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Antioxidant effect of zinc chloride against ethanol-induced gastrointestinal lesions in rats

Rafael Porto Ineu [a,*], Cláudia Sirlene Oliveira [a], Vitor Antunes Oliveira [a], Lucélia Moraes-Silva [a], Sônia Cristina Almeida da Luz [a,c], Maria Ester Pereira [a,b,*]

[a] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, Universidade Federal de Santa Maria – UFSM, 97105-900 Santa Maria, RS, Brazil
[b] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria – UFSM, 97105-900 Santa Maria, RS, Brazil
[c] Departamento de Patologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil

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ABSTRACT

The aim of the present study was to evaluate the possible effects of zinc chloride against the gastrointestinal lesions caused by oral administration of ethanol in rats. Rats were divided into five groups, namely, saline, ethanol, zn, zn + ethanol and ethanol + zn. Ethanol 70% (2 mL/kg) was administered by gavage in 36 h fasted rats. Zinc chloride (27 mg/kg, ~13 mg/kg of zinc) was given by gavage 1 h before or 1 h after the administration of ethanol. Oral administration of ethanol consistently induced damage in the rat glandular stomach and intestine. Zinc did not demonstrate effect *per se* and significantly reduced gastrointestinal lesions when administered either before or after lesion induction. Ethanol induced enhancement of thiobarbituric acid reactive substance and reactive species levels, diminished the ascorbic acid and total protein SH content as well as superoxide dismutase and catalase activity in stomach and intestine of rats. Zinc treatment prevented and reversed these alterations induced by ethanol. Stomach and intestine of rats treated with zinc presented higher zinc content than the tissues of rats treated only with ethanol. Non-protein SH content was not altered by any treatment. Results suggested that the gastrointestinal protective effect of zinc in this experimental model could be due to its antioxidant effect.

1. Introduction

Gastric mucosal erosion was reported to be associated with the imbalance between the aggressive factors in the lumen and protective mechanisms in the duodenal mucosa. Gastrointestinal problems have now become a global problem, and many studies were carried out towards fixing it (Alvarez-Suarez et al., 2011; Ineu et al., 2008).

The etiology of gastric damage caused by ethanol is still not fully understood; however, it has been shown to produce gastric damage by impairing gastric defensive factors such as mucus and mucosa circulation (Cemek et al., 2010; Savegnago et al., 2006; Szabo et al., 1992). In addition, the gastric damage caused by ethanol may be due to the increase of oxygen radicals and lipid peroxidation (Alvarez-Suarez et al., 2011; Mutoh et al., 1990). Reactive oxygen species (ROS) provoke severe changes at the cellular level leading to cell death because of their extreme reactivity. They attack essential cell constituents, leading to the formation of toxic compounds (Kahraman et al., 2003). Both superoxide anion radical (O_2^{-*}) and hydroxyl radical ($^*OH$) are involved in tissue damage through initiation of lipid peroxidation and disruption of the interstitial matrix (Gu et al., 2012; Hogg et al., 1992). Indeed, including our previous study and others, wide types of compounds have been tested to avoid the effects of ROS into the cells (Ineu et al., 2008; Nogueira et al., 2004; Savegnago et al., 2006).

Zinc homeostasis is important for the integrity of gastric mucosal cells and is a key factor for the preservation of the structural integrity of the intestinal barrier (Lambert et al., 2004). A reduction in zinc content of the mucosa is observed in patients affected by ulcerative colitis, which is associated with an increase in reactive oxygen intermediates (Faa et al., 2008). Several key enzymes present in the epithelial cells of the intestinal mucosa, such as carbonic anhydrase are metalloenzymes and require zinc for their action (Kiefer and Fierke, 1994). Also, zinc is a cofactor for enzymes like superoxide dismutase, collagenase, alcohol dehydrogenase, alkaline phosphate and in spermatogenesis process (Mei et al., 2009). Zinc complexes have also been shown to have antiulcer activity. Zinc–carnosine is an antiulcer drug commonly used in the treatment of gastric ulcers in Japan (Odashima et al., 2006). The zinc–indomethacin and zinc–naproxen complexes reduce more significantly the ulcerogenic effects compared with similar drugs, without affecting its therapeutic action (Sharma et al., 2003; Singla

* Corresponding authors. Address: Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria – UFSM, 97105-900 Santa Maria, RS, Brazil. Tel.: +55 3220 8799; fax: +55 3220 8978.
E-mail addresses: rafaelineu@gmail.com (R.P. Ineu), pereirame@yahoo.com.br (M.E. Pereira).

