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Differential Effects of Zinc Supplementation on the Anti-Inflammatory Activity of Ketoprofen in Rats

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Zinc, as one of the microelements, is of great importance to the human body. Because of the role of zinc, the scientists started research on beneficial therapeutic responses of zinc supplementation. After many studies the researchers were able to show that zinc has anxiolytic, antidepressive, anti-inflammatory, analgesic, antioxidative and gastroprotective properties. The first aim of this work was to test the hypothesis that zinc supplementation may have an impact on anti-inflammatory and analgesic activities of ketoprofen. The second objective was to examine the gastroprotective properties of zinc. To that end, two independent experiments were carried out. Wistar rats were administered zinc hydroaspartate (ZHA) at a dose of 30 mg/kg b.w., *i.p.* and *p.o.* for 28 days. At the end of the experiment, the animals received ketoprofen once, at doses of 5, 10 or 20 mg/kg b.w. Afterwards carrageenan was injected to their paws to activate inflammation. An increase in the volume of rat paws was measured one, two and three hours after the injection. Then the tolerance to pain was measured with an Ugo Basile analgesimeter. The results of the experiment confirmed anti-inflammatory properties of ZHA and its influence on anti-inflammatory activity of ketoprofen. ZHA turned out to be more effective after *i.p.* administration. Also the observation of the rat gastric mucosa confirmed gastroprotective properties of zinc. The research did not prove any analgesic activity of ZHA. Altogether, these data suggest that it is reasonable to search for a combination of non-steroidal anti-inflammatory drugs and zinc, which may give both better therapeutic effects and lesser adverse effects on the gastric mucosa. Differences in the anti-inflammatory activity between the 14-day and 28-day chronic administration of zinc hydroaspartate were observed.

Key words: inflammatory state, Wistar rats, zinc hydroaspartate, NSAID

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INTRODUCTION

Inflammation is defined as a dynamic, complex and orderly process that occurs in living vascularized tissues. To induce an inflammatory response there, a damaging stimulus action is necessary. It may be a chemical, physical or biological stimulus. An inflammatory process is divided into phases. In its first phase leukocytes adhere to the endothelium. During the next phase – rolling – leukocytes move over the surface of the blood vessel to the damaged tissue. These processes are regulated by selectins. During activation chemokines are activated which guide leukocytes. In the next step – diapedesis - white blood cells go from the vascular to the tissue fluid. These processes occur with the participation of integrins. This is how acute inflammation typically progresses. Acute inflammation may turn into chronic inflammation as a result of prolonged exposure to damaging factors, such as inflammation agents, long-lasting infection and autoimmune diseases (Simmons et al., 2004).

Inflammatory mediators are endogenous compounds. They can be divided into two groups: plasma-derived and cell-derived inflammatory mediators. Taking into consideration the mechanism of action of NSAIDs, the most important group consists of arachidonic acid derivatives. NSAIDs are one of the most commonly used drugs (Rao and Knaus, 2008). They are prescribed by doctors and purchased by patients without a prescription (Rainsford, 2007). NSAIDs have a wide field of applications, however, they are not devoid of adverse effects resulting from the mechanism of action. The mechanism of action of NSAIDs is related to an inhibition enzyme called cyclooxygenase (COX). There are three types of COX: COX-1 is called the constitutive enzyme and it is connected with homeostasis, COX-2 is called the pathological enzyme because of its role in the inflammatory process, and COX-3 is localized in the central nervous system (Smith et al., 2000; Mizushima, 2010). Side effects of NSAIDs result from the nonselective inhibition of isoforms. Inhibition of COX-1 leads to damages in the digestive tract or kidneys, hypertension or inhibition of platelet aggregation (Vane et al., 1998; Schwab et. al., 2003; Goldstein, 2004).

Zinc is one of the trace elements which are most important for living organisms (Prasad, 2008a; Prasad, 2009). Its anti-inflammatory activity is scientifically proven, as well as its role in wound healing; especially as regards gastric ulcers. It acts in numerous processes such as proper functioning of enzymes, cell proliferation and differentiation. Zinc acts in metabolic processes, regulates the activity of the immune system and endocrine system (Prasad, 2008b). Studies have revealed that supplementation of zinc improves the anti-inflammatory activity of NSAIDs and simultaneously protects the gastric mucosa against ulceration (Mei et al., 2009). Even some compounds of zinc have anti-edematous activity, especially in the early phase of inflammation response (Safieh-Garabedian et al., 1996; Nozaki et al., 2011).

