

The glutathione system in bone tissue under the action of copper-zinc ore components and antioxidants administration

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Abstract

Aim. To study changes in the glutathione system in bone tissue during chronic intoxication with elements contained in copper-zinc pyrite ore and antioxidant vitamin administration.

Methods. 36 mature male white rats were divided into three groups (control, comparison, experimental). The rats of the experimental and comparison groups received intragastrically copper-zinc pyrite ore powder in a 2% starch solution as a suspension at a dosage of 60 mg/100 g bodyweight daily for three months. During the last month, the experimental group received an antioxidant vitamin preparation (the complex of vitamins with a trace element) containing α -tocopherol, β -carotene, ascorbic acid and selenium. The content of reduced glutathione, free thiol groups in proteins, the activity of glutathione peroxidase, glutathione transferase, glutathione reductase, gamma-glutamyl transferase, and glucose-6-phosphate dehydrogenase were determined in homogenates derived from femoral epiphysis. The statistical analysis of the results was performed using Statistica 6.0 software. The median (Me) and percentiles (Q_1 and Q_2) were calculated, a non-parametric Mann–Whitney U test was carried out to compare study groups.

Results. Chronic intoxication with elements contained in copper-zinc pyrite ore causes impairment of the glutathione system in bone tissue. Intoxicated rats showed a decrease in the reduced glutathione content to 71.9% ($p=0.014$) and free sulfhydryl groups of proteins to 77.8% ($p=0.0143$), inhibition of glutathione-dependent antioxidant enzymes activities, and disruption of the glutathione reduction system in tissues, compared to the control group. Antioxidant vitamin administration increased the levels of reduced glutathione and free thiol groups of proteins, activated the enzymes involved in the glutathione system: the reduced glutathione content increased to 94.8% ($p=0.2132$), glutathione peroxidase activity to 85.7% ($p=0.0432$), glutathione transferase — up to 94.3% ($p=0.5251$), glutathione reductase — up to 86.1% ($p=0.0442$) compared to the control group.

Conclusion. Chronic intoxication with metals contained in copper-zinc pyrite ore leads to decreasing the content of reduced glutathione and free thiol groups of proteins in bones along with reducing glutathione reductase and glucose-6-phosphate dehydrogenase activities, inhibition of glutathione peroxidase and glutathione transferase; an antioxidant vitamin administration increases the activity of glutathione reduction enzymes in bone tissue, the content of reduced glutathione and free sulfhydryl groups of proteins, the activities of glutathione peroxidase and glutathione transferase.

Keywords: bone tissue, glutathione system, effect of copper-zinc ore elements, antioxidant drug.

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Background. Glutathione (*L*- γ -glutamyl-*L*-cysteinyl-glycine) accounts for more than 90% of the reduced equivalents in the cell. It is the most abundant intracellular thiol and can reach millimolar concentrations in most tissues. It is detected not only in the cytosol but also in the mitochondria, nucleus, and endoplasmic reticulum. The glutathione system plays a key role in the detoxification of metals, xenobiotics, and endogenous toxic pro-

ducts; is integral to maintaining the redox pool; and serves as an important component of the multilevel protection of biomolecules from oxidative and nitroxide damage [1–3].

The toxic effect of metals is due to the disruption of metal-ligand homeostasis, the formation of complexes with functional groups of biomolecules, changing their conformation and biological activity. A special place in these mechanisms is occupied by

their interaction with compounds containing thio-, alkylthio-, thioether, and disulfide groups. Excessive intake, accumulation, or intracellular release of metals with variable valence, especially heavy ones, induces increased generation of reactive oxygen species in tissues and activation of free-radical oxidation processes [3,4].

Deposits and mining of non-ferrous metal ores represent potential sources of environmental pollution with heavy and other toxic metals. Workers of ore mining enterprises involved in the extraction of non-ferrous metals are exposed to highly dispersed mine dust, the release of which accompanies the main technological processes. As a result of the influx of metals and other elements from the industrial aerosol of the air in the working area, the elemental homeostasis in biological fluids and tissues of the body is re-formed. A significant excess of Cu, Zn, Fe, Cr, Mn, Cd, Pb, Hg, As, and other elements contained in the ore was found in the oral fluid, hard dental deposits, and hair of workers engaged in the extraction and processing of copper-zinc ores [5,6].

