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Protective effect of bovine milk against HCl and ethanol–induced gastric ulcer in mice

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ABSTRACT

The purpose of this study was to investigate the gastroprotective effects of bovine milk on an acidified ethanol (HCl-ethanol) mixture that induced gastric ulcers in a mouse model. Mice received different doses of commercial fresh bovine milk (5, 10, and 20 mL/kg of body weight) by oral gavage once a day for 14 d. One hour after the last oral administration of bovine milk, the HCl-ethanol mixture was orally intubated to provoke severe gastric damage. Our results showed that pretreatment with bovine milk significantly suppressed the formation of gastric mucosa lesions. Pretreatment lowered gastric myeloperoxidase and increased gastric mucus contents and antioxidant enzymes catalase and superoxide dismutase. Administration of bovine milk increased nitrate/nitrite levels and decreased the malondialdehyde levels and the expression of proinflammatory genes, including transcription factor NF-κB, cyclooxygenase-2, and inducible nitric oxide synthase in the stomach of mice. These results suggest that bovine milk can prevent the development of gastric ulcer caused by acid and alcohol in mice.
Key words: bovine milk, gastric ulcer, gastroprotective effect, antioxidant enzyme activity, anti-inflammatory effect

INTRODUCTION

Gastric ulcer is one of the common diseases, affecting more than 10% of the world's population (Liu and Cho, 2000). Etiological factors of gastric ulcer include alcohol abuse, smoking, stress, drug overuse, and microorganism infection (Kim et al., 2014). Of these factors, alcohol can perturb the gastric mucosa and induce numerous metabolic changes, leading to mucosal damages and lesions in the stomach (Szabo et al., 1985). Alcohol also increases the generation of reactive oxygen species (**ROS**) and lipid peroxidation while suppressing the activity of antioxidant enzymes such as superoxide dismutase (**SOD**), catalase (**CAT**), and glutathione peroxidase. These enzymes play important roles in protecting stomach against mucosa damages (Pan et al., 2008). Alcohol is an ulcerogenic substance that induces mucosal damage, which reduces intraluminal acid and promotes back diffusion of hydrogen ions. It also directly damages the oxyntic (parietal) cells and increases the gastric mucosal permeability by the increase in back diffusion and the transmucosal potential difference, which reflects surface cell layer exfoliation (MacMath, 1990; Keshavarzian et al., 1994). In addition, alcohol-induced mucosal damage can increase gut permeability, which allows the transport of bacterial endotoxins that are normally unable to cross the intestinal wall to enter the blood or lymph (Bode, 1980).

Milk contains various beneficial nutrients and, as such, it has been used as a valuable food source (Chung, 2010). Milk consumption is generally believed to be an important element in a healthy and balanced diet. Milk contains various bioactive peptides, including digestive health peptides (Mohanty et al., 2016); however, to the best of our knowledge, systemic assessment of a gastroprotective effect of bovine milk, especially against gastric damage induced by hydrochloride (HCl) and ethanol, has not been reported in an animal model such as the mouse. We hypothesized that bovine milk has protective effects against gastric mucosal damage via modulating antioxidant enzymes and anti-inflammatory activities. To test this hypothesis, we determined the gastroprotective effect of bovine milk against oxidative damages induced by HCl and ethanol in mice.

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Table 1. Experimental design, with milk administered as a pretreatment for 14 d

Group	Animals (n = 10)	Test substance[1] (mg/kg)
Normal control	Untreated mice	Distilled water at 20 mL/kg of BW, oral administration
HE control	Gastric ulcer mice	Distilled water and HE administration
Experiment group	Gastric ulcer mice	Milk 5 mL/kg and HE administration
Experiment group	Gastric ulcer mice	Milk 10 mL/kg and HE administration
Experiment group	Gastric ulcer mice	Milk 20 mL/kg and HE administration

[1]Milk = commercial fresh bovine milk (Na 100%, Seoul Dairy Corp., Seoul, Korea); HE = HCl-ethanol mixture (98% ethanol containing 150 m*M* HCl).

