THE INFLUENCE OF KETOPROFEN AND ZINC OXIDE NANOPARTICLES ON SERUM COPPER LEVEL IN RATS

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Abstract

The role of copper in anti-inflammatory response includes several mechanisms. Antagonism between zinc (Zn) and copper (Cu) and proper balance between the two elements in the organism may affect the course of inflammatory diseases. Copper is a component of Zn/Cu superoxide dismutase (Zn/Cu SOD) and other enzymes involved in the anti-inflammatory response of the organism. To investigate the serum copper level during inflammation and diseases, numerous researches were conducted. Copper deficiency or copper intoxication may lead to biological consequences. Copper deficiency may be caused by various factors, one of them is excessive zinc supplementation. The aim of the study was to investigate the alterations in the serum copper level after 2-week zinc oxide nanoparticles (NPs-ZnO) administration. The second aim was to investigate serum copper level alterations after 2-week NPs-ZnO and single ketoprofen administration. The inflammatory state was induced in each group by the carrageenan injection at the 15th day of the experiment. The results indicate for the decrease in serum copper level in group receiving NPs-ZnO compared to control. Moreover, in groups receiving NPs-ZnO as well as ketoprofen, a decrease in serum copper level was observed. We may conclude that NPs-ZnO administration and also ketoprofen administration acts as anti-inflammatory agents and may induce a decrease in serum copper level.

Keywords: serum copper level, zinc oxide nanoparticles, ketoprofen, zinc-copper antagonism, inflammation

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Introduction

Copper and zinc are trace elements, components in key enzymatic reactions in the human body. After iron and zinc, copper is the third, most abundant trace element. It is estimated that its body content is 70–80 mg, mostly localized in the muscles (~25%), in the skin (~16%), bone marrow (~15%), in liver (8–15%), in the brain (~9%), and others about 20%. The highest concentrations of copper per gram of tissue are found in the brain and in the liver [1, 2]. Copper is necessary in many enzymatic systems; therefore, its deficiency may influence several body tissues. Copper is a component of many enzymes in human body, such as ceruloplasmin, cytochrome c oxidase, lysyl oxidase, tyrosinase, dopamine-β-hydroxylase. As a component of lysyl oxidase, copper participates in the cross-linking of collagen and elastin. Collagen is an important structural protein, a component of the bones, skin, and of the connective tissue. It is the most abundant protein in the body and is the main component of the

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connective tissues, for example, vascular tissue, skin, joints, bones, or eyes. The deficiency of lysyl oxidase caused, for example, by the copper deficiency, may lead to the incorrect cross-binding that may cause vascular rupture, loose skin and joints [3, 4]. For the proper iron metabolism, copper is necessary [5], and it is also involved in the melanin synthesis, as a component of the enzyme tyrosinase. Copper deficiency may lead to anemia and lack of pigmentation. Copper is one of the factors as a component of dopamine-β-hydroxylase, which enables proper synthesis of catecholamines. Its deficiency may also lead to the neurological symptoms. Serum copper level is influenced by the inflammatory state [6]. Copper and zinc are necessary for the proper immune and inflammatory response. These trace elements are involved in the pathogenesis of the inflammatory state. Adequate zinc and copper levels in the organism are important for its functioning, hence evaluation of its body level, especially in pathological states, is important. Alterations in serum copper level are observed in many diseases and they may be connected with the progression of a disease. Changes is serum copper level may also correlate with the inflammatory state intensity in the diseases ongoing with the inflammatory state. Research indicates that in case of many diseases, for example, inflammation, diabetes, cancer, cardiovascular diseases, serum copper levels differ from healthy individuals [7]. For now, the copper blood concentration is a basic examination in the copper deficiency diagnosis and may be important while pathological state in organism occurs [8]. Also, zinc supplementation may lead to copper deficiency, and in this case, measurement of serum copper level is important. Zinc is known as an anti-inflammatory, anti-oxidative agent. Its role in the treatment of many diseases, especially diseases involving inflammatory state, is an important topic of the research. Positive effects of zinc supplementation were observed in many diseases, for example, rheumatoid arthritis, depression, viral or bacterial infections. What is more, the influence of zinc ions on the anti-inflammatory activity of the nonsteroidal anti-inflammatory drugs (NSAIDs) is being established [9]. Also, the experiments with NPs-ZnO supplementation and evaluation of its influence on the anti-inflammatory activity of ketoprofen (NSAID) were performed. Those results indicate that NPs-ZnO influenced the anti-inflammatory activity of ketoprofen [10]. Zinc oxide nanoparticles are being widely investigated due to their properties. Also, experiments on rodents, evaluating its influence on serum zinc and copper levels, were performed using different nanoparticles, different doses, and ways of administration. NPs-ZnO were administered in different time periods and duration [11]. The aim of this study was to investigate the differences in the serum copper level in rats receiving NPs-ZnO for 2 weeks in the following doses: 7 mg/kg and 14 mg/kg, p.o. or i.p.

