In vivo Experiments on Zinc Toxicity

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Even zinc is essential mineral for living organisms, in some cases can become toxic. The aim of our research was to perform in vivo study on Wistar rats, administrating (gavage) two different high doses of $ZnCl_2$ solutions, for a short time. Finally, we sampled different tissues (longissimus dorsi muscle, and also kidney, liver and brain) for determination of Zn concentration, by atomic absorption spectroscopy method. The results showed increased Zn concentration after Zn overdoses administration, with two exceptions: the kidney and the brain. Also, liver concentration of zinc was higher after overdosed $ZnCl_2$ administration compared to control group (which got gavage administration of plain water). This demonstrate that Zn overdoses (short term administration), decreases Zn concentration in kidney and brain, and increased concentration in liver and longissimus dorsi in case of Wistar rats experimental animals.

Keywords: longgisimus dorsi, kidney, liver, brain, Wistar rats, zinc

Minerals are essential elements for human, animal and plant organism. Their benefic role was established after years of *in vivo* and *in vitro* studies. One of the essential mineral nutrients is zinc; witch is present in the structure of different metabolites. Thus, zinc is involved of the structure of more then 300 enzymes from all six classes. Zinc is part of proteins' structure assuring the structure and stability of the metalloproteins (containing zinc element). Also, zinc is present as catalytic element (coenzyme) for various metabolic reactions. One of its very important catalytic roles is that zinc is part of the composition of superoxid dismutase enzyme that takes part to the oxidative stress process [1].

Plasma enzymes activity (more exactly in cell membranes) is directly affects by zinc, because this element forms stabilizing components that has important role to prevent oxidative processes. On the other hand, zinc is involved in the process of amyloid-b peptide enzymatic degradation, that can suffer an aggregation process with the formation of a neurotoxic species resulting in synaptic and memory deficits, such as, Alzheimer's Disease [2]. Zinc is implicated in the retinal synthesis (using retinol as precursor), being component of the catalytic enzyme: alcohol-dehydrogenase.

Every cell cycle is depending by zinc, being involved in DNA and RNA synthesis. RNA synthesis is 5000 times more susceptible to zinc divalent ion damage then DNA [3].

As well, zinc is present all over in nature, so it has high bioavailability. It is found in plants and fruits, but also is found in soil and water – as natural component or as a contaminant comes from different industries waste. Also, zinc is a component of different medical materials used in orthopedic or prosthetic implantations [4, 5].

Zinc is present in lots of products (medical drugs) used for different treatments, such as: dermatological diseases (eczema, psoriasis, acne); mental disorders (ADHD – attention deficit-hyperactivity disorders, Alzheimer's diseases, Autism, Down syndrome and others). In other medical conditions zinc can be used in various treatments for male infertility and erectile dysfunction; rheumatoid arthritis, osteoporosis and muscle cramps; herpes treatment and others [2,6]. Also, zinc salts as sulfate or citrate is used as eye irritation products or is component of toothpaste [20-22].

Talking about zinc chemical properties we can say that zinc has not exceptional reactivity if it is compared to others metals reactivity. If we consider the position of zinc in the periodical table, we see that zinc divalent ion (Zn^{2+}) has completed *d* orbital with electrons (d^{10}) and it is obvious that zinc doesn't participate to reduction-oxidation reactions, being a stable ion in biological systems [7, 8].

In our study we tried to establish the relation between administration of some overdoses of zinc as zinc chloride (in two different concentrations) to Wistar rats, for a short period of time, and the absorption of zinc with its distribution in some organs (kidney, liver, brain) and muscle (longissimus dorsi) – being part of much larger study.

Experimental part

Materials and methods

Our study was performed on three groups of *Wistar* rats (*Rattus norvegicus* breed), each group was formed from 10 adult animals (female and male), with alike somatic characteristics. The animals used for experiment were treated in good physiological conditions, in concordance with Romanian and European Union legislation [9-13].

We administrated – as gavage – two different concentrations of zinc chloride prepared in our laboratory. Thus we prepared 1.316mMol/L $ZnCl_2$ solution for the first experimental group (E_I) and 2.633mMol/L $ZnCl_2$ solution for the second experimental group (E_{II}). The solutions were administrated as gavage, and also to the control group we administrate as gavage drinking water to use the same procedure. We choose gavage method of administration because we wanted to be sure about the quantity of zinc that was ingested by every experimental animal. Also, we administrate as gavage method, drinking water for animals from control group because we wanted to simulate the same stress as for experimental animals. Before gavage administration the animals were anaesthetized with ketamine hydrochloride, administrated as intramuscular injection (commercial product: Calypsol – 500mg ketamine hydrochloride/10mL, produced by Gedeon Richter Ltd., Budapest, Hungary).

