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THE EFFECT OF ZINC CHLORIDE ADMINISTRATION ON SOME TRACE METALS IN WISTAR RATS LIVER

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Abstract

The aim of this study is to appreciate the effect of zinc over-dose on trace metals homeostasis in rat liver, considering Mn, Cu, Fe and Zn, after gavage intake of two different doses of RDIx2, and RDIx4 (RDI – Recommended Daily Intake) of zinc as zinc chloride solutions. The excess of zinc intake brings about an accumulation of this metal in liver comparing with control, from 24.1±1.5μg/g w.t. to 30.0±1.6μg/g w.t. in the first experimental group (RDIx2), and 43.7±1.8μg/g w.t. in the second experimental group (RDIx4). The study also revealed a strong disturbance of iron homeostasis.
***Keywords**: Zinc chloride, trace metals, liver*

Introduction

Zinc is very common element that occurring naturally in air, soil, water and all foodstuffs. In human organism the total amount of zinc are 2 or 3 g distributed mainly in muscles, bones and some organs (liver, kidney, prostate). Zinc plays an essential role in human metabolism, helping the functioning of more than 200 enzymes such as: carbonic anhydrase which regulates CO_2 exchange, superoxide dismutase, carboxypeptidase, isocitric dehydrogenase, alcohol dehydrogenase, and ceruloplasmin; and for the stabilisation of DNA

and the expression of genes: RNA polymerase (Badawy, 1987; Opresko, 1992; Mincu, 1993; Gârban, 1999).

Common compounds of zinc used for different study of necessary and toxicity include salts solutions as chloride, oxide, sulfate, and sulfide. After ingestion and absorption, zinc is transported in organism by metallothionein (Stokinger, 1981). Around 20-80% from ingested zinc is absorbed in gastrointestinal tract. This depends on the chemical compound as well as on zinc levels in the body, the concentration of zinc solutions, the period of time for entired experimental study, and dietary concentrations of other nutrients, and others (Bertholf, 1988; U.S.E.P.A., 1992).

High dietary levels of phytate, calcium, or phosphorus reduce absorption, but enhances protein uptake (Pup, 2002).

Homeostatic mechanisms for zinc involving the presence of metallothionein in the mucosal cells of the gastrointestinal tract that regulates zinc absorption and excretion (ATSDR, 1989).

In cases of acute intoxication with zinc the pacient accused nausea, abdominal cramps, diarrhea, and vomiting sensation. Zinc chloride ingestion with over-doses of zinc, decreases serum calcemia and increased the activity of amylase in pancreas (Murphy, 1970; Chobanian, 1981).

But chronic oral exposures to zinc in some studies have resulted in hypochromic anemia associated with hypoceruloplasminemia, hypocupremia, and neutropenia in some individuals (Hoffman et al., 1988). Anemia and pancreatitis were the major adverse effects observed in chronic animal studies (Johnson and Sauberlich, 1982; Allen et al., 1983).

Experimental

The experiment was made on three groups of 10 Wistar rats each, one control group (C) and two experimental groups E_1 and E_2 respectively. In the fourth day and in the seventh day of the experiment, solutions of $ZnCl_2$ in concentration which offer in 1 ml with an amount of 0.428 mg/kg b.w. (RDI x2), and 0.856 mg/kg b.w (RDI x4) were administered through gavage to both experimental groups. In the fourteen day of the experiment animals were killed and liver samples were taken in order to determine some trace elements (Vincu, 1999).

Trace metals analysis were made with an AAnalyst 800 Atomic Absorption Spectrometer, by electrothermal atomization for Mn and Cu, and flame technique for Zn, and Fe.

Statistical data were obtained with Excel descriptive statistics and Student test evaluation.

Results and Discussions

The very active chemical environment of liver, determine this organ being capable of detoxification. Hepatic cells represent a very active reaction site for different metabolic processes. One of these processes is iron storage. Among the iron from hemoglobin, a large amount of iron is found in liver as ferritine. Hepatic cells contain large amounts of a protein called apoferritine, which is capable to bound different iron quantities. When the iron is present in extra-cellular fluids into large amounts, this is combined with apoferritine and stored in liver until somewhere in the body a necessary of iron is needed.

Table 1. Trace elements content in liver (μg/g w.t), after $ZnCl_2$ administration

Working group	Mn	Cu	Zn	Fe
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD
Group C	1.43 ± 0.25	4.10 ± 0.25	24.1 ± 1.5	121 ± 9
Group E_1 $ZnCl_2$ (RDIx2)	1.25 ± 0.14*	4.05 ± 0.19	30.0 ± 1.6*	130 ± 12*
$\Delta\overline{X}$	- 0.18	- 0.05	+ 5.9	+ 9
Group E_2 $ZnCl_2$ (RDIx4)	1.14 ± 0.11*	3.38 ± 0.13*	43.7 ± 1.8*	405 ± 11*
$\Delta\overline{X}$	- 0.29	- 0.72	+ 19.6	+ 284

*$p < 0.05$

It can be observed that hepatic distribution of biometals in control group is:

$$Fe > Zn > Cu > Mn$$

Zinc accumulates in liver after zinc chloride administration, from 24.1 in control to 30.0μg/g w.t. in the first experimental group (RDIx2) and 43.7 in the second (RDIx4), corresponding to 24 % and 81% increase concentration.

Observing the table, cooper decrease after zinc over-dose in both experimental groups, comparing with control (Fig. 1).

