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Lipoic acid protects gastric mucosa from ethanol-induced injury in rat through a mechanism involving aldehyde dehydrogenase 2 activation

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ABSTRACT

Numerous studies demonstrate that reactive aldehydes are highly toxic and aldehyde dehydrogenase 2 (ALDH2)-mediated detoxification of reactive aldehydes is thought as an endogenous protective mechanism against reactive aldehydes-induced cell injury. This study aims to explore whether lipoic acid, a potential ALDH2 activator, is able to protect gastric mucosa from ethanol-induced injury through a mechanism involving clearance of reactive aldehydes. The rats received 60% of acidified ethanol through intragastric administration and held for 1 h to establish a mucosal injury model. Lipoic acid (10 or 30 mg/kg) or Alda-1 (a positive control, 10 mg/kg) was given 45 min before the ethanol treatment. The gastric tissues were collected for analysis of gastric ulcer index, cellular apoptosis, 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) contents, and ALDH2 activity. The results showed that acute administration of ethanol led to an increase in gastric ulcer index, cellular apoptosis, 4-HNE and MDA contents concomitant with a decrease in ALDH2 activity; these phenomena were reversed by lipoic acid or Alda-1. The gastric protection of lipoic acid was attenuated in the presence of ALDH2 inhibitor. Based on these observations, we conclude that lipoic acid exerts the beneficial effects on ethanol-induced injury through a mechanism involving, at least in part, ALDH2 activation. As a dietary supplement or a medicine already in some countries, lipoic acid can be used to treat the ethanol - induced gastric mucosal injury.

1. Introduction

Gastric mucosal ulcer is a common gastrointestinal disease worldwide. Chronic gastric ulcer is closely related to infection with bacteria (such as Helicobacter pylori) (Almeida et al., 2015), irregular eating habits (Guo, Zhao, & Zhang, 2011), chronic bile reflux (Abe et al., 2005), stress or certain autoimmune disorders (Konturek, Brzozowski, & Konturek, 2011), whereas acute gastric ulcer is usually caused by excessive consumption of alcohol or high dose of nonsteroidal anti-inflammatory drugs (such as aspirin) (Choi et al., 2014; Wang, Zhang, Zhu, Ma, & Wang, 2015). There is evidence that high concentration of pure ethanol (40–80% v/v) can cause human hemorrhagic gastric ulcers in 30 min after consumption (Franke, Teyssen, & Singer, 2005). Ethanol is easily absorbed by gastrointestinal mucosa. In addition to direct damage to gastric mucosa, ethanol can also sensitize the mucosa to damage when it is no longer in contact with the mucosa. Ethanol-induced lesions can appear within 30 min, and reach a maximum after 60 min. Over consumption of some alcoholic beverages can also produce acute gastric ulcers (Knoll, Kolbel, Teyssen, & Singer, 1998; Luo et al., 2013a). Although numerous studies focus on alcohol-induced gastric mucosal injury, the underlying mechanisms are still not fully elucidated.

The mechanisms by which alcohol hurts the mucosa are multifaceted, such as impairment of the mucosal barrier, intercellular junction disorders, and alterations in epithelial transport, etc (Bujanda, 2000; Luo, Liu, Dai, Yang, & Peng, 2013b). Recently, it has been shown that ethanol-induced oxidative stress and lipid peroxidation contributes to the pathogenesis of acute gastric ulcer (da Silva, Boeing, Somensi, Cury, Steimbach, Silveria, & et al, 2015; Li et al., 2011). Usually, the generation of reactive oxidative species (ROS) in gastric tissue maintains at a basic level because of the balance between prooxidant and antioxidant system. However, the balance is disturbed in many situations including alcohol consumption. A recent study has demonstrated that alcohol is an oxidative stressor for gastric epithelial cells because cellular ROS

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levels were dramatically elevated after exposure to ethanol for 15 min (Tamura, Matsui, Kaneko, & Hyodo, 2013). Lipid peroxidation, a process in oxidative degradation of lipids in cell membranes, is a major mechanism responsible for oxidative injury caused by ROS. The main end products of lipid peroxidation are reactive aldehydes, including 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) (Calamaras et al., 2015). Reactive aldehydes, 4-HNE in particular, are highly toxic and can react with proteins to form various adduct, resulting in protein dysfunction and cellular damage (Gomes et al., 2015). The mitochondrial enzyme aldehyde dehydrogenase 2 (ALDH2) plays a major role in detoxification of reactive aldehydes in a range of organs and cell types (Luo, Liu, Ma, & Peng, 2014). Thus, ALDH2 might be a novel target for prevention of ethanol-induced gastric mucosal injury.

Lipoic acid, also known as α-lipoic acid, is an excellent antioxidant in many foods or naturally-made in our bodies. For many years, high doses of lipoic acid supplements have been used in parts of Europe for certain types of diseases such as type 2 diabetes and nerve pain (Cakici, Fakkel, van Neck, Verhagen, & Coert, 2016; Rochette, Ghibu, Muresan, & Vergely, 2015). Most of studies attribute the beneficial effects of lipoic acid to its antioxidative properties. Actually, in addition to acting as an antioxidant, lipoic acid can function as a co-factor for ALDH2 (Walters, Porter, & Brookes, 2012). Considering the major role of ALDH2 in detoxification of reactive aldehydes, we speculate that lipoic acid can protect gastric mucosa from ethanol-induced injury either through eliminating free radicals (by antioxidative activity) or reactive aldehydes (by activation of ALDH2).