The aim of the study was also to determine the serum copper level in groups receiving NPs-ZnO for 2 weeks and single ketoprofen in the following doses: 5 mg/kg, 10 mg/kg, 20 mg/kg, p.o.

Materials and Methods

Drugs

Zinc oxide nanoparticles (Sigma Aldrich, Germany) were suspended in deionized water. Ketoprofen (Sigma Aldrich, Germany) was dissolved ex tempore in distilled water.

Animals

Male albino Wistar rats, weighing between 150 and 250 g, were used for the tests. The animals were housed and fed in a laboratory and kept at a constant temperature of 22°C under standard conditions (12:12 h L:D cycle, standard pellet diet, tap water). The rats were divided into five or six groups, with one of them being a control. Each group consisted of 6–7 animals. 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.

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

Experimental Protocol

Rats were divided into the following five or six groups: zero group, control group, and experimental groups. Animals in experimental groups received NPs-ZnO in the doses of 7 and 14 mg/kg for two weeks, p.o. or i.p. On the 15th day, rats in experimental groups were given ketoprofen by gavage in doses 5, 10, or 20 mg/kg (p.o.). One hour after the administration of ketoprofen, carrageenan-induced hind paw edema test was performed in control and experimental groups [12]. In order to produce edema, 0.1 ml of 1% carrageenan solution in water was injected into hind paw subplantar tissue of rats.

Using the plethysmometer (Ugo Basile, Italy), the development of edema was measured. The paw volume were measured and recorded after the 1st, 2nd, and 3rd hour from the carrageenan injection. The percent of edema inhibition was calculated according to the following formula:

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

where N is the paw volume measured 1, 2, and 3 hours after injection of carrageenan to the control group (paw volume at the beginning) and N′ the paw volume measured 1, 2, and 3 hours after injection of carrageenan to the test groups (paw volume at the beginning).

Rats were decapitated after 24 hours, the blood and tissues were collected. The blood was centrifuged in 2500 rpm, 15 min, at 4°C. The serum was collected until analysis.

Scheme of the experiments:

Control group (with carrageenan) NPs-ZnO 14 days administration, 7 mg/kg or 14 mg/kg, p.o. or i.p.

NPs-ZnO administration (time, doses, route as in pt. 2) + single ketoprofen administration 5 mg/kg p.o.

NPs-ZnO administration (time, doses, route as in pt. 2) + single ketoprofen administration 10 mg/kg p.o.

NPs-ZnO administration (time, doses, route as in pt. 2) + single ketoprofen administration 20 mg/kg p.o.

Ctr-0 group – untreated healthy rats

In experiments with NPs-ZnO in doses 7 mg/kg p.o. and NPs-ZnO in doses 14 mg/kg p.o., additional zero groups (ctr-0) were involved in the experiments. The groups that received 0.9% NaCl saline, the carrageenan-induced hind paw edema test was not performed. Rats in groups 2–5 received NPs-ZnO for 14 days in doses 7 or 14 mg/kg, p.o. or i.p. Rats in groups 3-5 received NPs-ZnO for 14 days in doses 7 or 14 mg/kg, p.o. or i.p., and on 15th day of the experiment received single ketoprofen p.o. in doses 5, 10, and 20 mg/kg. One hour after administration of ketoprofen, carrageenan-induced hind paw edema test was performed in groups 1–5. On the next day, rats were sacrificed and blood was collected. Serum from whole blood was separated using centrifuge 5000 rpm at 15 minutes. After this, serum was frozen until analysis.

