studies have demonstrated that endotoxin-triggered hepatic inflammation plays a critical role in the pathogenesis of ethanol-induced liver injury (Louvet & Mathurin, 2015; Wang et al., 2013). Endotoxin is primarily composed of lipopolysaccharide (LPS) derived from cell membrane of Gram-negative bacteria. Significantly increased circulating endotoxin level was found in patients with alcoholic liver disease (ALD) (Fukui, Brauner, Bode, & Bode, 1991) and in experimental animals upon acute or chronic ethanol exposure (Fukui et al., 1991; Jokelainen, Reinke, & Nanji, 2001; Mathurin et al., 2000; Tsukamoto, Mkrtchyan, & Dynnyk, 2008), and the level of plasma endotoxin has been identified to be well correlated with the development of liver injury (Tsukamoto et al., 2008). In addition to the increased circulating endotoxin level, ethanol exposure sensitizes the liver to endotoxin-induced cellular injury and exacerbates inflammatory cytokine release in liver (Yang, Lin, & Diehl, 2001). Studies on the molecular events underlying endotoxin-triggered liver injury showed that circulating endotoxin activates hepatic Kupffer cells via Toll-like receptor-4 (TLR4)-mediated nuclear factor κB (NF-κB) signalling, resulting in overproduction of proinflammatory cytokines and chemokines, eventually leading to a vicious cycle of chronic inflammation in liver (Zhou et al., 2015). The circulating endotoxin is mainly originated from intestinal microflora, and gut leakiness caused by gut barrier dysfunction is considered as the major cause of ethanol-induced endotoxemia (Szabo, 2015). Indeed, ethanol and its metabolites, such as acetaldehyde, disrupt intestinal epithelial barrier and result in the increased intestinal permeability that facilitates the translocation of intestinal bacteria and bacterial endotoxin into portal vein (Bharrhan, Koul, Chopra, & Rishi, 2011; Mathurin et al., 2000). Recent studies have also indicated that ethanol exposure is associated with the dysbalance of pathogenic and beneficial bacteria in the intestinal microbiome (Fouts, Torralba, Nelson, Brenner, & Schnabl, 2012; Wang et al., 2015).

Low levels of n-3 polyunsaturated fatty acids (PUFAs) were found in blood and liver tissue biopsies from patients with ALD (Johnson, Gordon, McClain, Low, & Holman, 1985; Lakshman, 2004). The role of fish oil or long chain n-3 PUFA, e.g., eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), in ALD has been well documented (Huang et al., 2013; Song, Moon, Olsson, & Salem, 2008; Wada, Yamazaki, Kawano, Miura, & Ezaki, 2008). Alpha-linolenic acid (ALA, C18:3 n-3), a plant-derived n-3 PUFA, widely presents in flaxseed oil which is a traditional edible oil, and thus is more accessible and economical compared to EPA and DHA, although the conversion of ALA to EPA and DHA is limited (about 1–4%) (Taylor, Noto, Stringer, Froese, & Malcolmson, 2010). As a functional food ingredient, flaxseed oil or flax seed has been incorporated into baked foods, juices, dairy products, dry pasta products, and meat products (Kajla, Sharma, & Sood, 2015). However, little is known about the impact of ALA on ethanol-induced liver injury. Actually, ALA serves as the dietary precursor for EPA and DHA synthesis in mammals, and exhibits potent anti-inflammatory activity in numerous inflammatory conditions (Calder, 2006). The aim of present study, therefore, was to further investigate possible protective effects of ALA-rich flaxseed oil against ethanol-induced liver injury and the underlying mechanism related to gut-derived endotoxin-mediated inflammation in a mouse model of chronic-plus-single-binge ethanol feeding, which mimics acute-on-chronic ethanol-induced liver injury in patients (Bertola, Mathews, Ki, Wang, & Gao, 2013).

2. Materials and methods

2.1. Animals and treatments

Male C57BL/6 mice (8 weeks) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China), and were housed in institutional individually ventilated cage (IVC) system with 12-h light and dark cycles. A simple mouse model of alcoholic liver injury was induced by chronic ethanol feeding plus a single binge ethanol feeding (Bertola et al., 2013). Animals were initially fed the Lieber-DeCarli control diet *ad libitum* for one week to acclimatize them to liquid diet. Subsequently, mice were fed the modified Lieber-DeCarli liquid diets containing ethanol (EtOH-fed, n = 10) or isocaloric maltose dextrin as the control (Pair-fed, n = 10) with corn oil (CNO) or flaxseed oil (FDO) as fat for 10 days. The energy compositions of liquid diets were shown in Table 1. Thirty-five percent of total calories were provided by either corn oil (rich in linoleic acid) or flaxseed oil (rich in α-linolenic acid), respectively (Fig. 1). The liquid diets were freshly prepared from powder daily according to the manufacturer's instruction (TROPHIC Animal Feed High-tech Co., Ltd. Nantong, China). On day 11, EtOH-fed and pair-fed mice are gavaged with a single dose of 31.5 % (v/v) ethanol (5 g/kg BW) or isocaloric maltose dextrin, respectively. At the end of the experiment, mice were euthanized after fasting for 9-h, and their blood, entire liver and intestinal segments were collected for further analyses. All of the animal experiments were approved by the Animal Ethics Committee, Institute of Chinese Medical Sciences, University of Macau.

2.2. Measurements of plasma parameters

Plasma ethanol concentration was measured using an Ethanol Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). Plasma enzyme activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), and total bilirubin level were determined by the corresponding commercial assay kits (Nanjing Jiancheng

Table 1 – Caloric profile of the liquid diets.

	Corn oil		Flaxseed oil	
	Pair-fed	EtOH-fed	Pair-fed	EtOH-fed
Protein	18	18	18	18
Carbohydrate	47	19	47	19
Ethanol	-	28	-	28
Corn oil	35	35	-	-
Flaxseed oil	-	-	35	35

Values are expressed as the percentage of total calories.