Since the antioxidant properties of lipoic acid have been well recognized (Hiller et al., 2016; Shi et al., 2016), we thus focused on its potential role in activation of ALDH2 in the present study. By using a model of ethanol-induced gastric mucosal injury, we examined whether lipoic acid was able to protect the gastric mucosa against ethanol - induced injury; by using the specific activator of ALDH2 as well as the selective inhibitor of ALDH2, we investigate whether the beneficial effects of lipoic acid on gastric mucosa was related to preventing the accumulation of reactive aldehydes.

2. Methods

2.1. Animals

Male Sprague - Dawley rats (200–250 g) were obtained from Laboratory Animal Center, Xiang-Ya School of Medicine, Central South University, China. The animals were fasted for 24 h before the experiments, with free access to tap water. The study was performed following the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication, 8th edition, 2011), the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines and experiments were approved by the Central South University Veterinary Medicine Animal Care and Use Committee.

2.2. Ethanol-induced gastric mucosal injury

Gastric mucosal injury was induced by oral administration of 1 mL acidified ethanol (60% ethanol in 0.15 M HCl) via an orogastric feeding catheter. Gastric ulcer was formed 1 h after administration of ethanol.

2.3. Experimental protocol

The rats were randomly allocated to 7 groups (n = 6 per group). The control group (i) and the ethanol group (ii) received saline (0.9% NaCl, i.g.) and acidified ethanol (60% ethanol in 0.15 M HCl, i.g.), respectively. The remaining five groups were treated with low-dose lipoic acid (10 mg/kg, i.g.) (iii); high-dose lipoic acid (30 mg/kg, i.g.) (iv); Alda-1 (10 mg/kg, i.g. an activator of ALDH2, as a positive control) (v); high-dose lipoic acid plus daidzin (5 mg/kg, i.g., an inhibitor of ALDH2) (vi); or vehicle of lipoic acid, Alda-1 or daidzin (0.5% carboxymethyl cellulose sodium, i.g.) (vii), 45 min before ethanol treatment, respectively. All reagents were administered intragastrically. The optimal doses and time point for lipoic acid administration were determined by our preliminary experiments and the literature (He et al., 2012). Animals were sacrificed under anaesthesia (sodium pentobarbital, 60 mg/kg, i.p.) l h later after ethanol treatment. The stomachs of rats were removed for the assessment of gastric mucosal ulcer index first, and then saved for TUNEL assay and other measurements.

2.4. Evaluation of gastric mucosal ulcer index

An hour after ethanol treatment, rats were killed under anaesthesia and their stomachs were removed and photographed. A Guth scoring system with slight modification was used for ulcer index (UI) assessment (Guth, Aures, & Paulsen, 1979). In brief, lesion sizes (mm) were determined by measuring each lesion under magnifying glass. The greatest diameter was recorded in the case of petechial lesions. Four such lesions were considered to be the equivalent of 1 mm ulcer. The scores were relative value and were determined by the lesion size as follows: no lesion (score = 0), lesion <1 mm (score = 1), 1 < lesion<2 mm (score = 2), 2 < lesion<3 mm (score = 3), 3 < lesion<4 mm (score = 4). The scores for each sample were blindly evaluated and the mean UI of each group was obtained by dividing the total score for each group by the number of animals.

2.5. TUNEL assay

Apoptotic cells in gastric tissues were assessed by a terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. The fresh gastric tissues were cut into 1–2 mm-thick sections, which were subjected to a general protocol for paraffin embedment. The procedure for TUNEL assay in the paraffin-embedded gastric tissue sections was carried out following the manufacturer's instruction (Roche, Nutley, NJ, USA). Briefly, dewax and rehydrate the tissue sections according to standard protocols, then incubate them with Proteinase K Working Solution at room temperature for 20–30 min. After rinsing the slides with PBS, apply 50 μl TUNEL reaction mixture (or 50 μl Control Label solution for negative control) to each slide. After rinsing the slides 3 times with PBS, add 50 μl Converter-AP (alkaline phosphatase) on sample and incubate the slides at 37 °C for 30 min. At the end, add 50–100 μl substrate solution (NBT/BCIP, nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate) and incubate the slides for 10 min at room temperature in the dark. Slides were examined under microscope at 400× magnification and imaged by a high-resolution digital camera (Nikon Eclipse 80i, Tokyo, Japan). Dark blue-stained cells were identified as TUNEL-positive cells. Twenty high-power fields from each sample were randomly chosen and blindly quantitated. The number of TUNEL-positive cells was presented as percentage of the total cells.

2.6. Measurement of the activities of caspase −3 and ALDH2 in gastric tissue

Assays of the activities of caspase-3 (Beyotime, Shanghai, China) and ALDH2 (Abcam, Cambridge, MA, USA) were performed according to the manufacturer's instructions. For the measurement of caspase −3 activity, 10 μL of tissue homogenate was mixed with