Serum trace element levels' measuring

Using Perkin_Elmer 5100 ZL apparatus, copper serum levels were measured by flame atomic absorption spectrometer (F-AAS) method, and serum samples were diluted 5 times with deionized water (Millipore) before analysis. Measured concentration for trace element was calculated based on calibration curve. For copper, calibration curve was obtained on the standards 0:00 0.25; 0.50; 1.00, and 2.00 mg/l. Wavelength for Cu analysis was λ=324.8. Using control serum SeronormTM Human, the correctness of measurements was verified.

Statistical analysis

For serum copper concentration in different groups, the one-way ANOVA was applied followed by Bonferroni multiple comparison test. P<0.05 were considered significant. For comparison between groups receiving single ketoprofen and groups receiving NPs-ZnO and ketoprofen in Table 1, the two-way ANOVA was applied followed by Bonferroni multiple comparison test. P<0.05 were considered significant.

Results and discussion

Figure 1 and Table 1 summarize the results concerning serum copper concentration in experimental groups. Serum copper levels were established in the following groups: zero group (ctr-0); control (ctr); group receiving NPs-ZnO for 14 days; NPs-ZnO for 14 days, and single ketoprofen in doses 5, 10, 20 mg/kg p.o. (ket 5 mg/kg p.o., ket 10 p.o., ket 20 p.o.). The results indicate that there is a statistically significant increase in serum copper concentration in the control group, compared to the zero group (Fig. 1-C, D). The statistically significant decrease of serum copper level was observed in groups receiving NPs-ZnO in the following doses: 14 mg/kg i.p., 7 mg/kg p.o., 14 mg/kg p.o. and ketoprofen in the dose 20 mg/kg p.o., compared to control (Fig. 1-A, C, D).