Administrated doses for zinc were calculated as overdoses of zinc, having in view the Recommended Daily Intake for

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zinc (RDI_{Zn}) and using as references the Expert Group on Vitamin and Minerals Meeting – Nutrition, 2002 (Food Standards Agency U.K.). Their recommendations admitted that the optimum amount for zinc daily intake is 0.171-0.214mg/kg body weight, and we multiply this dose with two (1.316mMol/L $ZnCl_2$ solution) and multiple with four (2.633mMol/L $ZnCl_2$ solution) to obtain our concentration of administrated solutions.

At the beginning of the experiment the animals were grouped in control group animals, experimental I group (E_I), and experimental II group (E_{II}). Each group (control and experimental) consisted in 10 adult animals. Administration of $ZnCl_2$ solutions for experimental animals and drinking water for control group animals was performed in the fourth and seventh day of experiment and the scarification was performed in the fourteenth day of experiment. First days of experiment we let the animals in an adjustment period, to adapt to the experimental conditions, ensuring adequate nutritional and shelter conditions. Also, during the experiment period we closely monitor the animal behavior in order to see if there are changes in behavior or if there are any effects associated with administration of zinc.

In the last day of experiment, after anesthesia, we took samples of muscle (*longissimus dorsi*) and organs (liver, kidney and brain) with surgical, sterile instruments, and the samples were transferred in clean glass containers and then were freezing (-18°C) until were analytical testing the samples in laboratory.

For Atomic Absorption Spectroscopy (AAS), the samples were defrosted at room temperature, and then were digested in a Millestone Microwave System applied a program for fast exothermic reactions. For digestion we used 65% HNO_3 solution (Merk) and 1mL 30% H_2O_2 solution, and for dilution to the final volume (for AAS) we used double deionised water (<5μS/cm). AAS was performed with a spectrometer produced by Perkin-Elmer that has Zeeman effect for background correction and transversal heating of graphite tube. Zinc concentration was determined in air-acetylene flame (AA). Before we made the analytical determination of zinc from our samples, the AAS was calibrated using a standard single element solution (Merck), and final results were calculated in μg/g wet tissue.

After we obtained the final concentration of every sample, we used descriptive statistics (Excell), and the results were reported as * symbol in case of significant modifications of analyzed parameters of experimental animals groups compared to control animals ($p<0.05$; P=95%).

Results and discussions

We performed the quantitative analysis for zinc concentration and presented the results graphically, as distribution in organs and muscle. Thus, figure 1 shows the distribution of zinc after administration of zinc overdoses as zinc chloride solution as gavage to Wistar rats.

Liver, as a detoxifier organ, cumulated zinc during our experiment. Concentration of zinc was significantly increased with 24.48% for animals from E_I group ($(p<0.05)$ and with 81.32% for animals from E_{II} group compared (also significantly, $p<0.05$) to control group (C). Administrated zinc was absorbed along the intestinal tract, then entered into the blood system, and was transported to the organs that are responsible with the detoxification (liver) and excretion (kidney) of toxics in organism.

Kidney was another organ that was in our attention. After zinc administration at the experimental animals we also wanted to determine the quantitative zinc from kidney – having in view that one way of overdoses excretion is renal. But, both experimental groups showed a decrease of zinc concentration after over intake. Thus, the quantum of zinc in kidney for first experimental group (E_I) decreased with 17.76% and the second experimental group (E_{II}) was lower with

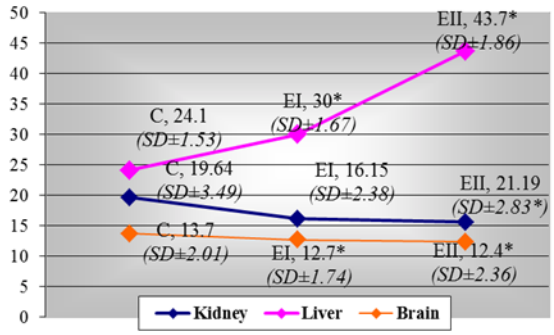

Fig. 1. Zinc quantum in different organs after $ZnCl_2$ administration (μg / g w.t.)

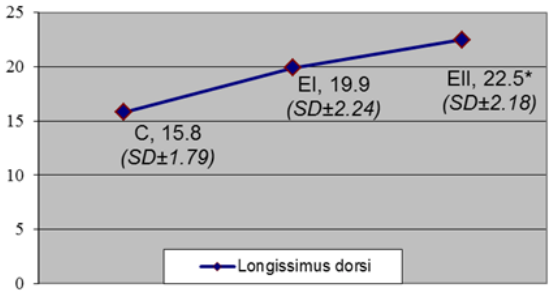

Fig. 2. Zinc concentration in Longissimus dorsi after $ZnCl_2$ administration (μg / g w.t.)

20.41% compared to control group (C). So, we can confirm that in rats, for a short time administration of zinc over intake, kidney zinc concentration decreases in the opposite way with the quantity of zinc administration (higher zinc administration lead to lower renal zinc concentration).

In the same direction with kidney, the brain analysis for zinc concentration presented also a decreased quantum of this element after acute overdoses of zinc administrated to Wistar rats. Thereby, brain analysis showed that concentration of zinc from first experimental group (E_I) was lower with 7.29% compared to control group and for second experimental group (E_{II}) the concentration of zinc decreases also compared to control group with 9.48% and compared to first experimental group with 2.36%.