Analysis of the general and occupational morbidity in ore mining and processing operations reveals that diseases of the musculoskeletal system and connective tissue rank high [7]. In miners working at these operations, osteopenia and osteoporosis are detected 2–4 times more often than in ground workers, and a decrease in bone strength is already detected at a young age (depending on the duration of employment) [7, 8].

Previous studies simulating chronic intake of components of copper-zinc pyritic ore in rats showed that the elements contained in the ore accumulate in the bone tissue of experimental animals and have an osteotoxic effect, causing the intensification of free-radical oxidation processes and incoordination of remodeling with the prevalence of osteoresorption [8–10].

The study of the state of the glutathione system in bone tissue in the pathochemical mechanisms of the development of osteopenia and osteoporosis under the action of elements of polymetallic ores [11], as well as the possibility of correcting changes with the administration of antioxidants, is not only of interest in connection with the role of glutathione as a cofactor in detoxifying reactions catalyzed by glutathione peroxidase and glutathione transferase [2]; the importance of this system in the regulation of the redox potential of cells, the activity of enzymes, receptors, and transcription factors [2,3], as well as its influence on the course of the cell cycle, proliferation and differentiation, and apoptosis of cells is also of interest [12, 13].

The study aimed to analyze the changes in the glutathione system in bone tissue during the mode-

ling of chronic intoxication with elements contained in copper-zinc pyrite ore and to evaluate the effect of administration of an antioxidant vitamin preparation.

Materials and methods of research. The experiments were conducted on 36 sexually mature male white rats weighing 220–240 g. The studies were approved by the local ethics committee of the Bashkir State Medical University (protocol No.6 of 12/07/2016).

The animals were distributed into three groups (control, comparison, and experimental; 12 rats in each).

The suspension of finely ground powder of copper-zinc pyrite ore of the Uchalinsky deposit (Bashkortostan, Uchalinsky mining and processing plant) in a 2% starch solution was injected intragastrically using a special probe into rats of the experimental group and the comparison group daily for 3 months, at a rate of 60 mg per 100 g body weight, taking into account the main routes of entry of mine dust into the bodies of miners under conditions of underground ore mining [6,7].

Ore comprises 3.5% copper, 5% zinc, 15%–45% sulfur, 0.1%–3.0% arsenic, 0.2%–7% barium, 0.015%–0.5% antimony, 0.15%–0.3% lead, and 0.06%–0.12% cadmium. Such elements as mercury, chromium, silver, fluorine, and selenium are constantly present as accompanying elements [7]. The calculation of the administered dose of ore powder was based on the minimum toxic dose of copper, which is 2.1 mg per 100 g of the animal's body weight [4], and its average content in the ore is 3.5%.

During month 3 of the experiment, an antioxidant vitamin preparation (a complex of vitamins with a trace element) was injected intragastrically daily into the animals of the experimental group in the form of a suspension in a 2% starch solution at a dose of 50 mg/kg of body weight. One capsule (a complex of vitamins with a trace element) contains 40 mg of α -tocopherol, 10 mg of β -carotene, 100 mg of ascorbic acid, and 50 μ g of selenium in combination with powdered dry yeast. The daily dose of a complex of vitamins with a trace element was calculated for rats using a dose conversion factor according to the guidelines for conducting pre-clinical studies of drugs [14].

The animals were kept under identical conditions on a standard diet (complete dry mixed feed for laboratory animals Chara, Assortiment-Agra) with free access to water. The rats were withdrawn from the experiment by euthanasia under Rausch anesthesia.

In homogenates of the epiphyses of the tubular bone, spectrophotometric methods (spectrophotometer SF-56, Spectr, Russia) were used to deter-

Table 1. Indicators of the glutathione system in the bone tissue of rats with intragastric administration of a suspension of copper-zinc pyrite ore powder and an antioxidant vitamin preparation, Me [Q₁–Q₃]