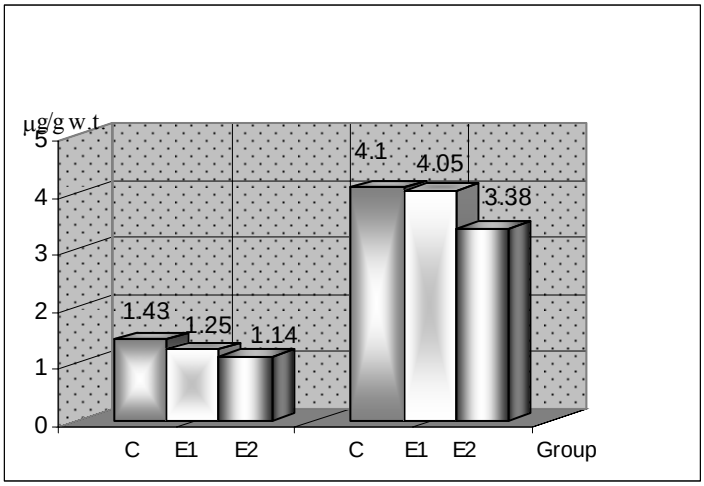

Fig. 1. Mn and Cu concentration in Wistar rats liver after $ZnCl_2$ administration

Experimental studies showed that metalothioneine from stomach, favorite zinc absorption. Copper bound metalothioneine stronger than zinc but in a case of zinc excess, copper absorption is decreased. Generally, zinc absorption is favorites by the presence of animal proteins and small molecule compounds as amino acids and hydroxiacids.

After absorption in the body, zinc is bound to the most important protein complexes: metallothioneine, which is acting as a transporter. Oral chronic exposure at zinc can lead to syderoblastic or hypochromic anemia, associated with hypoceruloplasminemia, hypocupremia (Broun, 1990; U.S.E.P.A., 1992). It is well known that excess of zinc intake through diet or supplements, can affect iron absorption (Kannan,

2003). Iron demobilization from deposits signifficantly raise nonhemic hepatic iron. The iron irreversible stored in liver, have an important role in initialization and catalyze of different reactions of reactive species of oxygen – ROS (Aust, 1985, cited by Dejica, 2000). Except Haber-Weiss reactions which produce hydroxyl from superoxyde radical, the iron can react with superoxyde and hydrogene-peroxyde, producing many other oxidants having role in lipoperoxidation.

Physiologically, the iron is stored as ferritine in liver without chemical reactivity. Free iron complexes, nonferritine iron, capable to initiate in vivo ROS formation, are linked to metal binding compounds as ATP, ADP, or citrate, and might be present in microsomal membranes (Miotti, 1989, cited by Dejica, 2000).

Based on these aspects is obvious that zinc can interfere with iron metabolism, and that can explained the increase of iron accumulation in liver following the two administrations (Fig. 2).

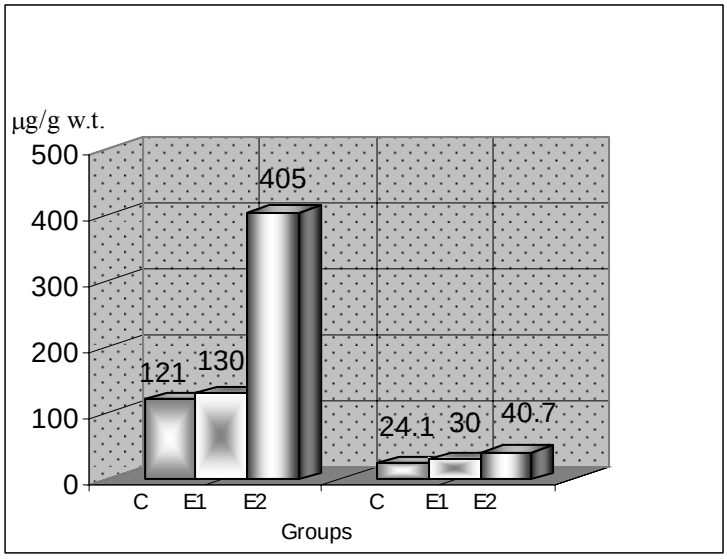

Fig. 2. Changes of Fe and Zn concentration in liver after $ZnCl_2$ administration

Spectacular increasing of hepatic iron after administering RDIx4 dose can be due to iron release from deposits and its accumulation under zinc excess effect. This study presents a manganese significant, but low decrease in both experimental groups, comparing with control.

Anyway, is obvious that zinc accumulation in liver, in conditions of the present experiment, determine a small decrease in cooper and manganese content of this organ and the increase of iron amount.

Zinc is the metal that we administrated in excess as zinc chloride to Wistar rats. Because of this, the aspects of antagonistic – synergistic effects may be evaluated comparing to manganese, cooper and iron. Owing to modifications were registered for metals with major implications in enzymes function we can presume that zinc excess administered to rats determine in liver changes in antioxidant protection system of the body represented by CuZnSOD, MnSOD, FeSOD.

Conclusions

Increasing $ZnCl_2$ dose administered in experimental groups comparing with control, zinc accumulates in liver with 24% after $ZnCl_2$ RDIx2 and with 81% after $ZnCl_2$ RDIx4, suggesting a strong disturbance of Zn homeostasis in this organ. Excess intake of Zn determines a small decrease of hepatic amount of Cu and Mn, suggesting possible antagonist behavior between those trace elements, in present experimental conditions.

The increase of hepatic iron after zinc excess intake, led to conclusion of a synergic relationship between iron and zinc, but this accumulation of hepatic iron can determine an intensification of cell oxidative reactions and an oxidative stress appearance. After $ZnCl_2$ intake in dose of RDIx4, hepatic iron had an increase of 235% comparing with control.

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