International Immunopharmacology 34 (2016) 183–188

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Preliminary report

Gastroprotective effect of 2-mercaptoethane sulfonate against acute gastric mucosal damage induced by ethanol

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ARTICLE INFO

Article history:
Received 31 January 2016
Received in revised form 28 February 2016
Accepted 4 March 2016
Available online 9 March 2016

Keywords:
MESNA
Inflammation
Antioxidant
Gastric mucosal damage
Ethanol

ABSTRACT

Gastric mucosal damage induced by ethanol is a serious medical problem. Recent evidences suggest that reactive oxygen species and inflammatory mediators play a key role in the destruction of gastric mucosa. The present study was aimed to evaluate the potential beneficial effect of MESNA (2-mercaptoethane sulfonate) against ethanol-induced gastric mucosal damage in mice. The animals were orally pretreated with vehicle or MESNA and then treated with acidified ethanol to induce gastric mucosal damage. One hour after ethanol ingestion mice were euthanized and stomach samples were collected for biochemical analysis. Macroscopic and histopathological evaluation of gastric mucosa showed that pretreatment with MESNA attenuated gastric lesions induced by ethanol. Administration of MESNA significantly increased glutathione content and superoxide dismutase and catalase activity in the gastric tissues. In addition, MESNA markedly reduced ethanol-induced lipid peroxidation, myeloperoxidase activity, tumor necrosis factor-alpha, interleukin (IL)-1β, IL-6, and monocyte chemotactic protein-1 levels. These findings suggest that the thiol-containing compound MESNA is able to decrease alcohol-induced oxidative stress and inflammation in the gastric tissue. It seems that MESNA may have a protective effect against ethanol-induced gastric mucosal damage.

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1. Introduction

Gastric ulcer is the most prevalent gastrointestinal disorder that affects many people worldwide. Although the mechanism of gastric ulcer has not been completely elucidated, it is shown that the gastric mucosal injury results from an imbalance between aggressive and protective factors. The risk factors that contribute to the formation of gastric lesions include consumption of alcohol, non-steroidal anti-inflammatory drugs (NSAIDs), increased secretion of hydrochloric acid, *Helicobacter pylori*, stress, and tobacco [1]. It has been established that ethanol as an exogenous irritant damages the gastrointestinal mucosal cells and leads to gastric ulcers by different molecular mechanisms. Experimental and clinical studies showed that the reactive oxygen species (ROS) and inflammatory mediators induced by ethanol contribute to the pathogenesis of ethanol mediated gastric lesions. After entering cells, ethanol is metabolized and facilitates production of the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl free radical ($HO^{\bullet}$). Generation of highly reactive oxygen species induces lipid peroxidation of cellular membranes and leading to cell death and epithelial damage. Furthermore, in the gastric mucosal cells, ethanol significantly reduces the levels of major antioxidants that can eliminate reactive oxygen species [2,3]. It has also been demonstrated that neutrophil infiltration into the gastric mucosa and release of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and chemokine MCP-1 are involved in the pathogenesis of gastric mucosal injury [4–7].

Recently, many studies have focused on novel approaches for prevention of gastric mucosa from alcohol-induced injury. Several compounds with antioxidant and anti-inflammatory activity have been studied for their gastroprotective effects [3,5,6,8,9]. 2-Mercaptoethane sulfonate (MESNA) is a small, synthetic, sulfhydryl-containing molecule that has the ability to scavenge reactive oxygen species through its antioxidant effects. MESNA is frequently used with cyclophosphamide and ifosfamide to reduce hemorrhagic cystitis induced by acrolein, a toxic urinary metabolite of oxazaphosphorine-alkylating agents. Sulfhydryl group present in MESNA binds to acrolein within the urinary tract and forms nontoxic stable thioether that is safely eliminated in the urine. Adverse effects associated with MESNA are few and well tolerated by most patients. Several studies have been shown that MESNA is effective in the treatment of oxidative stress induced disorders. It has been reported that MESNA prevents doxorubicin-induced plasma protein oxidation, cisplatin-induced ovarian damage and ischemia reperfusion-induced oxidant damage of the intestinal mucosa [10–12]. The present study was aimed to investigate the effectiveness and the possible mechanisms of action of MESNA in a mouse model of ethanol-induced gastric mucosal damage.

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http://dx.doi.org/10.1016/j.intimp.2016.03.006
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2. Material and methods

2.1. Animals

Experiments were performed on male Swiss albino mice weighing 26–30 g. Animals were kept in our animal house under controlled conditions and allowed free access to tap water and a standard diet. Mice were fasted overnight with free access to water before the induction of gastric mucosal lesions. All animal procedures were carried out in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH publication 85–23, revised 1996).

2.2. Materials

5,5′-Dithiobis-2-nitrobenzoic acid, tetramethylbenzidine, glutathione reduced, Tris–HCl buffer, hydrogen peroxide, thiobarbituric acid, trichloroacetic acid and 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich. MESNA was obtained from Baxter (Germany). Ethanol was purchased from Taghtir Co. (Iran). Protease inhibitors (Complete Mini tablets) were purchased from Roche (Germany).