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and Wadhwa, 1995). Studies have revealed that a significant fraction of ingested alcohol is oxidized in gastric mucosa cells by a zinc-dependent enzyme, alcohol dehydrogenase (Mutoh et al., 1990). This metabolic step is known as the first-pass metabolism of ethanol and is considered a gastrointestinal barrier against the systemic toxicity of ethanol (Gu et al., 2012; Julkunen et al., 1985).

It has been known that an excessive intracellular increase of zinc can be cytotoxic and cause neuronal degeneration (Choi and Koh, 1998). However, recent studies have shown that zinc can be used as an important treatment against several toxicants, such as toxic metals. In line with this, previous studies from our group demonstrated that zinc acts as a protector agent against several mercury toxic effects in rats (Franciscato et al., 2011; Peixoto et al., 2003).

The aim of the present study was to evaluate the gastrointestinal protective effects of zinc chloride ($ZnCl_2$) against the lesions caused by oral administration of ethanol in rats and the possible antioxidant effects of $ZnCl_2$ against damage induced by ethanol in stomach and intestine of rats. In line with the previous results about zinc, this is the first time that the gastrointestinal benefits effects of $ZnCl_2$ against lesions caused by ethanol in rats are checked.

2. Materials and methods

2.1. Animals

A total of 34 male adult Wistar rats (225 ± 10 g) from our own breeding colony were used (6–7 animals per group). Each cage contained five animals, on a 12-h light: 12-h dark cycle, at a room temperature of 22–24 °C, and with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria RS, Brazil.

2.2. Materials

Reagents used were obtained from Sigma Chemical Co. (St. Louis, MO) and standard commercial suppliers. The commercial kit for biochemical dosages was obtained from Labtest Diagnostica SA, Brazil.

2.3. In vivo and ex vivo experiments

2.3.1. Ethanol-induced gastric lesions and zinc treatment

The gastric lesions were induced with ethanol according to the method described by Robert (1979). The lesions were induced in 36 h fasted rats. For this purpose the rats were kept in a cage equipped with a gate apparatus that hindered the animals feeding, allowing only water. Absolute ethanol (etOH) was diluted at 70% in distilled water and administrated by gavage (v/v, 2 mL/kg, p.o.). The $ZnCl_2$ at dose of 27 mg/kg (1 mL/kg) (~13 mg/kg of Zn^{2+}) (Peixoto et al., 2003) was dissolved in saline 150 mM and administered (p.o.) 1 h before or 1 h after etOH administration. The animal groups were following:

Group 1 – saline + saline

Group 2 – etOH + saline

Group 3 – zinc + saline

Group 4 – zinc + etOH

Group 5 – etOH + zinc

In the zinc pre-treatment protocol, 1 h after the administration of ethanol, rats were sacrificed by decapitation. In the zinc post-treatment protocol, animals were sacrificed also by decapitation 1 h after Zn administration. The tissues were immediately removed to measure the biochemical parameters and stomachs were used to determine the gastric lesion index.

2.3.2. The gastric lesion index

The stomachs were removed, opened along the greater curvature and fixed to determine the gastric lesion index. The ulcerative lesion index of each animal was calculated according to Gamberini et al. (1991) and using score as follows: loss of mucosal folding, mucosal discoloration, edema or hemorrhage (score 1 each); ulcers/cm^2 less than 1 mm (score = number of ulcer × 2); ulcers more than 1 mm/cm^2 (score = number × 3); perforated ulcers (score = number × 4). The observer of gastric lesions was blinded to the treatment. The stomachs of the rats from all five groups were removed and examined morphologically and histological. The gastric tissue was fixed in 10% neutral formalin and embedded in paraffin. Sections from tissue blocks were stained with hematoxylin–eosin (HE).

