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INFLUENCE OF ZINC OVER-DOSES ON SOME METALS IN WISTAR RATS BRAIN

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Abstract

Zinc is an essential trace element and plays essential roles in metabolisms from human and animal organism. But over-doses of zinc can translocate other metals and disturbing the minerals' homeostasy. This experiment presents the concentration of some trace elements (Cu, Mn) and potential toxic metals (Al, Pb) after administration of zinc over-doses compare to Recommended Daily Intake (RDI), analyzed from brain of Wistar strain rats. We had three experimental groups and we administrated by gavage $ZnCl_2$ solution (2xRDI for Zn) at rats from E_1 group, $ZnCl_2$ solution (4xRDI for Zn) for E_2 group and tap water for control group (C). After experiment, concentration of Cu (3.42±0.79μg/g for E_1 and 2.40±0.45μg/g for E_2), Mn (0.54±0.16μg/g for E_1 and 0.53±μg/g for E_2), Al (3.69±1.05μg/g for E_1 and 3.72±1.18μg/g for E_2) and Pb (undetectable for E_1 and E_2), decrease at experimental groups (E_1 and E_2) compare to control group (Cu 3.98±0.97μg/g, Mn 1.25±0.60 μg/g, Al 6.17±1.76 and Pb 0.52±0.17 concentrations for control group).

***Key words**: rat brain, trace elements.*

Introduction

Zinc is an essential mineral, being involved in catalysis of different reactions and having structural functions. As a highly hydrophilic ion (Zn^{2+}) it crosses biological membranes by specialized mechanisms with cellular uptake and release. There are characteristic and specialized systems of zinc absorption, transport and excretion in human organism. Zinc concentration in organism depends of many factors as: time,

concentration, pH, temperature, presence of others minerals and others. The synergic and antagonist effects of minerals with zinc influence the quantum of zinc in a certain organ.

First reports of scientists about zinc deficiency and his essential role in organism were found in young Iranian men (Insel et al., 2004). They were diagnosed with severe growth-retarded, anemia, hypogonadism and some people could not see well in the dark. Researchers found that their diet was mainly wheat bread, without animal proteins and they often eat clay. One hypothesis was based on high phytate intake from diets, which impaired absorption of zinc and iron.

Referring to excess of zinc and health dysfunctions, some studies showed that altered zinc concentration is implicated in an extensive number of disorders of the central nervous system, including alcoholism, Alzheimer-type dementia, amyotrophic lateral sclerosis, Down's syndrome, epilepsy, Friedreich's ataxia, Guillaine-Barré syndrome, hepatic encephalopathy, multiple sclerosis, Parkinson's disease, Pick's disease, retinitis pigmentosa, retinal dystrophy, schizophrenia, and Wernicke-Korsakoff syndrome (Ebadi et al., 1995).

Dietary Recommendations for zinc are 15 mg/day for men presented by National Research Council in 1989, 12mg/day for women. As good nutritional sources for zinc: red meat, seafood products and unrefined whole grains.

Experimental

This study presents the gavage administration of zinc over-doses – as zinc chloride – to Wistar rats and the quantum of some elements (trace and potential toxic elements) in brain. Thus, after administration of two different over-doses of zinc we try to establish the relation between zinc and copper, manganese, aluminum, and lead concentration in rat brain. Thus, experiments were performed on three groups of animals as follow: one control group (C) and two experimental groups (E_1 and E_2). Each group has 10 animals (females and males) with an average weight of 100 $\pm$ 10 g. After anesthesia a total amount of 1 mL solution / 100 g body weight (b.w.) was administered to Wistar rats by gavage on days 4th and 7th of experiment. To the group C we administrated tap water, to group E_1 – $ZnCl_2$ solution (two times the RDI for zinc for humans, i.e. $4 \cdot 0.214$ mg / b.w.) and to animals in group E_2 – $ZnCl_2$ solution (four times the

RDI for zinc for humans, i.e. 4 · 0.214 mg / b.w.) – Derek, 1995; Delvin, 1999; Vincu et al., 2000; Vincu, 2004.

