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Short communication

Chronic treatment with zinc hydroaspartate induces anti-inflammatory and anti-ulcerogenic activity in rats

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ABSTRACT

Background: The previous study indicated the enhancement of the anti-inflammatory effect of ketoprofen by acute and sub chronic administration of zinc hydroaspartate.
Methods: The present study examined anti-inflammatory, anti-ulcerogenic and analgesic activity induced by chronic (14 days) administration of ZHA (30 mg/kg, *po*), with a combination of a single administration of ketoprofen, in rats. Moreover, the zinc concentration in serum and stomach mucosa was also determined.
Results: Chronic ZHA *po* administration exhibits anti-inflammatory activity and enhanced the effect induced by ketoprofen. Likewise, ZHA administration demonstrated anti-ulcerogenic activity. While ZHA alone did not exhibit analgesic action, it enhanced the effect of ketoprofen.
Conclusions: The present study demonstrated for the first time that chronic treatment with zinc salt exhibits anti-inflammatory activity. Besides, anti-ulcerogenic activity and the enhancing properties of zinc to ketoprofen induced anti-inflammatory and analgesic activity were also shown.

Introduction

Zinc is involved in the immune system's functioning properly. Zinc deficiency impairs both innate and acquired immunity [1]. It leads to a reduction in the activity of NK cells, reduces the chemotaxis and phagocytosis processes, as well as the activity of granulocytes, monocytes and macrophages. Acquired immunodeficiency disorder manifests itself in the functioning and maturation of T and B cells [2]. The latest data show that the addition of zinc for the treatment of pneumonia shortens the duration of the illness. In addition, it is believed that zinc supplementation, when strictly controlled, can be used in the treatment of autoimmune diseases such as multiple sclerosis, due to the influence of the micronutrient on the activation and proliferation of T lymphocytes and is not kept by the production of cytokines. The anti-inflammatory properties of zinc are closely related to its antioxidant activity [3].

The most common adverse effects of NSAIDs include disorders of the gastrointestinal tract [4]. Mild symptoms such as nausea, diarrhea, abdominal pain, loss of appetite can be demonstrated. More serious symptoms include severe damage to the upper gastrointestinal tract manifested by bleeding, ulceration, erosions and perforations (often leading to death) [5]. The lower part of the gastrointestinal tract may also be subject to such damage. It leads to the protein-losing enteropathy or ulcers, colitis resembling ulcerative colitis (UC). The risk of damage increases during *Helicobacter pylori* infection as a result of excessive alcohol consumption and the use of other drugs with an ulcerogenic effect, such as glukocorticosteroids [6]. NSAIDs are among the most

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common drugs that cause these presented results, hence a traveled peptic ulcer being a clear contraindication to their use. An explanation of the cause of such serious damage should be clarified in order to protect the gastric mucosa. The mechanism of healing of such gastrointestinal damage may involve the production of mucus by the epithelial cells. It is a barrier coating between the hydrochloric acid contained in the food, and gastric mucosa. The factors that stimulate the production of mucus are PGE2 and PGI2. These eicosanoids by stimulating the secretion of bicarbonate can modify the composition of mucus, thereby improving its protective function. The integrity and possibility of regeneration depend on the amount of oxygen delivered via blood. Another important mechanism for the protection of epithelial cells is impermeability to ionized substances. Hydrogen ions are not able to access the lumen of the vessels and the sodium ions are not able to access gastric lumen. Our previous studies indicated that ketoprofen had a strong damaging effect on the lining of the stomach [7]. Moreover, we demonstrated that zinc (as hydroaspartate salt) in single or subchronic administration enhanced the anti-inflammatory activity of ketoprofen in rats [8].

The aim of the present study was to assess the analgesic, anti-inflammatory and anti-ulcerogenic activity of zinc hydroaspartate administered chronically (for two weeks) and its impact on ketoprofen effect in rats.

Materials and methods

Animals

Male albino Wistar rats, weighing between 150 and 250 g, were used for the anti-inflammatory and analgesia tests. The animals were housed and fed in a laboratory and kept at a constant temperature of 22 °C under standard conditions (a 12:12 h L:D cycle, standard pellet diet, tap water). Treatment of laboratory animals in the present study was in full accordance with the respective Polish and European regulations and was approved by the Local Ethics Committees.

