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# CHANGES IN ENZYMES ACTIVITY SUPEROXID DISMUTASE, GLUTATHIONE REDUCTASE, REDUCED GLUTATHIONE AND QUANTITY OF MALONIL DIALDEHYDE AFTER ACUTE ALUMINUM POISONING

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*The important and increasing application of aluminum motivated us to determine whether and in what way does the antioxidative status of erythrocytes in aluminum acute poisoned animals change by studying the metabolism of superoxid dismutase (SOD), glutathione reductase (GR), reduced glutathione (GSH) and malonil dialdehyde (MDA). The experiment involved desert mice (gerbils) who received intraperitonally an aluminum chloride solution at a dose of 3.7 g/kg BM. The experimental groups of animals were sacrified after 24, 48, 72 and 96 hours from the beginning of the experiment by cardial punction or by punction of the abdominal artery. To the control group saline solution was applied. It has been noticed that during the course of the experiment SOD activity increased. We assume this represents a defense mechanism of the organism to increased quantities of free radicals. Further, aluminum considerably reduces the glutathione reductive system capacity affecting its enzymatic component, glutathione reductase which leads to a further decrease in reduced glutathione. The lipid peroxidation index expressed through MDA as an indicator of membrane status remains unchanged. This could mean that malonil dialdehyde is measured in erythrocytes not jet structurally damaged.*

*Key words: aluminum poissoning, enzymes activity, superoxid-dismutase, glutathione reduktase, lipid peroxidation*

## INTRODUCTION

Since aluminium was discovered in 1827, its production and applications have been in constant increase. Maximum increase has been noted after the Second World War, late seventies and beginning of the eighties (Osinska *et al*., 2004).

The first data on aluminum adverse effects on health, were recorded in 1947, when Shaver and Ridell described a pulmonary disease in workers

employed at processing of boxite. The disease was called aluminosis or Shaver's disease, that is connected to inhalation of very fine quartz particles, that are present in up to 30-40% of $Al_2 O_3$ production process. Similarly, Hamilton and Hardy described in 1949 cases of pulmonary diseases similar to silicosis among workers that were exposed to aluminum dust in the German military industry during the Second World War.

Recently, special attention has been devoted to testing the connection of aluminum exposition and the changes occurring in the central nervous system. This is of special interest to Alzheimer disease and changes in bone tissue which lead to microcitic hypochromic anemia (Farina *et al*., 2005; Suwalsky *et al*., 2001).

Aluminium can be introduced into the organism by means of ingestion, inhalation or percutaneous resorption. The most common aerosol particles which can be inhaled are aluminium oxide, sulphate and silicate.

Submicron particles are being fagocited by lung alveolar macrophages and transported into lymph nodes. It was proved that aluminum from macrophage lisosomes is released, thus penetrates into tissues. The aluminium daily intake by ingestion of food and water is in the range of 10-160 mg. Aluminum is excreted by feces.

The soluble form is absorbed by a mechanism which is not completely explained. It was established that parathyroid hormone and citric acid increase aluminum intestinal absorption (Ersley *et al*., 1985).

Aluminum distribution has not been adequately studied due to defect of the stabile radioactive substance. In experiments with rats that were orally or paranterally poisoned with aluminum and then sacrificed, high concentrations of this metal were found in the liver, kidneys, adrenal glands, brain, muscles, bones and bone marrow. In humans the greatest concentrations were found in the lungs, increasing with age (in contrast to other organs) and probably this represents deposits of aluminum inhaled particles.

Some data indicate that aluminum concentration in the brain increases with age (Harrison *et al*., 1972). In patients with chronic renal insufficiency, aluminum is accumulated in some organs up to two hundred times more than it normal values. Aluminum elimination is through the kidneys. Concentrations in the urine in adult normal individuals vary from 3 to 30 μg/L, and they are neither in reciprocal relationship with aluminium concentrations in the blood nor affected by age and smoking habits.

There is also no effect of concentration mechanisms or urine dilution on aluminum levels in the urine. In workers exposed to aluminum, urine secretion is considerably greater depending on the intensity and duration of exposure. Values vary from 44 to 300 μg/L. Within short exposure half-time of urinary elimination is 8 hours.

After exposure to high aluminum concentrations, there is a linear correlation between aluminum concentration in the blood and urine (Vittori *et al.*, 1999).

Intracelluraly, aluminum is localized in the lysosomes, most probably in the form of aluminum phosphate. Lysosomes contain a great number of enzymes which, besides other properties, concentrate metals such as gallium, indium and aluminum. Aluminum from exracellular liquid, where it exists in small

concentrations in a soluble form, passes through the lysosomal membrane, most probably by active transport. Acid phosphatase plays a significant role in the process (Bommer *et al.*, 1983). Intralysosomal precipitation of insoluble aluminum phosphate represents a detoxication phenomenon, because it is considered that the insoluble aluminum form is non toxic. However, long-lasting great aluminum quantities accumulation in lysosoms is incompatible with cell life. Cells are protected from free radicals in several ways. First of all, by enzymatic systems which neutralize superoxide anions, hydrogen peroxide and lipid peroxides: superoxide dismutase, catalase and peroxidase. Cell enzymes that enable adequate antioxidative enzyme effects are important, too: glutathione reductase, glucoso-phosphate dehydrogenase etc. Cells also have endogen free radical neutralizators: tocopherol, ascorbic acid and peptides with the tiol group (Ikeda *et al*., 1990). Cell structure being also significant, thus enabling free radical separation (Siesjo, 1981).

*Superoxide dismutase* (SOD) is a metal-enzyme cell complex, which appears in the mitochondria in the form of a tetrameric manganese-enzyme complex, and as a dimeric copper-zinc-enzyme complex in the cytoplasm.