Figure 2 presents the quantum of zinc after AAS analysis from longissimus dorsi collected from Wistar rats.

Other analysis consists in determination of zinc concentration from longissimus dorsi collected from control and both experimental groups of animals (C, E_I, E_{II}). After AAS analysis we calculate zinc quantum from sampled muscle and the results presented an increased concentration for both experimental groups. More precise, the quantum of zinc for E_I animals showed increases of zinc with 25.49% and for the second experimental animals the concentration of zinc was significantly higher with 42.40% ($p<0.05$) compared to control animals.

In single cells endogenous zinc is significantly involved in cytotoxic events, but exogenous zinc acts like a regulatory mechanism preventing the uptake of toxic doses for cells [14].

Transport of zinc is possible by some metalloproteins, and the most important is metallothioneines – proteins rich in cysteine and with low molecular weight. These proteins carriers of zinc are localized in the membrane of Golgi apparatus. Metallothioneines are specialized proteins involved in detoxification process because have the capacity to bind biominerals (especially zinc and copper; but also selenium and others) and xenobiotics (like mercury, cadmium, arsenic and silver). The relation between the response of zinc to oxidative stress and the metallothioneine is demonstrating that is benefic in safe anticancer therapies [15].

Zinc overdoses administration to rats increases also the concentration of different enzyme in liver. Thus, concentration

of serum GPT (glutamic-pyruvate transaminase or alanine aminotransferase), GOT (glutamic oxaloacetic transaminase or aspartate aminotransferase), ALP (alkaline phosphatase) and AMY (amylase) appear increased after zinc overdoses. The concentration of presented enzymes was correlated with the administrated doses (increased dose of zinc was followed by increasing the quantum of enzymes). This demonstrates that activity of liver was altered after intoxication with zinc [16].

As much, liver presents significantly increased zinc concentration (with 24.48% for E_I experimental group and with 81.32% for E_{II} experimental group) after administration of zinc chloride in two different concentrations to Wistar rats. This is explainable maybe because liver is the organ responsible for detoxification and the excess of zinc was transported to the liver to find a way of reduce and excretes the zinc overdose.

Kidney is the organ responsible for elimination of excess of zinc (in our study). But for short time administration of zinc excess to rats, the zinc distribution in kidney presented a decrease concentration. Thus, compared to control animals, the quantum of zinc for the first experimental group was with 17.76% lower, and for E_{II} group decreased with 20.41%.

Another goal of our experiment was the analysis of some renal blood test, such as: creatinine, uric acid and blood urea nitrogen – analyzed from blood serum at the final of experiment. The concentration of uric acid increased significantly after acute toxicity with zinc for both experimental groups. The quantum of creatinine and blood urea nitrogen was higher compared to control group but was not significant increased for experimental groups [17].

Brain analytical test presented also a decrease of zinc concentration for both experimental animals. Thereby, the concentration of zinc for E_I group decreases with 7.29%, and for second experimental group the quantum of zinc was lower with 9.48%, compared to control group of rats.

For long term administration brain cumulates metals, but for short term administration of metals does not increased the quantum of administrated metals. The biological damages for brain after zinc chronic intoxication are severe, and these affects the brain cells with serious lesions that are reflected to the nervous system. On the other hand, brain accumulates zinc after intoxication for long term with this element, acting on different molecular regulators involved in apoptosis. As a consequence of zinc intoxication, brain concentrates free zinc leading to cytotoxicity [14].

Different studies reveal the cumulative effects of metals in muscles after an over intake for a short or long term administration [18].

Zinc analysis from *longissimus dorsi* muscle showed significantly increased zinc concentration after zinc overdosed to Wistar rats. As much, zinc quantum significantly increased in the same way with increases administrated zinc doses: for the first experimental group zinc concentration was with 25.49% higher, and for second experimental group zinc quantum was with 42.40% higher compared to control group.

Tizioto et al. (2015) presented the results of their study that mineral content of *longissimus dorsi* muscle in *Bos indicus* cattle was influenced by various small-effect quantitative at alleles' level [19]. The allele is one of possible forms of a gene. Quantitative trait loci are stretches of DNA that are linked or are connected to genes that substantiate a quantitative trait, refers to phenotypes related to polygenic effects. This study brings important information revealing that the most genes presented in the quantitative trait loci regions are involved directly in signal transduction, transcription regulation and also metal ion binding.

Conclusions

Zinc is essential for biochemical pathways, but in overdoses can become toxic for organism. Zinc chloride solution administrated to Wistar rats can be used for evaluation of zinc toxicity for short term in vivo experiments.

Concentration of zinc in liver increased, while in kidney and brain tissues decreased, after zinc overdoses for experimental animals compared to the control animals.

In *longissimus dorsi* muscle zinc increased after zinc chloride administration in excess for a short time in both experimental Wistar rat groups compared to control group.

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