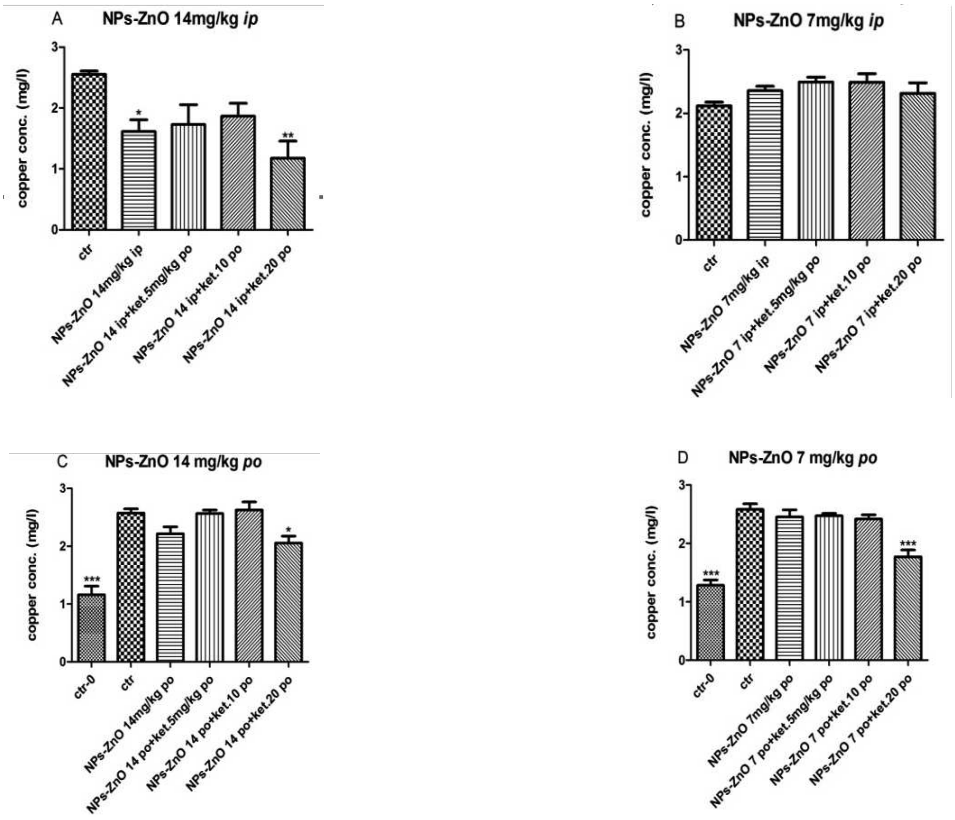

Fig. 1. Serum copper concentration (mg/l) in experiments with chronic administration of NPs-ZnO in the following doses: A—14 mg/kg i.p.; B—7 mg/kg i.p.; C—14 mg/kg p.o.; D—7 mg/kg p.o. Serum copper concentration in the following groups: control (ctr); experimental groups receiving NPs-ZnO; NPs-ZnO and single ketoprofen in the doses—5 mg/kg p.o. (ket. 5 mg/kg p.o.), 10 mg/kg p.o. (ket. 10 p.o.), 20 mg/kg p.o. (ket. 20 p.o.). For C,D, additionally serum copper level for the zero group (ctr-0). Data expressed as means ± SD. (*) p<0.05; (**) p<0.01; (***) p<0.001.

Table 1. Serum copper concentration in groups receiving single ketoprofen in the following doses: 5, 10, and 20 mg/kg p.o. (without previous supplementation) compared to serum copper concentration (mg/l) in experiments with chronic administration of NPs-ZnO in the doses of 7 and 14 mg/kg i.p. Data expressed as means ± SD. (*) p<0.05; (**) p<0.01.

Ketoprofen 5 mg/kg p.o.	Ketoprofen 10 mg/kg p.o.	Ketoprofen 20 mg/kg p.o.	NPs-ZnO 7 mg/kg i.p.+ketoprofen 20 mg/kg p.o.	NPs-ZnO 14 mg/kg i.p.+ketoprofen 10 mg/kg p.o.	NPs-ZnO 14 mg/kg i.p.+ketoprofen 20 mg/kg p.o.
2.28±0.26	2.551±0.32	1.73±0.26	2.314±0.41*	1.87±0.52**	1.175±0.626*

In case of the experiments with the administration of NPs-ZnO in the doses 7 mg/kg p.o. and NPs-ZnO 7 mg/kg i.p., there were only slight differences between the experimental groups and the control group in serum copper level (Fig. 1, B, D). Those data are mostly not statistically significant. Only in case of NPs-ZnO in doses 7 mg/kg i.p. and ketoprofen 20 mg/kg p.o. administration, statistically significant decrease in serum copper level was observed. More variations were observed in case of the administration of NPs-ZnO in doses 14 mg/kg p.o. and NPs-ZnO in doses 14 mg/kg i.p. In both cases, the decreases in serum copper level were observed in the experimental groups, compared to control. Statistically significant decreases in serum copper level were observed in the experiment with the administration of NPs-ZnO in doses 14 mg/kg i.p. (Fig. 1, A). Surprisingly, in case of the administration of NPs-ZnO in doses 7 mg/kg i.p. and ketoprofen in the dose 20 mg/kg p.o., no changes (data not significant) in serum copper level were observed.

The first aim of our study was to investigate the copper serum level after 14 days of administration of NPs-ZnO. The second aim was to investigate the serum copper level after 14 days of NPs-ZnO administration and also single ketoprofen administration. In these groups, the inflammatory state was induced by the carrageenan injection at the 15th day of the experiment.

Copper is an element that has both pro-inflammatory and anti-inflammatory activity [**13**]. Zinc, as an anti-oxidant and ant-inflammatory agent, is one of the factors that enables proper response of the organism to the pro-inflammatory factors. The inflammation is the basic response of the organism to the negative factors, such as bacteria, injury, radiation; the importance of these trace elements in the pathogenesis and response to the inflammation needs to be fully established. The role of copper and zinc in the pathogenesis and development of inflammation is important and hence the observed alterations in the blood levels of these elements in various inflammatory states [**8**]. For now, the blood or serum copper and zinc levels are the basic examinations which reflects body levels of these trace elements. Copper deficiency may be manifested by the decreased serum copper level; for this purpose, measurement of serum copper level is the basic examination for copper deficiency. Indication of the serum copper/zinc ratio is also very important [**14**]. The value of copper/zinc ratio seems to be correlated with the inflammatory state factor levels in serum [**14**, **15**]. Increased copper/zinc ratio may be observed in various inflammatory diseases and correlates with the inflammatory state intensity. Increased

