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Oleanolic acid co-administration alleviates ethanol-induced hepatic injury via Nrf-2 and ethanol-metabolizing modulating in rats

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ABSTRACT

Alcoholic liver disease (ALD) is one of the leading causes of death in the world. Oxidative stress plays an important role in the pathogenesis of alcohol-induced liver injury. Our previous results have found that oleanolic acid (OA), a liver protective agent, plays a potent antioxidant activity in hepatocyte. In the present study, the protective effects of OA co-administration on ethanol-induced oxidative injury in rats were investigated through detecting hepatic histopathology, antioxidant enzymes, ethanol metabolic enzymes and inflammatory factors. Preventions of ethanol-induced oxidative injury by OA were reflected by markedly decreased serum activities of AST, ALT and significantly increased the hepatic ATP level. In addition, the increase of the hepatic TG content, MDA level and the decrease of hepatic GSH level, SOD activity, CAT activity induced by ethanol were significantly inhibited by OA co-administration. Furthermore, OA could also elevate the protein expressions and nuclear translocation of antioxidant transcription factor Nrf-2 and then up-regulated antioxidant enzymes expressions of HO-1, SOD-1 and GR. Moreover, OA co-administration can significantly reduce the activity and expressions of CYP2E1 and ADH, which has characteristic of generation ROS mediated oxidative stress and acetaldehyde respectively. Furthermore, OA co-administration could inhibition of the generation of inflammatory factors TNF-α and IL-6. Those above results indicated that OA co-administration can protect rats against ethanol-induced liver injury by induction Nrf-2 related antioxidant to maintain redox balance and modulating the ethanol-metabolizing and inflammatory pathway.

1. Introduction

It is generally believed that excessive drinking is harmful to private and public health. Alcoholic liver disease (ALD) has become one of the leading causes of death in the world [1]. Long term alcohol consumption can induce liver damage and cause alcoholic fatty liver, liver fibrosis, cirrhosis and even hepatocellular carcinoma. How to prevent alcohol-related liver injury as an important public health issue is urgent need to address [2]. Reactive oxygen species (ROS) are involved in a variety of diseases and the excessive generation of ROS could be induced by the situation of toxic compound uptake including ethanol abuse [3]. In hepatocytes ethanol can be metabolized via ethanol metabolism enzymes or cytochrome P450 2E1 (CYP2E1) system with the generations of ROS [4]. When the cellular antioxidant defense systems can't adapt to the ROS over-generations, it will bring about oxidative stress with pathologic consequences. As we know, pathological dose of ROS could induce damnification to the biological macromolecules such as protein, lipid and DNA. Oxidative stress is considered to be an important factor involved in the pathogenesis and development of ALD [5]. Under normal conditions, the body has a complex defense system to eliminate the damage of oxidative stress. ROS can be scavenged by the antioxidant defense system including antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT),

Abbreviations: ALD, alcoholic liver disease; ROS, reactive oxygen species; OA, oleanolic acid; EtOH, ethanol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; TG, triglyceride; ATP, adenosine triphosphate; GSH, glutathione; GSSG, oxidized form glutathione; Nrf-2, NF-E2-related factor-2; CAT, catalase; GR, glutathione reductase; SOD, superoxide dismutase; HO-1, heme oxygenase-1; ARE, antioxidant response element; MDA, malondialdehyde; LPO, lipid peroxidation; CYP2E1, cytochrome P4502E1; ADH, alcohol dehydrogenase; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; DMSO, dimethyl sulphoxide; PBS, phosphate buffered saline.

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glutathione reductase (GR) and nonenzymatic antioxidants such as glutathione (GSH) [6]. Nuclear factor erythroid 2-related factor 2 (Nrf-2) as a nuclear transcription factor plays an important role in the regulation of many defensive genes encoding detoxifying enzymes and antioxidant enzymes through interaction with antioxidant response element (ARE) [7]. Mild oxidative stress could induce the uncoupling of Nrf-2 and Keap1 and then Nrf-2 transfers into nuclear to play the role of antioxidant protection, but when excessive and severe ROS may lead to Nrf-2 activation disorder with the results of cell dysfunction, apoptosis or necrosis. It is reported that the activation of Nrf-2 could exert good protective effects against ethanol-induced apoptosis in nerve cells [8]. The level of inflammatory cytokines such as TNF-α and IL-6 was always increased in ALD models. The activation of inflammatory pathways is involved in the pathogenesis of ALD, and anti-inflammatory therapy could improve the symptoms of ALD in a certain degree [9]. Chronic ethanol consumption can induce the expression of CYP2E1 form of cytochrome P450 enzyme, which could metabolize many toxicological or exogenous substrates, including ethanol. Alcohol dehydrogenase (ADH) is a key enzyme in the metabolism of ethanol and is involved in the formation of acetaldehyde, a more toxic than ethanol. CYP2E1 and ADH play a pivotal role in ethanol metabolism and their over-expressions contribute to oxidative stress and the development of ALD [10]. The pathological process of ALD is very complicated and involved in a series of other factors such as abnormal nutrition metabolism, lipid metabolism disorders, mitochondrial dysfunction and endoplasmic reticulum stress. It is very important to develop new treatments oriented from the pathophysiological targets of ALD.