On the 15th day of experiment, after laparoscopy, the brain samples were taken from all experimental animals for quantitative analysis. Using a Perkin Elmer "Analyst-100" apparatus, the concentration of Cu, Mn as well as of Al, Pb in rat brain samples were determined by atomic absorption spectroscopy (AAS).

Data obtained in this study were statistically proceeding and mean values ($\overline{X}$) and standard deviations (SD) were calculated for each element. We also calculated data variation compare to control ($\Delta \overline{X}_i$).

Results and Discussions

Over-doses of some trace elements – that in common conditions are essential for human organism – can become sometimes-potential toxic elements. Thus, the present experimental study tries to investigate a possible translocation of zinc overdoses to the rat brain. This experiment on animals presents similitude with morpho-physyological and biochemical processes in the human organism (Underwood, 1977; Ghergariu, 1980).

Today the role of zinc for organism is uncontestable. Thus, zinc is essential in a large number of enzymes and metalloproteins (e.g.: some carboxypeptidase, phosphatase-alkaline, various dehydro-genases), having stabilizer function of the molecular structure from sub cell membrane. In the cytoplasm, zinc and copper are the most important components of superoxide-dismutase (an enzyme that accelerates antioxidant reactions and protect cells from free radical damage). Zinc participates to the synthesis and degradation of glucoses, lipids and proteins and also nucleic acids (in severe zinc deficiency cells fail to replicate). Zinc is vital for a good immune system, for gene regulation and is involved in vision (dark time). Also, zinc interacts with a number of hormones, increase the affinity of hemoglobin for oxygen, participate to perception and appetite regulation, and can induce process of apoptosis. Soon it has been discovered that Zn is playing an essential role in all liquids and tissues of the body (Gârban, 1999). Zinc is an essential nutrient in the development of neurons of the normal brain. Rats Zn deficient in prenatal and early postnatal periods (gestational-lactational) develops abnormal brains (Insel et al., 2004).

Total zinc content of the body is between 1.5 and 2.5 g. In the case of bone absorption and tissular catabolism Zn can be released and reused in a large amount. Experimental studies in humans having diets poorly in Zn, show that zinc concentration can be maintained in normal limits during several months – which indicate a certain available amount of zinc. In 1997 World Human Organization presented the total amount of zinc in the body, as classified it in an intermediate position between bio minerals, in the sense that it is much higher than copper or manganese and about 50 % inferior than iron.

Zinc is present in all tissues, and the highest concentrations occur in the prostate gland (Stokinger, 1981), but in kidney, liver, heart, and pancreas is also high concentration of zinc (Bertholf, 1988 – in Agency for Toxic Substances and Disease Registry – Toxicological Profile for Zinc, 1989). This repartition of high-level zinc concentration in different organs compare to brain is normal, because in zinc is present usually in organs with important protein content and brain has significant lipid content.

Zinc bioavailability – antagonistic effects of zinc. Scientists found that the phytate content of the food ratio affects negatively – in some conditions – the absorption of zinc. Sensitivity to phytates as zinc antagonists, is not significant except to the diets based on raw cereals and high calcium intake from dieta. This antagonistic efect was reported by World Human Organization in 1997.

Experiments on animals show that calcium in excess becomes an antagonist of zinc only in the presence of phosphorus excess, effect not demonstrated to humans (Ghergariu, 1980). The influence of calcium in zinc bioavailability to humans is controversial, but calcium might be in some conditions an antagonist of the zinc (Internet).

Also, copper in excess inhibits the zinc and manganese absorption (Mincu, 1993; Gârban, 1999; Ghizdavu, 2000).

Cadmium is a well-known antagonist of zinc, inhibiting not only its transfer from the intestine wall through the blood, but even interferes with other metabolic processes. Zinc has a protection role against cadmium toxicity (Ghizdavu, 2000; The Scientific basis for mineral utilization – Internet).

Zinc compounds have an antiseptically, astringent and antifungal role, as a result of a competition mechanism of the zinc with

magnesium and manganese, being essentials for metabolically activities of bacteria and fungus (Scvortov, 1951).

The intake for a long period of time of iron supplements, especially in aqua-matrix, can induce an unbalance between iron and zinc. Also, excesses of zinc supplements affect negatively the iron absorption and blood iron has an adverse effect upon zinc absorption (Ghizdavu, 2000; Srimathi – Influencing Iron and Zinc Bioavailability, Internet).