copper/zinc ratio during inflammation is mostly caused by the elevated level of copper and decreased level of zinc. Adequate level of zinc and copper in the organism and its proper balance is important for the proper functioning of the organism. The presented results concerning alterations in serum copper level after zinc supplementation may be due to antagonism of copper and zinc. Zinc oxide nanoparticles administration may lead to the alterations in serum copper level. Impaired level of copper is observed in various diseases, and the exact mechanism is not fully established; but there are several hypotheses. Inadequate level of copper in the organism is being connected with the development of many diseases, such as cardiovascular diseases, diabetes, cancer, neurodegenerative diseases, that may lead to the disease progression. The mentioned diseases are becoming more commonly diagnosed; it is important to recognize the exact mechanisms of those diseases and factors involved in their development. More research is concerned to evaluate the treatment of those diseases, also some research involves examination of serum copper level and level of other trace elements. The role of copper in the inflammation includes several mechanisms, one of them involves Zn/Cu SOD. Besides zinc and manganese, copper is a part of the Zn/Cu SOD, which is a strong antioxidant. It has an important role in the protection of the cells and tissues from the damage caused by the reactive oxygen species (ROS) [**16**]. Superoxide dismutase neutralizes ROS, which are responsible for the oxidative damage of the cells and has a role in the pathogenesis of the inflammatory states. In case of the diseases ongoing with the inflammatory states, proper synthesis of SOD is important. Superoxide dismutase acts in the anti-inflammatory response of the organism. Copper is a component of many other enzymes, also involved in the anti-oxidative response of the organism. Table 2 summarizes the roles of copper in inflammation. Both copper deficiency and copper intoxication may lead to the biological and pathological consequences.

Table 2. Role of copper in inflammation [17, 18, 19]

COPPER IN INFLAMMATION			
COPPER-ZINC ANTAGONISM	ROLE IN IMMUNE FUNCTION	ENZYMES COMPONENT	COPPER BODY LEVEL
Increase in copper/zinc ratio in serum: increase in serum copper level and decrease in serum zinc level: 1. Activation of NF-kB pathway in inflammation - increased ZIP14 (zinc transporter) production in liver - zinc influx in the hepatic cells - enhanced expression of MT in liver (and other tissues) - decrease of zinc serum level. 2. Increased production of ceruloplasmin in liver- increase in serum copper level. 3. Zinc compartmentalization due to an inflammatory response - decrease in zinc level in serum. 4. Increase in serum of free copper.	1. Important for proper immune function. 2. Influence on T lymphocytes function. 3. Increased copper transport to macrophages (INFγ- increased production of CTR1-copper transporter- in macrophages), increase in copper accumulation in phagosomes. 4. Copper influences the function of macrophages against pathogens. 5. Influence of copper in ROS induction and ROS-dependent pathogens killing.	Copper is the component of enzymes that function in antioxidative and anti-inflammatory response: 1. Component of Zn/Cu SOD. 2. Glutathione peroxidase. 3. Activity of NO2-oxidase. 4. Ceruloplasmin (acute phase protein).	1. Altered copper compartmentalization. 2. Increased metabolic demand for copper 3. Increased copper excretion.

Changes of copper level are observed in malabsorption syndromes, total parenteral nutrition, and also in Wilson disease. Moreover, inadequate zinc supplementation may lead to the copper deficiency that may cause anemia, increased risk of infections, edema, neurological symptoms. The proper ratio of zinc to copper in diet is 10:1. Zinc deficiency in diet is quite common, and the excess copper also may increase its deficiency [**20**]. The exact mechanism of copper deficiency after zinc supplementation is not yet fully established. The mechanism involves metallothioneins (MT), which are a group of metalloenzymes. MT are main proteins, which bind zinc and copper in the cell. Copper and zinc are absorbed mainly in the duodenum. In the enterocytes, zinc and copper

are bound to the MT. In case of zinc excess in the duodenum, zinc ions induce the production of MT in the enterocytes. Copper has more affinity to MT than zinc and displaces zinc from connections with MT. Enterocytes with copper and MT are then exfoliated.