Indicators	Group of animals		
	Control, n = 12	Comparisons, n = 12	Experiential, n = 12
Reduced glutathione, $\mu\text{mol/mg protein}$	2.10 [1.98–3.15]	1.51 [1.19–2.03] $p=0.014$	1.99 [1.77–2.31] $p=0.2132$ $p_1=0.0481$
Free sulfhydryl groups, $\mu\text{mol/mg protein}$	7.8 [6.2–10.5]	6.08 [5.25–7.96] $p=0.0143$	7.24 [6.17–8.03] $p=0.4281$ $p_1=0.0345$
Glutathione peroxidase, $\text{nmol/min} \times \text{mg protein}$	2.10 [1.54–2.56]	1.49 [1.34–1.70] $p=0.0235$	1.80 [1.44–2.22] $p=0.0432$ $p_1=0.0486$
Glutathione transferase, $\text{nmol/min} \times \text{mg protein}$	15.8 [12.9–19.0]	13.5 [9.8–18.4] $p=0.0383$	14.9 [14.4–17.8] $p=0.5251$ $p_1=0.5054$
Glutathione reductase, $\text{nmol/min} \times \text{mg protein}$	4.52 [3.69–4.68]	3.75 [2.82–4.03] $p=0.0282$	3.89 [3.09–4.81] $p=0.0442$ $p_1=1.0$
Glucose-6-phosphate dehydrogenase, $\text{nmol NADPH/min} \times \text{mg protein}^1$	3.7 [3.0–3.9]	3.03 [2.9–3.62] $p=0.0486$	3.16 [2.9–3.68] $p=0.0409$ $p_1=0.7784$
γ -glutamyl transpeptidase, $\text{nmol/min} \times \text{mg protein}$	13.4 [11.9–14.2]	8.3 [8.0–9.6] $p=0.0096$	11.9 [10.3–14.0] $p=0.0476$ $p_1=0.0355$

Note: p is the level of statistical significance of differences with the control group, and p_1 is the level of statistical significance of differences with the comparison group.

mine the content of reduced glutathione and free thiol groups of proteins using 5,5-dithio-bis-(2-nitrobenzoic acid) [15], activity of glutathione peroxidase (enzyme classification (EC) 1.11.1.9) using the GLUTATHIONE PEROXIDASE reagent kit made by Randox Laboratories Ltd. (Denmark), glutathione transferase (EC 1.1.1.18) [13], glutathione reductase (EC 1.6.4.2) [16], glucose-6-phosphate dehydrogenase (EC 1.1.1.49) [17], and γ -glutamyl transpeptidase (EC 2.3.2.2) [18].

To obtain a homogenate, a weighed portion of the femoral epiphyses was crushed with scissors and ground in a glass homogenizer with 0.1 M potassium phosphate buffer (pH = 7.4) in a ratio of 1:5, and then Triton X-100 was added to a final concentration of 0.1%, left for 30 min, and centrifuged at 2800 rpm for 10 min (Hitachi Himae CT15RE centrifuge, Japan). All operations were performed at a temperature of 2°C–4°C. The supernatant was used for the study. The protein content in the samples was determined by the Lowry method [19].

The results were processed using the Statistica 6.0 program for Windows with determination of the median (Me) and percentiles (Q₁ and Q₃). Statistical differences in the intergroup parameters were assessed using the Mann–Whitney U-test.

¹NADPH, nicotinamide adenine dinucleotide phosphate.

Results and discussion. Chronic intake of heavy metals and other toxic elements of copper-zinc pyrite ore causes a decrease in the amount of reduced glutathione and free thiol groups of proteins in the bone tissue of animals (comparison group) (Table 1). The level of reduced glutathione in the femoral epiphyses decreased by almost a third, and the levels of free thiol groups of proteins decreased by up to 78% compared with animals of the control group. Simultaneously, a statistically significant decrease in the activity of glutathione-dependent enzymes, namely glutathione peroxidase, glutathione transferase, and γ -glutamyl transpeptidase, was registered.

The results of the determination of a number of metals by atomic absorption spectrometry and volt-amperometric analysis revealed that, after daily intragastric administration of a suspension of copper-zinc ore powder for 3 months, the levels of Cu, Zn, Fe, Mn, Sr, Cd, Cr, and Hg increased significantly in the bones of rats, while that of Mg decreased. The level of Cu in this case increased by more than 30 times, those of Pb, Zn, and Hg by 20, 5, and 4 times, respectively, and those of Mn and Fe by more than 2 times [8].

The primary cause of the decrease in reduced glutathione and free sulfhydryl groups of proteins under these conditions is likely their complexation

with heavy metals. In addition, toxic elements such as Pb, Cd, and Hg can displace essential elements from metal-containing biocomplexes, thereby disrupting their structure and functional activity, and elements with variable valence (Fe, Cu, Mn, Cd, Pb, Hg) can stimulate the formation of reactive oxygen species and intensify significantly the processes of free-radical oxidation [3,4], causing intensive consumption of antioxidants and depletion of physiological antioxidant resources.