Chemicals

Ketoprofen (Sigma–Aldrich, Germany) and zinc hydroaspartate (ZHA) (Farmapol, Poznań) for *po* administration were suspended *ex tempore* in 0.9% NaCl in saline.

Determination of anti-inflammatory activity of the investigated compounds using the carrageenan-induced hind paw edema test

Animals fasted for 24 h before the experiment were used in the hind paw edema test. The tested compounds were administered to fasted rats having free access to drinking water. After one hour and in order to produce inflammation, 0.1 mL of 1% carrageenan solution in water was injected into the hind paw subplantar tissue of the rats, according to method of Winter et al. [9]. The development of the paw edema was measured pletysmographically (Ugo Basile, Italy). The paw diameters were measured and recorded prior to the carrageenan injection and after the 1st, 2nd and 3rd hour, while the percentage of the edema inhibition was calculated according to the following formula:

$$\textit{Edema inhibition}\,\% = \frac{(N - N' \times 100)}{N}$$

where: N is the paw diameters measured 1–3 h after injection of carrageenan to the control group – paw diameters at the beginning. N' is the paw diameters measured 1–3 h after injection of carrageenan to the test groups – paw diameters at the beginning.

Analgesic activity test

The analgesic activity was evaluated according to Randall's and Selitto's method [10]. The pain threshold in the hind paw of rat affected by inflammation was measured using an analgesimeter 4 h after administration of the compounds. The mean pain thresholds were calculated for both the treated and control groups and the percentage change in relation to the control was then determined.

Irritant action on the gastric mucosa according to Komatsu

The ulcerogenic effect was determined by the method devised by Komatsu et al. [11]. The tested compounds were administered to fasted rats having free access to drinking water. Twenty-four hours after administration of the compounds, the rats were sacrificed, the stomach was removed and, after incision along the lesser curvature, rinsed with a tap along with a warm (37 °C) saline soak, then spread on a cork board and pinned down. The mucosa of the glandular part of the stomach was inspected using a binocular microscope (10-fold magnification). The mucosal lesions were evaluated using the five-point scale (0 – no lesions, 1 – erythema, 2 – punctiform ulcers, 3 – small ulcers, 4 – large ulcers, 5 – perforation).

Sample preparation for determination of Zn in stomach tissue

The tissue samples were thawed, rinsed with ultrapure water (18.2 MΩ cm) obtained from a WG-HLP deionization system (Wigo, Poland), drained and cut (using a Teflon scissor). Next, after a careful and accurate weighing, the samples (max 0.5 g) were placed into digestion vessels and 6 mL of 65% HNO_3 (Suprapur, Merck, Germany) was added. The digestion of the stomach samples was carried out in a Multiwave 3000 microwave oven (Anton Paar, Austria) with the 8-position rotor. The conditions of the three-step digestion procedure were as follows: power 1200 W, ramp 15 min, hold 20 min, cooling 40 min, maximum pressure 50 bar and maximum temperature 200 °C. The obtained digested samples solutions, after removal of the gasses, were transferred quantitatively to a 10 mL volumetric flasks, and diluted by deionized water before being finally transferred to polyethylene vials. The samples were stored at 4 °C until further analysis.

Zinc determination in serum samples and mineralized stomach tissue samples

Zinc determination in serum (without mineralization) and in mineralized stomach tissue was performed by a slightly modified flame atomic absorption spectrophotometry (F-AAS) method previously described [12]. The SOLAAR 939 (ATI UNICAM) spectrometer with deuterium background correction was used. The chemicals used were: zinc standard solution 1000 mg/L (Wzormat), zinc serum controls (LGC Standards), zinc tissue controls (LGC Standards), and deionized water. The F-AAS assay parameters were: wavelength 213.8 nm, monochromator width 0.5 nm, lamp amperage 4 mA, and fuel acetylene-air with burner length 10 cm. Calibration with standards series method was used. Aqueous standard solutions were prepared from zinc standard solution of 1000 mg/L. The linear calibration equation was $y = (5.907\text{E}{-}0.04)x + (3.345\text{E}{-}0.004)$, and the limit of linearity was 0.1–0.4 mg/L with a correlation coefficient of 0.999. The relative standard deviation (RSD) of the method did not exceed 2.4% and the mean recovery of the zinc was 99% (SD 0.78).

Statistical analysis

In the carrageenan-induced hind paw edema test the obtained data was evaluated by a one-way analysis of variance (one-way