Commonly observed symptoms, such as anxiety, insomnia, fatigue, depression, mood swing, hair loss, acne, allergies, memory problems, also problems such as polycystic ovaries, may also be related to the excessive copper (hypercupremia), and as a consequence, zinc deficiency. Copper acts also as a neurotoxin, and excessive copper may lead to the hormonal disruption, involving impairment in levels of dopamine, noradrenalin, or histamine [**21**]. By its influence on the level of neurotransmitters, copper may cause many disorders, when its body level is elevated [**22**]. For this purpose, more research is being conducted to examine the mechanism by which copper may be involved in the pathogenesis of mentioned diseases and inflammatory states.

Increased copper levels beyond the known Wilson disease are linked with diseases such as Alzheimer and Parkinson diseases, schizophrenia and depression [**23**, **24**]. Elevated copper levels were also established in the patients suffering from cancer [**25**]. Inadequate copper/zinc ratio, also caused by the elevated level of copper, may lead to various diseases such as vascular diseases as follows: hypercholesterolemia, vascular and heart muscle damage [**26**]. The role of copper in the pathogenesis of the cardiovascular diseases is not yet fully established, but it is known that copper deficiency may be an important risk factor of the atherosclerosis. It may lead to the incorrect collagen synthesis, main component of the extracellular matrix of the vascular wall. Recent results have shown that Zn/Cu SOD plays an important role as the anti-inflammatory agent and in reducing the vascular endothelium dysfunction in case of cardiovascular diseases [**18**, **27**]. Zn/Cu SOD depends on copper for its enzymatic activity [**16**]. On the contrary, copper may catalyze LDL oxidation, which is an important risk factor in the development of the atherosclerosis. These observations may lead to the conclusion that copper deficiency or its increased organism level is one of the factors that may lead to several diseases such as cardiovascular disease [**28**].

Previous results indicate the increase of serum copper level after induction of inflammation [**29**]. In most cases, inflammation is connected with an increased copper level in serum [**30**]. Inflammatory disease markers such as interleukins (ILs) may upregulate the synthesis of ceruloplasmin. Ceruloplasmin binds copper and transports it in the serum [**29**]. Of about 90% of serum copper is transported by the ceruloplasmin and the remaining 10% by the albumin and erythrocuprein. There is a difference in the serum copper level in zero groups and control groups in the presented results. It may be related to the enhanced ceruloplasmin synthesis after carrageenan injection in the control groups. Ceruloplasmin is an acute phase protein, and its concentration may be increased after induction of the inflammatory state by the carrageenan injection. Copper serum level may correlate with the acute phase protein level in the inflammatory state, such as ceruloplasmin. These observations may also indicate the important role of copper in the pathogenesis and development of the inflammatory state. Other results concerning serum copper level, for example, in rheumatoid arthritis (RA) may also confirm our hypothesis. In this systemic inflammatory disease, serum copper level is usually increased compared to healthy control. It is also suggested that increased copper level is increased because of upregulation of synthesis and secretion of ceruloplasmin during disease [**19**]. Copper level correlates in RA with inflammation, and elevated serum copper level may be a risk factor for that disease [**13**]. Other results indicate the enhanced inflammatory state after zinc and copper excessive absorption [**31**]. This

may lead to the hypothesis that the presented results concerning serum copper level may also be correlated with the intensity of the inflammatory state after carrageenan, NPs-ZnO, and ketoprofen administration.