2.3. Experimental design

Intragastric administration of ethanol/HCl has long been used as an experimental method to induce gastric mucosal damage in animals. HCl in ethanol solution accelerates the progress of ulcerogenesis and enhances ethanol-induced gastric mucosal toxicity [13,14]. Mice were randomly allocated into four groups (8 mice each). The first group received normal saline orally and served as the control group. The second group of mice was given MESNA alone. The third group of mice was given a single dose of acidified ethanol. The fourth group was given 400 mg/kg MESNA and acidified ethanol. MESNA administered orally 1 h before application of the ulcerogenic agent. One hour after treatment with MESNA the animals received acidified ethanol (60% ethanol/0.3 M HCl, 0.1 ml/10 g body weight, orally) to induce gastric mucosal injury. The dose of MESNA used in this study (400 mg/kg) was selected based on our preliminary experiments and previous studies [15,16].

2.4. Sample collection

One hour after administration of ethanol/HCl mice were anesthetized with ketamine and xylazine and their stomachs were excised. The stomachs were opened along the greater curvature and gently washed with cold saline solution to remove the gastric contents. The gastric tissue samples were frozen in liquid nitrogen and kept at −80 °C for biochemical determinations.

2.5. Determination of gastric mucosal injury index

Macroscopic scoring of gastric mucosal damage was performed by light microscopy. The severity of gastric damage induced by ethanol/HCl was expressed as an ulcer index (UI) and calculated based on the number and diameter of the lesions [13]. $UI = \Sigma(A) + (2B) + (3C)$ (A is the number of small lesions up to 1 mm; B is the number of lesions up to 3 mm; and C is the number of linear lesions >3 mm). The mean ulcer index was calculated for each group and then statistically analyzed.

2.6. Histopatological analysis

A small specimen of each stomach was fixed in 10% formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E). The histopathology examination was performed under light microscopy by an experienced pathologist according to the method as described previously [17]: (1) hemorrhage (score 0–4), (2) mucosal edema (score 0–4), (3) epithelial cell loss (score 0–3), and (4) inflammatory cell infiltration (score 0–3).

2.7. Preparation of gastric tissue homogenate

The gastric tissues were cut into small pieces and homogenized in ice-cold Tris–HCl buffer (pH 7.4, containing protease inhibitor cocktail) using a homogenizer (Heidolph, Germany). The homogenates were centrifuged at 20,000 ×g for 20 min in a refrigerated centrifuge at 4 °C. The supernatants were collected and stored at −80 °C until analysis.

2.8. Measurement of malondialdehyde (MDA) levels

Gastric tissue MDA level was determined using the thiobarbituric acid method as described in our previous study [4]. Briefly, 100 μl of the supernatant was added to a reaction mixture containing 100 μl of 20% (w/v) trichloroacetic acid (TCA) and 100 μl of TBA (0.1 M). The mixture was incubated in a boiling water bath for 60 min to complete the reaction. After cooling, the samples were centrifuged at 10,000 ×g for 5 min at 4 °C and the supernatants were separated from the pellets. The absorbance of the supernatants was measured at 532 nm using a 96-well microplate reader (BioTek, USA).

2.9. Measurement of reduced glutathione (GSH) content

To determine GSH, 50 μl of 10% trichloroacetic acid (TCA) was added to 50 μl of the supernatant to precipitate the proteins. The mixture was centrifuged at 10,000 ×g for 5 min at 4 °C and the supernatants were separated from the pellets. 50 μl of the resulting supernatant was added to 150 μl of DTNB (5,5′-Dithiobis-2-nitrobenzoic acid) in phosphate buffer (0.2 M, pH 7.6, 1 mM EDTA). The intensity of the yellow color formed was read at 412 nm using a microplate reader.

2.10. Measurement of superoxide dismutase (SOD) activity

The activity of superoxide dismutase in gastric supernatant samples was measured using a SOD assay kit (BioVision) according to the manufacturer's protocol.

2.11. Measurement of catalase (CAT) activity

CAT activity was assessed according to the method as described previously [18]. Briefly, 10 μl of the supernatant was added to a cuvette containing 0.5 ml of phosphate buffer (50 mM, pH 7). Reaction was started by the addition of 0.5 ml of H_2O_2 (30 mM). The rate of change in absorbance was measured at 240 nm and CAT activity was defined as the quantity of enzyme degrading 1 mmol of H_2O_2 per minute.

2.12. Measurement of myeloperoxidase (MPO) activity

The activity of MPO in gastric tissue samples was determined according to our previous study [4]. Briefly, The reaction mixture for analysis consisted of 50 μl sample, 50 μl of 15 mM tetramethylbenzidine (TMB) and 100 μl of hydrogen peroxide (25 mM) diluted in phosphate buffer (50 mM, pH 5.4). The assay was performed in a microplate and the reaction was started by adding hydrogen peroxide. The rate of change in absorbance was measured at 370 nm and MPO activity was defined as the quantity of enzyme degrading 1 μmol of hydrogen peroxide per min at 25 °C.

2.13. Measurement of pro-inflammatory cytokines

The cytokine levels of TNF-α, IL-1β, IL-6 and MCP-1 were measured by using mouse ELISA kit from eBioscience according to the manufacturer's instructions.

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