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Expression of apoptosis-associated microRNAs in ethanol-induced acute gastric mucosal injury via JNK pathway

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ABSTRACT

MicroRNAs (miRNAs) have been shown to be closely associated with cellular apoptosis, but their involvement in response to ethanol-induced gastric mucosal epithelial cell apoptosis remains largely unknown. The purpose of this study was to investigate the expression profile of apoptosis-associated miRNAs in ethanol-induced acute gastric mucosal injury and the mechanisms underlying injury. Gastric mucosal injury was induced in rats by oral administration of ethanol, and gastric tissues were collected for analysis of gastric ulcer index, apoptosis ratio, caspase-3 activity, and miRNAs expression. Cell cultures of human gastric mucosal epithelial cells (GES-1) were incubated with ethanol to induce apoptosis. Mimics or inhibitors of miRNAs or c-Jun N-terminal kinase (JNK) inhibitor were added to the cell culture medium. GES-1 cells were collected for analysis of apoptosis ratio, caspase-3 activity, miRNAs expression, and protein phosphorylation levels of JNK, p38 mitogen-activated protein kinase (p38MAPK), or extracellular signal-regulated kinase (ERK). In the animal experiments, gastric ulcer index, cellular apoptosis, and caspase-3 activity were significantly increased, accompanied by up-regulation of miR-145 and down-regulation of the microRNAs miR-17, miR-19a, miR-21, miR-181a, and miR-200c. In the human cell culture experiments, the anti-apoptotic effects of miR-19a and miR-21 or pro-apoptotic effect of miR-145 were confirmed by their corresponding mimics or inhibitor; the ethanol-induced GES-1 apoptosis as well as the changes in miRNAs expression were significantly attenuated in the presence of JNK inhibitor. These results demonstrated that miR-145, miR-19a, and miR-21 were the apoptosis-associated miRNAs in gastric mucosal epithelial cells. The regulation of expression of these 3 miRNAs in ethanol-induced GES-1 apoptosis involved the JNK pathway.

Introduction

Gastritis is a very prevalent, global gastrointestinal disease. Chronic gastritis is closely related to infection with bacteria (primarily *Helicobacter pylori*) (Gulcan, Ozen, Karatepe, Gulcu, & Vitrinel, 2010), chronic bile reflux (Oh et al., 2005), and stress or certain autoimmune disorders (Melcescu et al., 2012). The main causes of acute gastritis are excessive consumption of alcohol or prolonged use of nonsteroidal anti-inflammatory drugs (also known as NSAIDs), such as aspirin (Chamberlain, 1993; Katsinelos et al., 2006). It has been reported that high concentrations of pure ethanol (40–80% v/v) are able to damage the human gastric mucosa (to the point of hemorrhagic gastritis) in only 30 min after application (Franke, Teyssen, & Singer, 2005). The lesions can appear within 30 min, and reach a maximum after 60 min. Similar effects were also found in some alcoholic beverages (Knoll, Kölbel, Teyssen, & Singer, 1998). However, the pathophysiological mechanisms for ethanol-induced acute gastric mucosal injury are not completely understood.

Alcohol is easily absorbed by gastrointestinal mucosa and exerts detrimental effects on gastric mucosa. In addition to direct damage to gastric mucosa, alcohol can also sensitize the mucosa to damage from other factors when the alcohol is no longer in contact with the mucosa. The mechanisms by which alcohol damages the mucosa are multiple, such as altering epithelial transport, triggering intercellular junction disorders, and impairing the mucosal barrier (Bor, Bor-Caymaz, Tobey, Abdulnour-Nakhoul, & Orlando, 1999; Bujanda, 2000). Recently, there has been evidence that ethanol-induced gastric epithelial cell apoptosis also contributes to gastric mucosal damage (Li, Luo, et al., 2012; Yamamoto et al., 2012; Ye et al., 2012). Although it is known that ethanol-induced cellular apoptosis is involved in inflammation, oxidative stress, chemical stimulation,