Numerous studies have revealed that zinc supplementation increases anti-inflammatory activity of NSAiDs and, at the same time, decreases the risk of ulceration (Bulbena et al., 1993; Rodriguez de la Serna and Diaz-Rubio, 1994; Varghese et al., 2009; Ueda et al., 2009; Sivalingam et al., 2011; Gaweł et al., 2013; Gaweł et al., 2014). Thus, the "hind paw edema" and Randall's analgesia tests were performed to investigate the effects of chronic administration of zinc hydroaspartate on anti-inflammatory activity of ketoprofen and its effects on the gastric mucosa.

The aim of the work was the comparative assessment of the impact of 14-day and 28-day zinc supplementation on the anti-inflammatory activity of ketoprofen in rats.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (150-200 g) were used for anti-inflammatory and analgesia tests. The animals were housed and fed in a laboratory kept at constant temperature of 22°C under standard conditions (12:12 h L:D cycle, standard pellet diet, tap water). Each experimental group consisted of 8 animals/dose and all the animals were used only once. Treatment of the 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.

The control group in each experiment received 0.2 ml/100 g of 0.5% methylcellulose (MC) solution *p.o.* Experimental groups were given *i.p.* ketoprofen at doses of 5, 10 and 20 mg/kg suspended in MC (0.5% solution of methylcellulose) and *p.o.* or *i.p.* zinc hydroaspartate (ZHA) dissolved in water. The experiment with ZHA suspended in MC had to be excluded because of disorders in ZHA absorption caused by MC. In the first experiment ZHA was administered chronically for 14 days before testing its anti-inflammatory and analgesic activities, and in the second experiment ZHA was given for 28 days before the test.

Determination of anti-inflammatory activity (Carrageenan-Induced Hind Paw Edema Test)

Animals fasted for 24 hours before the experiment were used in the hind paw edema test. The rats were given the investigated compounds according to the above description. After one hour, in order to produce inflammation, 0.1 ml of 1% carrageenan solution in water was injected into the hind paw subplantar tissue of rats, according to the modified method of Winter (1962) The development of paw edema was measured plethysmographically (Ugo Basile, Italy). Paw diameters were measured and recorded prior to carrageenan injection and after one, two and three hours, and the percentage inhibition of edema was calculated according to the following formula:

$$\% \text{ inhibition of edema} = \frac{(N - N') \times 100}{N}$$

where: N – paw diameters measured 1, 2 and 3 h after injection of carrageenan to the control group – paw diameters at the beginning. N' – paw diameters measured 1, 2, and 3 h after injection of carrageenan to the test groups – paw diameters at the beginning.

To analyze the anti-edematous activity of ZHA and the influence of ZHA on this activity of ketoprofen (given at three doses), two-way ANOVA and Bonferroni post-test were used. To analyze the analgesic activity of the examined compounds, one-way ANOVA and Turkey test were used.