Zinc influences the concentration of chrome from organism. Thus, zinc decreases the chrome absorption (Ghergariu, 1980; Mertz and Mertz, 1994).

Some dietary compounds as oxalates, dietary fiber, EDTA and tannins represent substances with antagonistic effects upon the zinc absorption (Srimathi – Influencing Fe and Zn Bioavailability, Internet).

Also, lead – as a potential toxic element – has inhibition effects upon the zinc absorption in human organism (Gârban, 1999).

Zinc bioavailability – synergic effects of zinc. For diets rich in phytates researchers recommend intake of animal proteins for helping zinc absorption.

In the human organism zinc helps to the vitamin A (retinol) absorption – Valnet, 1986).

An increase zinc intake from diet or nutritive supplements also may increase manganese level in plasma (Ghizdavu, 2000).

The relatively high absorption of zinc from breast milk compared to cow's milk or soy protein-based infant formula has been explained by the presence of zinc binding proteins in human milk which are more easily digested than casein in cow's milk. Alternatively, the higher content of amino acids (histidine and cysteine) in breast milk may also provide an answer to this question (Internet).

The results for investigated biometal concentrations, analyzed from rat brain for experimental groups (E_1; E_2) and control group (C), are presented in the Table 1.

Between zinc and copper there is a competition because their presence in some enzymes. Thus, zinc and copper are the most important component of superoxide dismutase and this could be an explanation for the antagonistic effect of zinc with copper. After a moderat zinc exces (RDI · 2 for Zn) concentration of copper decreases with 14.07% for animals from E_1 group and with about 40% for E_2.

Table 1. Trace elements concentration in rat brain after administration of $ZnCl_2$ (μg/g)

Group Specification	n	Cu	Mn
		$\overline{X} \pm SD$	$\overline{X} \pm SD$
Group C	10	3.98 ± 0.97	1.25 ± 0.60
Group E_1	10	3.42 ± 0.79	0.54 ± 0.16
$\Delta \overline{X}_1$		*- 0.56*	*- 0.71*
Group E_2	10	2.40 ± 0.45	0.53 ± 0.18
$\Delta \overline{X}_2$		*- 1.58*	*- 0.72*

n – number of animals per each working group

Manganese concentration analyzed from Wistar rat brain decrease after excess of zinc intake. So, when concentration of Zn intake is higher than recommended, Mn quantum in certain organs is drastically reduced (56.8% for group E_1 and 57.6% for group E_2) and this shows a competition and antagonistic effect of Zn with Mn.

Studies about concentration of zinc in different anatomic regions from brain are correlated with some neural functions. Thus, the hippocampal Zn is higher in Pick's disease (degenerative dementia) than in Alzheimer's disease or controls. Similarly, the blood cells and urine of patients with Pick's disease contain more Zn than those of patients with Alzheimer's disease or controls (Insel et al., 2004).

Some researchers from Czechoslovakia have also analyzed the quantum of copper, manganese from brain and the results are similar with our results for control group (Cu 3.35 μg/g; Mn 0.58 μg/g) – Eybl et al., 2001.

Table 2 presents the results for potential toxic metals (e.g.: lead and aluminum) analyzed from rat brain after administration of $ZnCl_2$ compared to the control group.

As is known from different studies aluminum concentration in brain can become a reason of Alzheimer diseases. When quantum of aluminum in brain is much higher than normal, lesions and mental health problems can appear. Thus, as it presented in this study aluminum concentration decrease very much after an excess of zinc intake as zinc chloride solution and may contribute to reduce Alzheimer diseases incidence.

Table 2. Potential toxic metals concentration in rat brain after administration of $ZnCl_2$ (μg/g)

Group Specification	n	Al	Pb
		$\overline{X} \pm SD$	$\overline{X} \pm SD$
Group C	10	6.17 ± 1.76	0.52 ± 0.17
Group E_1	10	3.69 ± 1.05	<0.50
$\Delta \overline{X}_1$		*- 2.48*	undetectable
Group E_2	10	3.72 ± 1.18	<0.50
$\Delta \overline{X}_2$		*- 2.45*	undetectable

n – number of animals per each working group

Lead after over-doses of zinc from group E_1 and E_2 is lower then to control group, that demonstrate the antagonistic effect. Our experimental conditions made undetectable the quantum of lead in brain after administration of $ZnCl_2$ because the concentration was under the detection limit.