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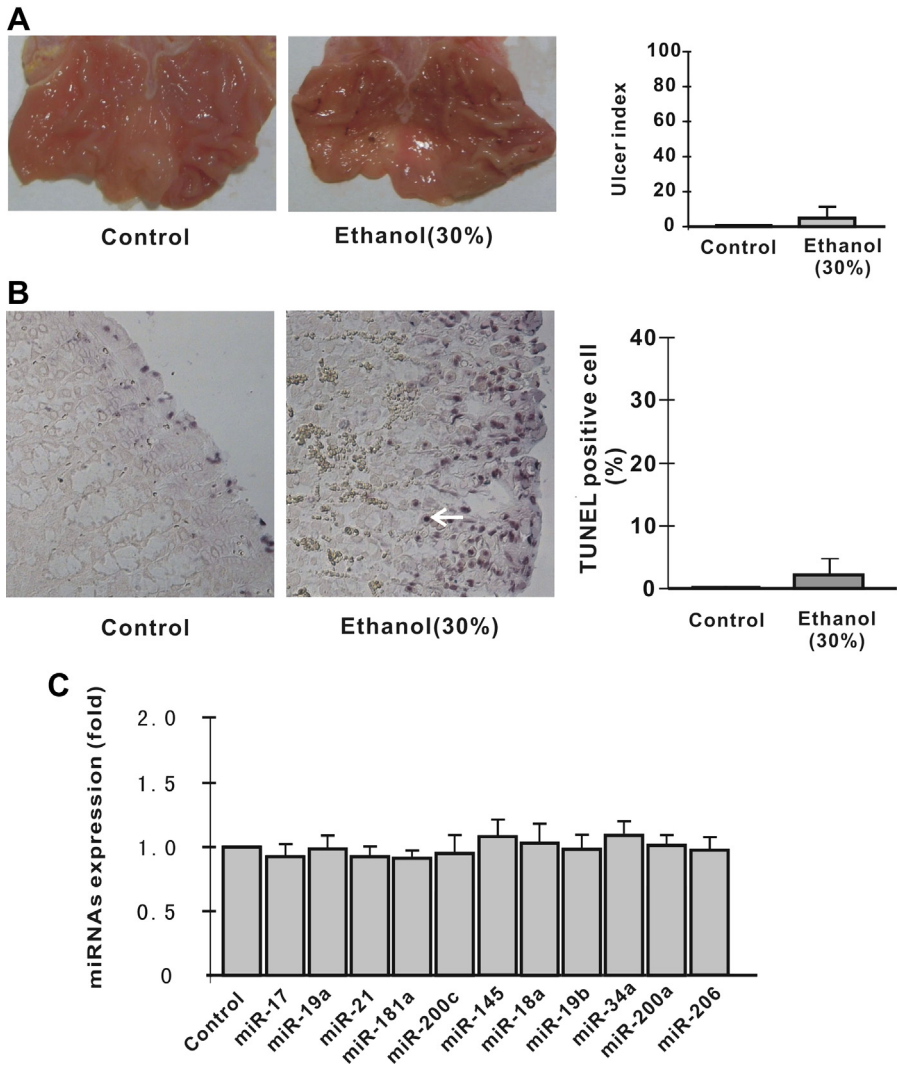

Fig. 1. Effects of low dose (30%) ethanol exposure on gastric mucosal damage, cellular apoptosis, and miRNAs expression. A. Left, representative images from each group, illustrating ethanol-induced gastric ulcer in stomach from 30% ethanol-exposed rat; right, the ulcer index in each group. B. Left, representative images from each group, illustrating ethanol-induced cellular apoptosis in stomach from 30% ethanol-exposed rat (positively stained cells are indicated by arrow); right, the percentage of TUNEL-positive cells in each group. C. Apoptosis-related miRNAs expression in gastric tissue from each group. Values are mean ± S.E.M. $n = 6$ in each group.

etc., the exact mechanisms for these phenomena are not well known.

miRNAs are small (~21 nucleotide), noncoding RNAs that participate in post-transcriptional regulation of target mRNAs, usually resulting in translational repression or target degradation and gene silencing (Pritchard, Cheng, & Tewari, 2012). Due to their abundant presence, miRNAs have many different physiological functions in cell differentiation, proliferation, metabolism, etc., and are involved in many human diseases and pathophysiological processes (Singh, Brand, & Mehla, 2012). It has been reported that some miRNAs (such as miR-17, miR-19a, and miR-21) are anti-apoptotic, while some (such as miR-181a, miR-200c, and miR-145) are pro-apoptotic (Li, 2010; Yang, Lu, & Wang, 2009). The anti-apoptotic miRNAs mediate their effects by targeting pro-apoptotic mRNAs or their positive regulators, whereas the pro-apoptotic miRNAs exert their effects by targeting anti-apoptotic mRNAs or their positive regulators (Yang et al., 2009). Although the connection of miRNAs to apoptosis is well established, their precise function in response to ethanol-induced gastric epithelial cell apoptosis remains largely unknown.

To find the answer, we carefully selected 11 apoptosis-associated miRNAs for the following studies. First, we examined the expression profiles of the apoptosis-associated miRNAs in the gastric mucosa by using a rat model of ethanol-induced acute gastric mucosal injury. Then we verified the correlation between the identified miRNAs and apoptosis in cultured human gastric mucosal epithelial cells. Finally, using a cell model of ethanol-induced apoptosis, we explored the correlation between the verified miRNAs and the JNK pathway, a well-recognized signaling pathway involved in cellular apoptosis.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 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 carried out according to the Guide for the Care and Use of