Results indicate that serum copper level is decreased in groups receiving NPs-ZnO and ketoprofen in the dose 20 mg/kg. It may be caused by the ketoprofen, which acts as an anti-inflammatory drug. Reducing of the inflammatory state after administration of ketoprofen in the highest dose in the experiments results in the decrease of the serum copper level. Another hypothesis may involve different copper body sequestration as a response for the different intensity of the inflammatory state after administration of NPs-ZnO and ketoprofen. Copper may also be transported to the inflamed tissue and used to synthesize, for example, MT, superoxide dismutase enzyme (Zn/Cu SOD), and glutathione. Experiments concerning copper level changes in serum in inflammatory state after NSAIDs administration were performed previously. The results suggest that copper level may differ in individuals and may also be related with pro-inflammatory state factor synthesis, for example, Il-1, Il-6 [**32**].

A broad spectrum of experiments were also performed with zinc supplementation. Positive effects of zinc supplementation were observed in many diseases, also ongoing with inflammatory state. The exact mechanism of zinc ions' action in treating some diseases is not fully established, but it is suggested that it is correlated with zinc anti-inflammatory and anti-oxidative activity. Zinc administration can influence disease in several mechanisms, for example, stimulation of IL production, inhibition of NF-kB pathway [**33**], and inhibition of complement activation [**34**]. There are different serum copper levels in experimental groups receiving NPs-ZnO in two doses and two ways of administration in the presented results.

Zinc oxide nanoparticles have different physicochemical properties and are more reactive than its standard form. Nanoparticles may cause oxidative stress and oxidative damage of the cells and tissues [**35**]. On the contrary, it may cause positive biological effects, not observed, when administering the standard form of ZnO. In case of NPs-ZnO administration in the dose 14 mg/kg i.p., the statistically significant decrease in the serum copper level was observed, compared to the control group. It may indicate the possible interactions of NPs-ZnO with endogenous copper or with proteins that bind copper, but these possible interactions are not established. For now, the influence of NPs-ZnO administration on the organism is investigated, as well as its anti-oxidative or pro-oxidative activity [**36**]. The experiments on cell lines indicate that the cells responses after NPs-ZnO administration may differ from each other. The cytotoxic or anti-inflammatory activities were observed [**37**, **38**]. The different results may be related to the various size, shape, and physicochemical properties of NPs-ZnO that were used in the experiments.

We could not exclude pro-inflammatory or anti-inflammatory activity of administered nanoparticles in our experiments. Different doses and different ways of administration may cause various biological effects. The results may also be related to the different body response for the inflammatory state (induced by the carrageenan injection) and the NPs-ZnO interaction with the inflammatory factors, which may be responsible for the different copper level in the experimental groups from four experiments. The other studies were focused on the administration of the complexes of copper or zinc with NSAIDs. The aim of those studies was to evaluate the possible enhancement of the anti-inflammatory activity of NSAIDs after administration of the mentioned complexes compared to the anti-inflammatory effect after administration of the NSAIDs [**39**, **40**]. Other studies conducted with the administration of the zinc hydroaspartate may also

confirm that zinc may influence the anti-inflammatory activity of ketoprofen [**41**]. Experiments involving NPs-ZnO in chronic administration in the dose 14 mg/kg i.p. and single ketoprofen administration also indicate that NPs-ZnO may influence the anti-inflammatory activity of ketoprofen. Zinc oxide nanoparticles in the dose 14 mg/kg i.p. caused statistically significant reduction of the inflammatory state compared to control [**10**]. In case of NPs-ZnO administration in the dose 14 mg/kg i.p., a decrease in the serum copper level was observed, compared to the control group. The serum zinc level in the group receiving NPs-ZnO in the dose 14 mg/kg i.p. is also increased compared to control (data not shown). The results indicate the reduction of the inflammatory state after NPs-ZnO administration. Zinc oxide nanoparticles in the mentioned dose were probably easily absorbed and easily penetrated to the body circulation, which resulted in the biological activity not observed in case of administration of standard form of zinc oxide. Observed slight changes or no changes in serum copper level in groups receiving NPs-ZnO in the dose 7 or 14 mg/kg p.o may be caused by slight absorption of NPs-ZnO from gastrointestinal tract and its small influence on the copper level. Probably NPs-ZnO was absorbed on dietary fiber and this way was extracted from organism. The serum zinc level in the mentioned groups also did not differ from the serum zinc level in the control group (data not shown). Comparison between groups receiving ketoprofen and NPs-ZnO and groups receiving only ketoprofen were also performed. Results indicate that statistically significant interaction between groups was observed only in three cases. In case of administration of NPs-ZnO in the dose 7 mg/kg i.p. and ketoprofen in the dose 20 mg/kg and NPs-ZnO in the dose 14 mg/kg i.p. and ketoprofen in the doses 10 mg/kg and 20 mg/kg, statistically significant interaction was observed (Table 1). In other cases, there was no difference between groups receiving NPs-ZnO and ketoprofen and groups receiving only ketoprofen (data not shown).

