Manganese and Zinc Overdose - Risk of Oxidative Stress Appearance

MIRELA AHMADI1*, MIHAELA PUP 2, LUCIA OLARIU3, HORIA VERMEASAN4, RADU PREJBEANU1

1 University of Agricultural Sciences and Veterinary Medicine of Banat Timisoara, Faculty of Food Products Technology, Department of Biochemistry and Human Nutrition, 119 Calea Aradului, 300 645, Timisoara, Romania
2 University of Agricultural Sciences and Veterinary Medicine of Banat Timisoara, Faculty of Veterinary Medicine, Department of Biochemistry, 119 Calea Aradului, 300 645, Timisoara, Romania
3 University of Medicine and Pharmacy "Victor Babeș" Timisoara, Departament of Orthopaedy, 1-2 Square Eftimie Murgu, Timisoara, Romania

The study presents the effect of Zn and Mn overdoses in relation with other trace metals in rat's liver. All studied metals are involved in some enzymes activity, which are implicated in antioxidant defense system of the body. But, in some conditions – our case in overdoses – an imbalance can appear, and antioxidant character of a metal can become prooxidant. In this case, antagonistic or synergic behaviour in relation with other trace metals is obvious. The observed modifications are the increasing of zinc and manganese level in liver after solutions administration, and the amplification effect over the hepatic iron, with the possibility of oxidative stress appearance.

Key words: zinc, manganese, oxidative stress, liver

One of the paradoxes of life on this planet is that the molecule that sustains aerobic life, oxygen, is not only fundamentally essential for energy metabolism and respiration, but it has been implicated in many degenerative diseases. Atmospheric oxygen in its ground-state is distinctive among the gaseous elements because it is a biradical, having two unpaired electrons. This feature makes oxygen paramagnetic; it also makes oxygen very unlikely to participate in reactions with organic molecules unless it is “activated”. A free radical can be defined as any chemical species that can independently exist and contains one or more unpaired electrons [1]. Reactive oxygen species (ROS) include oxygen containing radicals as well as non-radical derivates of oxygen, and they are: superoxide (O2·-), hydrogen peroxide (H2O2), hydroxyl radical (HO·) [2, 3]. In biological life, free radicals may attack biological molecules such as DNA, proteins and phospholipids, and thus cause damage at the cellular level. Transition metals have a variable oxidation number, which allows them to participate in single electron transfer reactions [4]. These transition metals are incorporated as functional redox centers in antioxidant enzymes such as catalase and superoxide dismutase. These metals are classified as antioxidant nutrients [5]. However, transition metals may also be considered pro-oxidant nutrients because of their capability to facilitate free radical reactions by converting H2O2, which is a product of normal cell physiology, via Fenton reaction (1):

\[ M^{n+} + \text{H}_2\text{O}_2 \leftrightarrow M^{(n+1)+} + \text{OH}^- + \text{OH}^- \]  \hspace{1cm} (1)

The Fenton reaction is the first step of the Haber-Weiss reaction (2), which results in the formation of hydroxyl radical (HO·), and other ROS:

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \leftrightarrow \text{HO}^- + \text{HO}^- + \text{O}_2 \]  \hspace{1cm} (2)

This reaction may be catalyzed by Fe, Cu, Cr and also other transitional metals.

Due to the fact that radicals have capacity to react in an indiscriminate manner leading to damage to almost any cellular component, an extensive range of antioxidant defenses are present to protect cellular components from free radical induced damage.

The antioxidant enzymes as catalase (CAT) and as superoxide-dismutase (SOD) are discussed further on. Catalase, a manganese or heme-containing enzyme, forms rapidly H2O2 and then water and oxygen.

\[ 2 \text{H}_2\text{O}_2 + \text{CAT} \leftrightarrow 2 \text{H}_2\text{O}_2 + \text{O}_2 \]  \hspace{1cm} (3)

Superoxide dismutases are Zn, Cu or Mn containing enzymes (ZnCu-SOD, Mn-SOD) that scavenge O2·- by a rapid dismutation reaction (4):

\[ 2 \text{O}_2^- + 2 \text{H}^+ + \text{SOD} \leftrightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]  \hspace{1cm} (4)

The ability of zinc to retard oxidative processes has been recognized for many years. Zinc has never been shown to interact directly with an oxidant species but rather prefers to exert its effects in an indirect manner. We are talking about the well known antagonism between zinc and iron and cooper, with respect to their abilities to promote formation of HO· from H2O2 and superoxide [6]. The acute effects of zinc are generally manifested in the presence of a demonstrable short term increase in levels of this metal. Increase of iron or copper amount in reaction site, can activate the formation of ROS. There are food products or pharmaceutical products with antioxidant role, acting as inhibitors of free radicals or ROS [7, 8].