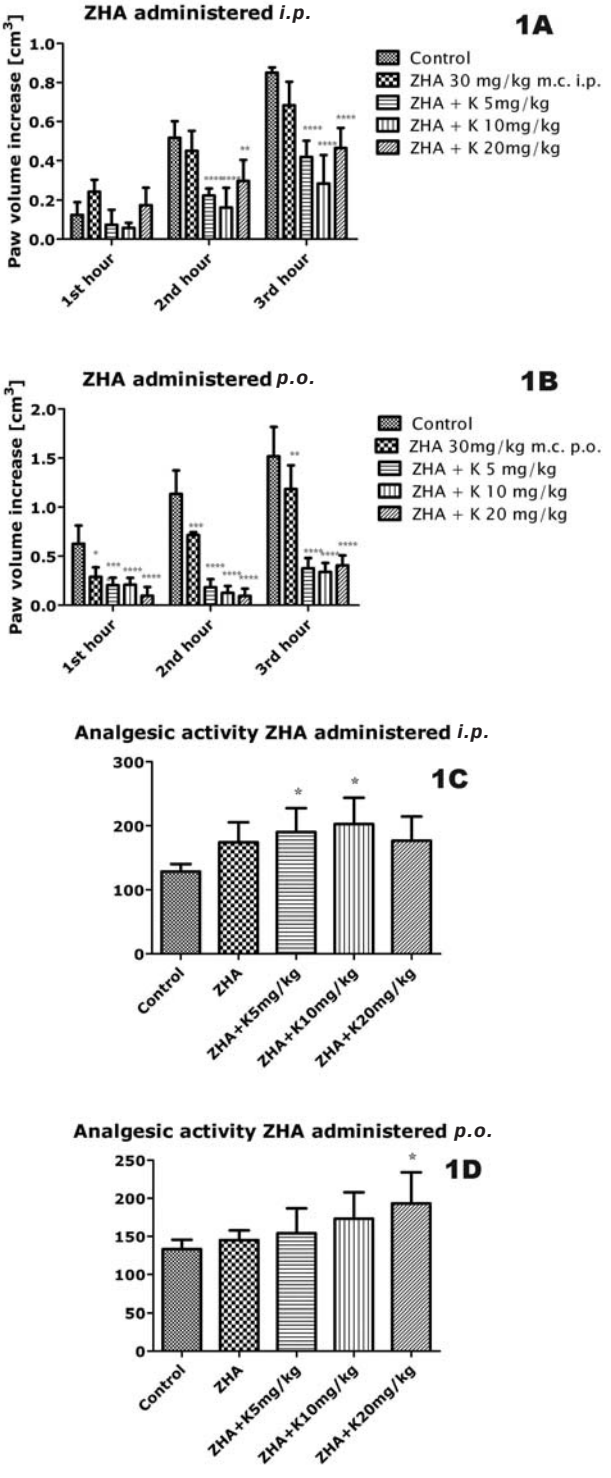

Fig. 1. The effect of 14-days chronic supplementation zinc hydroaspartate (*p.o.* and *i.p.*) on anti-inflammatory activity in rats
Graphs A and B refer to anti-edematous studies,
Graphs D and D refer to analgesic activity of ketoprofen.
The results are presented as mean ± SEM (n = 8).
*P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001