To conclude, serum copper levels differ in four experiments after administering NPs-ZnO and ketoprofen. It may be related to different biological effects after administering NPs-ZnO in two doses and two ways of administration. Ketoprofen in three doses also induce anti-inflammatory effects. The administration of both NPs-ZnO and ketoprofen may be responsible for the different serum copper levels in the experimental groups. The administration of NPs-ZnO in two doses and in two routes (p.o. or i.p.) probably leads to the different absorption of zinc to the circulation, and the antagonism of zinc and copper may also be the contributing factor. Excessive loss and metabolic demand for the copper may also be possible [**8**]. Observed results may also be related to the different organism reactions and to the interactions of the NPs-ZnO with the tissues and cells and with the endogenous copper. Different enhancement of the inflammatory state after NPs-ZnO influences the synthesis of ceruloplasmin and its release; it also leads to the different secretion of Il-1, Il-6, and other inflammatory factors [**19**]. Other hypotheses about redistribution of the copper in body tissues as a response to the inflammation and NPs-ZnO administration may also be possible. Previous results indicate the dual role of copper during inflammatory state and disease. During inflammation, serum copper level is increased, and carrageenan injection, which induces inflammation, also caused increase in serum copper level in the presented results. It is important to maintain proper copper serum level, also during pathological state of the organism. First, it was reported that after NPs-ZnO in the dose 14 mg/kg i.p. supplementation, serum copper level was decreased, compared to control. In case of NPs-ZnO in the doses 14 mg/kg i.p. and 7 in the doses 14 mg/kg p.o. and ketoprofen 20 mg/kg p.o. administration, serum copper level was decreased, compared to control. Results indi-

cate the anti-inflammatory activity of the NPs-ZnO and ketoprofen, which also influenced serum copper level. More research needs to be conducted to confirm that hypothesis.

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Resumo

La rolo de kupro en kontraŭinflama efiko inkluzivas kelkajn mekanismojn. Antagonismo inter zinko (Zn) kaj kupro (Cu) kaj taŭga ekvilibro inter la du elementoj en la organismo povas influi al la direkto de inflamaj malsanoj. Kupro estas komponanto de Zn/Cu superoksida dismutazo (Zn/Cu SOD) kaj aliaj enzimoj implikitaj en la kontraŭinflama respondo de la organismo. Por ekzameni la seruman kupran nivelon dum inflamo (inflama stato) kaj malsanoj, multaj esploroj estis efektivigitaj. Kupra manko aŭ veneniĝo per kupro povas evoluigi la biologiajn efikojn. Manko de kupro povas esti kaŭzita de diversaj faktoroj, unu el ili estas troa zinka konsumado. La celo de la studo estis esplori la ŝanĝojn de la kupra nivelo en la serumo post 2-semajna aplikado de nanopartikuloj de zinka oksido (NPs-ZnO). La dua celo estis determini ŝanĝojn de la kupra koncentriteco en serumo post aplikado dum 2-semajnoj NPs-ZnO kaj unuopa ketoprofena aplikado. La inflama stato estis induktita en ĉiu grupo per la injekto de karagenino dum la 15-a tago de la eksperimento. La rezultoj indikas la malkreskon de kupra koncentriteco en serumo en la grupo ricevanta NPs-ZnO kompare al kontrola grupo. Plie, en grupoj ricevantaj NPs-ZnO kaj ketoprofenon, malpliiĝis nivelo de kupro en serumo. Ni povas konkludi, ke aplikado de NPs-ZnO kaj ankaŭ ketoprofeno efikas kiel kontraŭinflamaj agentoj kaj povas kaŭzi malpliigon de nivelo de la kupro en serumo.

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