2.3.3. Tissue preparation

After 1 h of the last exposure, rats were euthanized by decapitation for blood and tissues collection. Serum was obtained by centrifugation at 2000*g* for 10 min (hemolyzed serum was discarded). Stomach, kidney and intestine (three portions of beginning, medium and end of intestine) were quickly removed, cleaned and homogenized in 10 volumes of 50 mM Tris–HCl, pH 7.4. The homogenate was centrifuged at 2000*g* at 4 °C for 10 min and a low-speed supernatant fraction (S1) was used for *ex vivo* assays.

2.3.4. Renal and hepatic metabolic parameters

Serum enzymes AST (aspartate aminotransferase) and ALT (alanine amino transferase) were used as the biochemical markers for the early acute hepatic damage and determined by the colorimetric method of (Reitman and Frankel, 1957)). Renal function was analyzed by determining serum urea (Mackay and Mackay, 1927) and creatinine levels (Jaffe, 1986) (LABTEST, Diagnostic SA, Minas Gerais, Brazil) by colorimetric method.

2.3.5. Zinc content determination

Zinc levels were determined by inductively coupled plasma atomic emission spectrometry (ICPE-9000; Shimadzu Scientific Instruments). The samples of wet tissue (0.5 g of stomach and intestine) were placed in vials and frozen at −20 °C until analysis. The digestion of samples and the determination of Zn content were conducted as described in detail by Prohaska et al. (2000) with some modifications. Samples were digested with concentrated HNO_3 in an overnight water bath (100 °C). After digestion, samples were diluted with deionized water to 25 mL and transferred to graduated poly-propylene vials and determined by ICPE-9000. The analytical standard zinc (Merck®) was used to make the curve and the results were expressed as (μg/g of tissue).

2.3.6. Lipid peroxidation

The low supernatant fraction (S1) of stomach, intestine and kidney were used for thiobarbituric acid-reactive species (TBARS) assay according to Ohkawa et al. (1979). Samples were incubated with 500 μL thiobarbituric acid (0.8%), 200 μL SDS (8.1%) and 500 μL acetic acid for 2 h at 95 °C. The amount of TBARS produced was measured at 532 nm (Spectrophotometer U-2001 Hitachi), using MDA as an external standard.

2.3.7. Reactive species measurement

Formation of reactive species (RS) was estimated according to a previous report by Ali et al. (1992) and adapted for stomach, intestine and kidney tissues. An aliquot of S1 was incubated with 10 μL of 2′,7′-dichlorofluorescein diacetate (DCFH-DA; 10 μM). The RS levels were determined by a spectrofluorimetric method. The oxidation of DCFH-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 525 and 488 nm of excitation 60 min after the addition of DCFH-DA to the medium.

2.3.8. Ascorbic acid determination

Ascorbic acid determination was performed as previously described by Carr et al. (1983) and Jacques-Silva et al. (2001) with some modifications. S1 protein content from stomach, intestine and kidney were precipitated in 10 volumes of cold 4% trichloroacetic acid solution and centrifuged (S2). The medium (final volume of 1 mL) was incubated for 3 h at 38 °C, after this was added 1 mL of H_2SO_4 65% (v/v). The reaction product was determined using a color reagent containing 4.5 mg/mL dinitrophenyl hydrazine and 0.075 mg/mL $CuSO_4$ at 520 nm and calculated using a standard curve (1.5–4.5 μmol/L ascorbic acid freshly prepared in sulfuric acid) (Spectrophotometer U-2001 Hitachi – Japan).

2.3.9. Thiol groups

Thiol group from stomach, intestine and kidney were determined as previously described by Ellman (1959). To determination of the total thiol groups the S1 was used, and to determine non-protein thiol (NPSH) the protein fraction of S1 was precipitated with 200 μL of 10% trichloroacetic acid followed by centrifugation. The colorimetric assay was carried out in 1 M phosphate buffer, pH 7.4. A standard curve using glutathione was constructed in order to calculate the SH in the tissue samples (Spectrophotometer U-2001 Hitachi – Japan).

2.3.10. SOD activity

Superoxide dismutase activity was assayed spectrophotometrically as previously described by Misra and Fridovich (1973). This method is based on the capacity of SOD to inhibit the autoxidation of epinephrine to adrenochrome. The supernatant (S1) (stomach, intestine and kidney) were assayed and the color