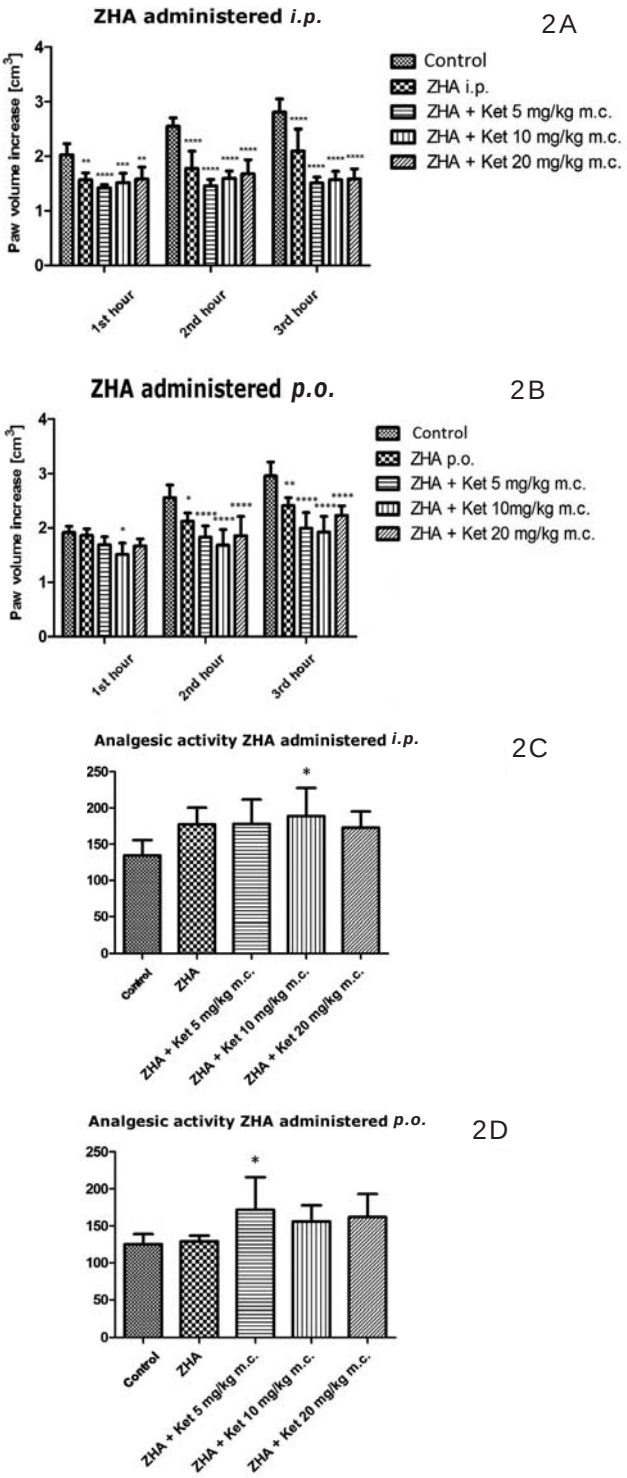

Fig. 2. The effect of 28-days chronic supplementation zinc hydroaspartate (*p.o.* and *i.p.*) on anti-inflammatory activity in rats
Graphs A and B refer to anti-edematous studies,
Graphs C and D refer to analgesic activity of ketoprofen.
The results are presented as mean ± SEM (n = 8).
*P< 0.05; **P< 0.01; ***P< 0.001; ****P< 0.0001

RESULTS

Different results were observed in 14-day studies and 28-day studies. In the 14-day supplementation experiment it was revealed that *i.p.* administration gave better results than *p.o.* administration. In comparison with the results obtained for the 28-day chronic supplementation, the anti-edematous activity of ketoprofen was much stronger in the 14-day experiment. Opposite results were obtained for *p.o.* chronic administration: the 28-day *p.o.* chronic supplementation increased the anti-inflammatory activity.

The performed studies have not given a clear answer as to which route of ZHA administration gives better results for anti-inflammatory activity of ketoprofen. It was observed that the 14-day supplementation of ZHA gives better results when the compound is administered *per os*. All the outcomes were statistically significant. Even the administration of ZHA only inhibited an increase in hind paw edema. Intraperitoneal supplementation of zinc hydroaspartate did not reveal statistically significant effects in the first hour of the experiment. In the 28-day studies it was observed that ZHA administered intraperitoneally gave better results. Zinc hydroaspartate administered that way inhibited an increase in paw edema even in the first hour of the experiment. All the obtained outcomes were statistically significant. When the compound was administered *per os*, the results were not so satisfactory. The influence on the analgesic activity of ketoprofen was not observed.

Chronic zinc hydroaspartate (ZHA) treatment (both routes of administration) did not result in the expected significant analgesic activity, however, it slightly enhanced the analgesic activity of ketoprofen (Fig. 1C, 1D, 2C and 2D).

DISCUSSION

The conducted experiments gave no clear answer as to whether an extension of time of ZHA administration to 28 days is better than 14-day supplementation with this compound. Also the route of ZHA administration seems to have a significant impact on the resulting inflammatory

response of ketoprofen. Absorption of zinc ions may be beneficial in the case of intraperitoneal administration, however, considering the possibility of using the tested treatments in therapy of human patients, administration by gavage should be taken into account.

Currently, these results indicate that the 28-day supplementation of zinc, using both routes of administration, provides the same anti-inflammatory effect of ketoprofen as the 14- day supplementation. Further studies should also consider the desirability of using other zinc salts, such as zinc picolinate and zinc citrate which may increase the bioavailability of zinc and enable a more favorable interaction of the effects of anti-inflammatory drugs. A statistically significant effect of ZHA chronic administration on anti-inflammatory activity of ketoprofen was shown, but there were no significant differences in the anti-inflammatory activity of ketoprofen depending on the time of chronic administration of zinc hydroaspartate (14 or 28 days). The study has shown that 28-day chronic administration of zinc hydroaspartate does not give better results than 14-day supplementation with this compound. However, to obtain a clear answer as to which route of zinc hydroaspartate chronic administration (*p.o.* or *i.p.*) is the best, it is necessary to carry out more investigations with different experimental models of anti-inflammatory states.

It has been found that a two-week extension of ZHA administration does not give the expected beneficial therapeutic effects. Therefore, to obtain a more favorable influence on the activity of zinc ions in anti-inflammatory drugs, it is necessary to search for such zinc compounds that will be characterized by better bioavailability regardless of the route of administration of zinc ions.

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