In mammalian cells, the main mechanism for maintaining the level of reduced glutathione, along with transport from the extracellular space and *de novo* biosynthesis, is reduction of the oxidized form [2]. A decrease in glutathione reductase activity under the action of ore elements in animals of the comparison group characterizes the inhibition of this process. One of the reasons for such inhibition is a decrease in the activity of glucose-6-phosphate dehydrogenase, which is a key enzyme of the pentose phosphate pathway of glucose oxidation, which is the main generator of NADPH in the cytoplasm, which is necessary for the functioning of glutathione reductase. Glucose-6-phosphate dehydrogenase belongs to the group of enzymes that are sensitive to the action of metals, forming strong complexes with the thiol groups of biomolecules [3, 4]. A decrease in the activity of γ -glutamyltransferase in animals of the comparison group (Table 1) also characterizes the inhibition of transmembrane transfer of a number of amino acids, including cysteine, in bone tissue cells [1].

When intoxication with elements contained in non-ferrous metal ores, the processes of reduction of sulfhydryl groups of proteins with the involvement of enzymes that catalyze these reactions, thioredoxin and glutaredoxin, the activity of which also depends on the state of the redox potential of cells, and the levels of reduced forms of nicotinamide coenzymes and glutathione, are also disrupted [13,20]. However, this requires a purposeful study.

The glutathione system is related to other antioxidant defense components. Previous studies have shown that the action of metals contained in poly-metallic ores in bone tissue cells significantly decreases the activity of superoxide dismutase and catalase, as well as the levels of α -tocopherol and ascorbic acid [8,10].

In this regard, it was of interest to evaluate the effect of an antioxidant preparation on the glutathione system during intoxication with elements of copper-zinc ore. For these purposes, a complex of vitamins with a trace element was used, containing in its composition α -tocopherol, ascorbic acid, β -carotene, and B vitamins in the form of dry

yeast. The choice of a complex of vitamins with a microelement for the experiment was also associated with the presence of selenium in its composition, which is part of glutathione peroxidase and glutathione transferase and is an antagonist of Hg, Cd, Pb, and As found in ore, which can inhibit the uptake of Se, as well as displace Se from proteins as a result of differences in the constants of the formation of their complexes with sulfur-containing amino acids [4].

The results (Table 1) indicate that the daily administration of a complex of vitamins with a trace element during month 3 of intoxication with a suspension of copper-zinc ore powder causes an improvement in the glutathione system in the bone tissue. In rats of the experimental group, compared with animals that did not receive the vitamin preparation (comparison group), the levels of reduced glutathione ($p_i = 0.0481$), free sulfhydryl groups of proteins ($p_i = 0.0345$), glutathione peroxidase activity ($p_i = 0.0486$), and γ -glutamyl transpeptidase ($p_i = 0.0355$) are increased. The levels of reduced glutathione, free SH-groups of proteins, and glutathione transferase activity did not differ from physiological parameters ($p = 0.2132$, $p = 0.4251$, and $p = 0.5251$, respectively). The activity of glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase increased but remained at lower values than in rats of the control group ($p = 0.0432$, $p = 0.0442$, and $p = 0.0409$, respectively).

The results obtained emphasize the essential role in the development of metabolic changes in bone tissue during the chronic intake of elements contained in non-ferrous metal ores, inhibition of the glutathione system with the activation of free-radical processes, and the need to use antioxidant vitamin preparations in the system of measures for the prevention of early development of bone system diseases in workers in contact with mine dust under industrial conditions for the extraction and processing of copper-zinc ores.

CONCLUSIONS

1. The complex of metals contained in copper-zinc pyrite ore, with prolonged intake into the body, leads to profound changes in the glutathione system in bone tissue. The levels of reduced glutathione and free thiol groups of proteins in bones decrease during a decrease in the activity of glutathione reductase and glucose-6-phosphate dehydrogenase, and, as a result, inhibition of the activity of glutathione-dependent enzymes of the antioxidant system (glutathione peroxidase and glutathione transferase) develops.

2. The administration of an antioxidant vitamin preparation in chronic intoxication with copper-zinc ore components has a positive effect, contributing to an increase in the activity of glutathione-reducing enzymes in the bone tissue; the levels of reduced glutathione and free sulfhydryl groups of proteins; and the activity of glutathione peroxidase, glutathione transferase, and the level of antioxidant protection.

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