MATERIALS AND METHODS

Animals

Forty male ICR mice (6 wk old, 34.34 ± 1.60 g) were purchased from OrientBio (Gyeonggi-do, Korea). Mice were housed in cages (20–25°C, 30–35% humidity, and a 12-h light/dark cycle). They were provided free access to water and standard rodent chow (38057, Purinafeed, Gyeonggi-do, Korea). After acclimation for 7 d, animals were randomly divided into 5 groups (n = 8 for each group, Table 1). All animal procedures were performed in accordance with guidelines issued by the Animal Care and Use Committee of Gachon University for the care and use of laboratory animals (approval number: GIACUC-R2016014).

Preparations and Administration of Test Materials

Commercial fresh bovine milk (Na 100%; Seoul Dairy Corp., Seoul, Korea) was purchased from a local market. Bovine milk was diluted with distilled water at a ratio of 1:4 and orally administered to mice once a day for 14 d. In our study, bovine milk was divided into 3 doses (5, 10, and 20 mL/kg of BW). The concentrations selected in the study were based on the daily milk intake of Koreans. The recommended intake of milk in South Korea is 400 mL, but, according to the Korean Nutrition Survey, the daily milk intake is only 66.4 mL (Korea Health Industry Development Institute, 2014). The highest dose of milk in the current study was 20 mL/kg, which is equivalent to half a cup of milk; the middle dose (10 mL/kg) is equivalent to the average daily intake of Koreans. The human equivalent dose of the highest dose of milk would be an intake of 112 mL/d for an individual of 70 kg of BW [human equivalent dose (mg/kg) = mouse dose (mg/kg) × mouse Km (3)/human Km (37), where Km is the factor for converting mg/kg dose to mg/m^2 dose (BW to surface area)], as calculated by the body surface area normalization method (Reagan-Shaw et al., 2008). In addition, the highest dose corresponds to the limit highest oral dosage volume in mice (Flecknell, 1996; Korea Food and Drug Administration, 2015), and 10 and 5 mL/kg were selected as middle and lower dose groups using a common ratio of 2. Equal volumes of distilled water (20 mL/kg) were administered orally to mice in the normal control group and HE control group (Table 1).

Induction of Gastric Mucosa Damages

Mice were deprived of food for 24 h in a cage with wide-mesh wire bottoms to prevent coprophagia before conducting the experiment. One hour after the last (the 14th) administration of vehicle, an HCl and ethanol (**HE**) mixture (98% ethanol containing 150 m*M* HCl) was orally administered to mice at 5 mL/kg of BW according to a previous report (Oyagi et al., 2010). Untreated control mice were administered an equal volume of distilled water instead of HE solution.

Quantification of Gross Lesion

Animals were euthanized at 1 h after treatment with the HE mixture by cervical dislocation under inhalation anesthetized with 2 to 3% isoflurane (Hana Pharm. Co., Hwasung, Korea) in the mixture of 70% N_2O and 28.5% O_2, using rodent inhalation anesthesia apparatus (Surgivet, Waukesha, WI) and rodent ventilator (model 687, Harvard Apparatus, Cambridge, UK). The abdomen of each mouse was cut with a median incision and the stomach was removed. The excised stomach was opened along the greater curvature and rinsed with cold saline solution to remove gastric contents and blood clots. After examining the ulcer area on stomach, digital images of the ulcer were acquired and analyzed using an automated computer-based image analyzer (iSolution FL ver 9.1, IMT i-solution Inc., Vancouver, Quebec, Canada) according to methods of Suleyman et al. (2009), Morais et al. (2010), and Oyagi et al. (2010) with some modifications. Ulcer lesions were measured to calculate gastric damage score. For this purpose, total areas of ulcerous stomach regions were calculated as millimeters squared of gastric mucosa.

Determination of Gastric Myeloperoxidase Activity

Tissue samples (about 0.2 g) were homogenized in 2 mL of ice-cold potassium phosphate buffer (50 m*M*

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