Previous studies have shown that moderate drinking of red wine has benefits for health and the important reason is that the red wine contains a lot of biological active substances such as resveratrol and other compounds which are believed to have antioxidant activities [11–13]. So it is a very promising approach to develop novel antioxidant and anti-inflammatory therapies for ALD. Among these therapies, natural antioxidants originating from traditional plants had attracted a lot of attentions because of their security and powerful biological activities [14]. This phenomenon noted us that co-administration the valid and innovative antioxidant with ethanol may prevent alcohol-induced liver injury and might have an important value in the actual application process. Oleanolic acid (OA), a triterpenoid saponin chemically named as 3β-hydroxy-olea-12-en-28-oic acid, exists widely in a lot of natural medicinal plants [15]. Drugs containing OA have been used for the treatment of hepatic diseases in traditional Chinese medicine with a long history [16]. We have previously reported that the OA possess potential antioxidant properties which could be associated with its alleged health benefits [17]. OA has been proved to advanced therapeutic activities to the oxidative stress relative diseases including diabetes [18], cancer [19] and inflammatory [20]. Moreover, the activation of Nrf-2 has been found to be involved in the antioxidant activity of OA [21]. OA increases the activities of many antioxidant enzymes account for its prominent cytoprotection effects against oxidative injury [22]. However, the solubility of OA is poor in water and the bioavailability is very low, which limiting its application in clinical practice [23]. It is noteworthy that OA can be dissolved in ethanol, which provides the possibility to increase its bioavailability by co-administration with ethanol. Although previous study have shown that suspension of OA at the dose of more than 50 mg/kg prepared in 1% gum tragacanth could possess hepatoprotective action, but that dose is too large for practical application process as drug. And the protective mechanism of OA is not very clear [24]. In order to improve the bioavailability of OA for better use in actual and study the protective mechanism, we chose to dissolve OA in ethanol in co-treatment way. The objective of this study was to analyze the protective effect and mechanism of oral OA co-administration at the dose of 10 mg/kg on alcohol-induced liver oxidative injury in rats. In this study, we prepared the OA dissolved in ethanol at the concentration of 1 mg/ml, which makes the solution is clear, colorless, odorless look and is easier to adapt the custom of drinking crowd in practice. We found that OA could ameliorate the pathological and toxicity effects associated with ethanol-induced oxidative stress and inflammatory reaction by interaction with antioxidant system and alcohol metabolizing enzymes.

2. Methods and materials

2.1. Chemicals and reagents

Oleanolic acid was purchased from Xi'an pharmaceutical research institute. Nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), glutathione (GSH) and oxidized form glutathione (GSSG) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The ATP, LDH, ALT and AST assay kits were purchased from Jianchen Corp (Nanjing, China). TG analysis kit was purchased from Biosino Biotechnology Corp (Beijing, China). The protein assay kit was from Thermo (USA). Nuclear fractionation isolation kit and RT-PCR one step cDNA synthesis kit were purchased from Beyotime Corp (Shanghai, China). RT-PCR amplification kit was purchased from TaKaRa Corp (Japan). GR, HO-1, SOD-1 and Nrf-2 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). ADH and Lamin B antibodies were purchased from Cell Signaling (Beverly, MA, USA). Tubulin and CYP2E1 antibodies were purchased from Abcam (Cambridge, MA). Anti-rabbit IgG and anti-mouse IgG were purchased from BOSTER Corp (Wuhan, China). All other chemicals were purchased from Tianjin Chemical Company (Tianjin, China) with highest purity commercially available.

2.2. Animals and experimental protocols

Adult Sprague-Dawley (SD) rats, weighing 180–200 g, were supplied by the Fourth Military Medical University Laboratory Animal Center. They were housed individually in plastic cages with free access to commercial rodent chow and water. Temperature (37 ± 1 °C) and humidity (60%, range 55–65%) were controlled by a completely automatic device. The dark/light cycle was 12/12 h (light on at 8.00 am; light off at 8.00 pm). Experimental rats were randomly divided into three groups as follows: (A) control group, negative control rats (n = 7), were orally given daily dose of saline (10 ml/kg) with an intragastric administration tube for 30 days; (B) ET group, positive control rats (n = 7), were orally given daily dose of ethanol (4 g/kg/day) with an intragastric administration tube for 30 days; (C) ETOA group, OA co-administration rats (n = 7), were orally given daily dose of OA (10 mg/kg/day) and ethanol (4 g/kg/day) for 30 days. Ethanol was diluted to 51.3% to prevent acute gastric irritation. For co-administration of OA with ethanol, OA was dissolved in ethanol and fed through intragastric injection. At the end of the experimental period, the final body weights of the animals were determined. All animal experiments were performed with the approval of the Fourth Military Medical University Animal Care and Use Committee.

2.3. Collection and preparation of tissues

The rats were killed by paralyzing after 12 h fasting. Fresh blood was immediately collected by cardiac puncture and plasma was obtained from the blood after centrifugation at 2000g for 20 min. The liver was quickly excised, minced in ice cold NaCl (0.9%), blotted on filter paper, weighed and then we calculated the ratio of liver weight and body weight. Part of livers homogenized in ice