Manganese absorbed from the body is avidly taken up by the liver and distributed to tissues. The mechanisms of absorption appear to be regulated in parallel with iron absorption (as antagonist effect) [9]. Some research studies showed that site-specific mechanism for free radical can induce biological damage. In fact it is about the essential role of redox-active transition metals.
Experimental part

For experiments, we had as laboratory animals, adult rats from Rattus norvegicus breed, line Wistar, different sexes but alike somatic characteristics. We used for administration the gavage, and the two solutions were zinc chloride and manganese chloride.

In the first stage of experiment we formed one control group (C) and four experimental groups (Ei), containing 10 animals each. Animals were maintained in good physiological conditions, according to Romanian and European Union laws [10 - 14] concerning animal protection in scientific researches. In the fourth day of experiment, we have administered first dose of solutions, and in the seventh day the second one, but only for experimental animals. Animals from control group were treated the same as experimental animals, but we have administrated as gavage drinking water.

Before gavage procedure a ketamine intramuscular injection was made to animals (Calypsol 10ml/500mg ketamine clorhydrate, produced by "Gedeon Richter Ltd.", Budapest, Hungary).

The reference for metal concentration of administrated solution was RDI - Recommended Daily Intake for manganese and zinc. From each salt solution, we had solution was RDI – Recommended Daily Intake for Budapest, Hungary). The reference for these concentrations of metals, we had the recommendations of Expert Group on Vitamins and Minerals Meeting- Nutrition [15], which are: 0.171-0.214 mg/kg b.w. of manganese daily and 0.171-0.214 mg/kg b.w. of zinc daily. So, for the first administered dose RDIx2 of Zn (E1 group), we prepared a 1.316mMol/L ZnCl2 solution, and for the second administrated dose RDIx4 of Zn (E2 group), we prepared a 2.633mMol/L ZnCl2 solution. Also, for the first administrated dose as RDIx2 of Mn (E3 group), we prepared a 1.550mMol/L MnCl2 solution, and for the second administrated dose RDIx4 of Mn (E4 group), we prepared a 3.110mMol/L MnCl2 solution.

After gavage procedure, animals were behavioral checked and no problems were registered. At the finish of experiment, after anesthesia with ketamine, animals were killed in the fourteenth day. After dissection, we sampled the liver. Sampling was made with sterile surgical instruments, and organs were stored in clean glass containers in refrigerator, at -18°C. The glass containers previously were washed with detergent solution, and rinsed well with water, then with acidic solution of 5%HNO3, then with distilled water, and dried to 105°C.

Before samples digestion tissues were weighted on a Mettler Toledo AG204 analytical balance with a tolerance of 0.1 mg. Digestion was made in a Milestone Microwave System with a special program for samples with fast exothermic reactions (containing a large amount of organic matter).

After wet digestion with 5mL of 65% nitric acid (Merck) and 1mL of 30%H2O2 solution, sample solution was transferred into a 50mL volumetric flask and then was diluted to volume with double deminized water. The equipment used for metal determination in solutions was atomic absorption spectrometer (AAS), produced by Perkin-Elmer, with Zeeman effect for background correction and transversal heating of graphite tube. Elements Zn, Fe, Mg were determined in air-acetylene flame (AA), and Cu and Mn by electro thermal atomization. We used appropriate ionization control substances for flame and matrix modifiers in graphite tube.

For instrument calibration, we used standard single element solutions of 1000mg/L, produced by Merck, making dilutions for calibration standards, to obtain a calibration curve in linear domain. The calibration curve control was made with a multielement standard solution (Merck). The results were obtained in µg/L in solution, and reported after calculations in µg/kg w.b., considering the initial weight of tissue and the volume of volumetric flask used (50mL).

Statistical data were obtained using descriptive statistics (EXCELL).

Results and discussions

Trace elements are necessary for normal function of the liver, and are therefore associated with morbid deficiency states. They are also commonly toxic when are presented in excess.

In figure 1 and figure 2 we presented studied trace elements status in liver, after administration of zinc excess as ZnCl2 solutions.

Hepatic distribution of metals in normal liver (liver from rats from control group) was: Mg > Fe > Zn > Cu > Mn.