Conclusions

Zinc over-doses administrated to Wistar rat decrease generally copper, manganese concentration in brain compare to animals from control group. After excess of zinc, administrated orally as zinc chloride, quantum of some potential toxic elements – aluminum and lead – from brain is lower then for the samples of control group. This method of administration over-doses of zinc may be used as treatment method in certain intoxicated persons with some trace elements or with some potential toxic elements.

References

Derek H.S. (1995). *Essential Nutrients in Supplements: Commissioned by European Federation of Associations of Health Product Manufactures*, Cambridge, UK.

Devlin T.M. – Ed. (1992). *Textbook of Biochemistry with Clinical correlation*, 3rd, ed., Wiley and Sons Inc. New York – Toronto.

Ebadi M., Iversen P.L., Hao R., Cerutis D.R., Rojas P., Happe H.K., Murrin L.C., Pfeiffer R.F. (1995). Expression and regulation of brain metallothionein, *Neurochem. Int*., 27(1), 1-22.

Eybl V., Kotyzová D., Koutenský J., Míčková V., Lešetický L., Jones M.M., Singh P.K. (2001). The influence of dithiocarbamate analogs on essential elements status in mice, *Supplement Symposium*, Vol. 4 (http://www.cechtuma.cz/bioenv/2001.html).

Gârban Z. (1999). *Biochimie: Tratat comprehensiv*, Vol. I, ed. 2-a, Ed. Didactică şi Pedagogică, R.A. Bucureşti.

Ghergariu S. (1980). *Oligominerale si oligomineraloze*, Ed. Academiei R.S.R., Bucuresti.

Ghizdavu L. (2000). *Chimie bioanorganică*, Ed. Poliam, Cluj Napoca.

Insel P., Turner Elaine R., Ross D. (2004). *Nutrition*, Second Edition, American Dietetic Association, Jones and Bartlett Publ., Sadbury, Massachusetts.

Mertz A., Mertz O. – Eds. (1994). *ILSI – Risc Assessment of Essential Elements*. ILSI Press, Washington DC.

Mincu I. (1993). *Impactul om alimentatie.* Editura Medicala, Bucuresti.

Scvortov V.I. (1951). *Manual de farmacologie*, Editura de stat pentru literature stiintifica si didactica, Bucuresti.

Underwood E.J. (1977). *Trace Elements in Human and Animal Nutrition*, 4th Edition, Academic Press, New York-London.

Valent J. (1986). *Tratamentul bolilor prin legume, fructe şi cereale*, Ed. Ceres, Bucureşti.

Vincu M., Precob V., Dumitru M., Daranyi G., Gârban Z. (2000). *Trace elements and biochemical homeostasis of some metal ions and enzymes in blood serum of laboratory animals. Note I. Preliminary data concerning the effects of Zn and Mn*. pp. 189-200, in New Perspectives in the Research of Hardly Known Trace Element Symposium, Budapest.

Vincu M. (2004). Oligoelemente metalice de interes biologic şi toxicologic în muşchi şi organe la animale, cu un model experimental al translocării, *PhD Thesis*, Universitatea Politehnica Timisoara.

* * * (1989). *Agency for Toxic Substances and Disease Registry (ATSDR) – Toxicological Profile for Zinc. Agency for Toxic Substances and Disease Registry*, ATSDR/TP-89-25, U.S.Public Health Service, Atlanta.

* * * (1989). *National Reseach Council (NRC) – Recommended Dietary Allowances*, 10th ed. National Academy Press, Washington, DC.

* * * (1997). *Aspects sanitaires et nutritionnels des oligo-éléments et des éléments en traces*. Ed. OMS, Genève, Publ. InfoPrint Singapour.

* * * Srimathi – *Influencing Iron and Zinc Bioavailability*, (http://www.garynull.com/Documents/Codex/Zinc.pdf); www.superlife.co.uk/healthlife.htm.