As shown before, the acute effects of zinc are generally manifested in a short term level increase of this metal in the body. In our case, zinc concentration in liver increase significant in both experimental groups comparing with control (24% and 81% respectively). Antagonist behavior is obviously in relation with manganese (not very important, even significant). Also, and with copper -
especially in group E2 - confirming again as in other research studies [5].

It is well known that excessive zinc intake (diet or supplements), can affect iron absorption by decreasing its absorption [16]. That means that iron will be removed from deposition sites and then will be eliminated.

Iron removal from deposits raise significantly the hepatic non-heme iron [17]. But iron reversible stored in liver had an important role in initialization and canalization of many ROS reactions, having an important contribution to tissue lesions induced by oxygen [18].

A strong augmentation of iron concentration in liver is visible in E group (over 300% comparing with control), leading to supposition that oxidative reactions in hepatic cells can be accelerate.

Manganese is an essential trace element for humans and animals having a protective role or toxic effect, depending on concentration. Transitional element, can have different oxidation states (+2; +3; +7) and as a consequence, different prooxidant and antioxidant properties. Transition of divalent to trivalent form, lead to an increase of oxidative capacity of manganese, determining ROS appearance, lipid peroxidation and cell membrane injuries. Experimental studies show that the body is protected by the toxic effects of manganese because of a low absorption rate and a fast elimination from liver, during bile excretion [19]. Hepatic levels of studied elements after manganese administration are presented in figure 3 and figure 4.

Observing the figure 4, we can see that for manganese in MnCl2 RDIx2 dose (E3 group), hepatic iron increase with 200% comparing with control group (C group), and increase very little when MnCl2 RDIx4 dose (E4 group) is administered. In the first case, the augmentation can be due to iron removal from deposits, being well known the antagonistic behavior between iron and manganese.

Conclusions

Considering zinc modifications in liver after zinc overdose intake, we can conclude that zinc accumulates in liver in both experimental groups, with almost 50% after RDIx2 dose (E1 group), and 100% after RDIx4 dose (E2 group). Manganese and copper concentration does not show important modifications. An important increase of iron in E2 group after RDIx4 dose of Zn was registered, so the modifications in oxidative processes in liver might be possible, and also the oxidative stress appearance.

Reviewing the results obtained concerning modifications induced by manganese overdose in liver, we can conclude that manganese accumulates in liver after an overdose intake, with 12% after RDIx2 (E3 group) and 47% after RDIx4 (E4 group), comparing with control group. Also, a synergic behavior between zinc and manganese appears in our experimental conditions. After this experiment we observed a different behaviour of manganese in relation with iron, depending on dose. The danger of oxidative stress appearance is correlated to augmentation of iron in liver.

Comparing the effect produced by zinc and manganese at the same overdose intake the results are very interesting. The modifications induced by manganese are more important than those of zinc at the same dose. The common modifications are the increasing the level of zinc and manganese in liver after administrations, and the effect on the hepatic iron with the possibility of oxidative stress appearance.

References

3. Diaconescu, C., Papuc, C., Rev. Chim. (Bucureºti), 57, nr. 8, 2006, p. 808

Fig. 3. Manganese and copper concentration in rats’ liver after manganese overdoses

Fig. 4. Zinc, iron and magnesium concentration in rats’ liver after manganese in overdoses

Significant increase of manganese in liver in respect with control group was registered. Copper seems to have a synergic behaviour with manganese in our experimental conditions.

A synergic relation between zinc and manganese in liver is obvious. Also, a strong increase of iron in liver after RDIx2 (E3 group) is visible (fig. 4).

There is a strong relation between iron and manganese absorption through intestinal wall, because of a competition for absorption sites. On the other hand, manganese and copper decrease iron absorption and that can explain low concentration in liver at the highest dose of manganese.

Other studies showed that at low concentrations, Mn2+ protect cells of oxidative stress. This protection effect seems to appear because of superoxide radical dismutation by the Mn2+. Research studies shown that during the oxidative stress Mn-SOD can be formed [20].
11. *** Romanian Regulation 37/2002, publ. in M.O. of Romania nr. 95/2.02.2002
12. *** Romanian Law nr 205/2004 (Art. 7, 8, 22), publ. in M.O. of Romania, Part I, Nr. 531/14.06.2004
13. *** Romanian Regulations no. 143/400/2002 (Art. 1, 2, 3), publ. in M.O. of Romania, Part I, nr. 697/24.09.2002
14. *** European Union Law 305/12.07.2006
18. DEJICA, D., Stresul oxidativ in bolile interne, Casa Cărţii de Știinţă, Cluj-Napoca, 2000, p. 73

Manuscript received: 12.12.2007