*Catalase and peroxidase* are significant in the regulation of hydrogen peroxide concentration in a cell. When hydrogen peroxide is quickly formed, catalase enables the reaction of hydrogen peroxide dismutation.

*Glutathione peroxsidase* catalyzes the hydrogen peroxide reaction with reduced glutathione (GSH), thus forming oxidized disulfide glutathione (GSSG) and water.

GSSG reduction is catalyzed by NADPH-dependent glutathione reductase.

NADPH is regenerated in the hexamonophosphate schant or in the cytosole reaction of isocitrate dehidrogenase (Halliwel *et al.*, 1985).

Having in mind the above mentioned facts, the basic aim of our research, was to establish the changes, if any, in concretations of oxsidized and reduced glutathione in erythrocytes, superoxide dismutase enzyme activity and quantity of malonil dialdehyde, by means of testing superoxide dismutase (SOD), glutathione reductase (GR), reduced glutathione (GSH) and malonil dialdehyde (MDA).

## MATERIAL AND METHODS

The experiment involved desert mice (Meriones unguiqulatus) adults of both sexes, of approximate body mass of 75 g raised in standard laboratory conditions, at constant room temperature with food and water *ad libitum*. Aluminum solution was applied to nonanesthetised animals in $LD_{50}$ dose of 3.7 g per kg of body mass, intraperitonally. The experimental protocol involved a control group treated with physiological solution only and four experimental groups treated with the above mentioned aluminum solution. The animals were sacrificed after 24, 48 and 72 hours from the application. Animals were sacrificed by cardial punction or by abdominal artery punction. Biological parameters (GR, GSH, SOD and MDA) were determined spectrophotometrically by a UV spectrophotometer.

*Erythrocytes superoxide dismutase* activity was determined as percentage of adrenalin autooxidation inhibition in the base environment by sample of non refined mitochondrial fraction.

*Glutathione reductase* activity was determined in the presence of NADPH, reducing oxidated glutathione, where as NADPH + H is oxidized to NADP. Enzyme activity was expressed in nMol NADP/mg protein/h.

*Reduced glutathione* content (GSH) in erythrocytes was determined by spectrophotometry, using 5,5-ditiobis-2-nitro-benzo acid (DTNB; Ellman's reagents).

*Lipid peroxidation index* was measured indirectly by measuring concentrations of tiobarbituric acid reaction with the stable product of degradation of unsaturated faty acids i.e. malonil dialdehyde (MDA).

## RESULTS AND DISCUSSION

*Changes in SOD enzyme activity in desert mice erythrocytes after aluminum application*

The effect of aluminum application on SOD enzyme activity in erythrocytes is indicated in Figure 1. On the first, second and third day, upon administration of the metal solution, a triple increase of SOD enzyme activity in relation to control values was evident. Thus representing a significant increase of enzyme activity, and in the fourth day of the beginning of the experiment, the enzyme activity returned to the control value.

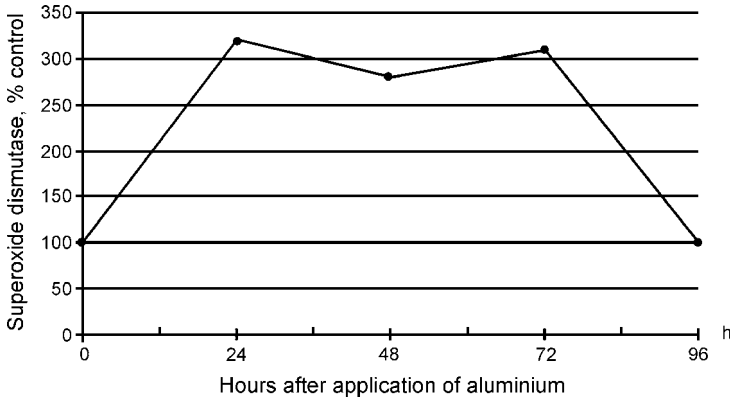


Figure 1. Activity of superoxide dismutase in μg/Hem in desert mouse erythrocytes after aluminium application
*Significant change in relation to the control group ($p<0.01$)

The appearance of the sudden rise of super oxide dismutase activity is paradoxal. The explanation could be given as a drastic glutathione reductase system reduction, whilist the defense from oxidative stress by superoxide dismutasis increased. The question is: could it be sufficiently effective?

Namely, superoxide dismutase affects already formed superoxides and accordingly there is always a possibility for some superoxides to avoid its effect. On the other hand, glutathione reductase prevents superoxide formation. The rise of superoxide dismutase activity does not exclude the possibility that the cell has already been damaged as there is a possibility that some formed superoxides had avoided the superoxide dismutase effect.

In his doctoral PhD thesis, Milovanović (1996), who was testing enzyme activities in the brain after aluminum poisoning, has come to a conclusion that in acute aluminum poisoning, there is a significant superoxide dismutase inhibition in the tested brain structures, the greatest being in the nucleus caudatus, less in the cortex, while the least was in the hippocampus. These values referred to total and mitochondrial superoxide dismutase, but a significantly greater sensitivity of mitochondrial superoxide dismutase after aluminum application, was observed. Mitochondria are the main spot of free radical formation in normal circumstances (Milovanović, 1996). Such finding showed mitochondria as the most sensitive spot in the cell. It should be pointed out that in erythrocytes it is exclusively in the cytosolic superoxide dismutase form.

The increase of superoxide dismutase activity found in erythrocytes is not sufficient to compensate the increased production of superoxidated radicals, which is the result of decreased glutathione reductase activity. It is to be studied whether it is a matter of the quantity of radicals and exceeding superoxide dismutase capacity, or the problem is in the type of radicals which can not be neutralized by superoxide dismutase (Gonsales *et al*., 2000).

*Changes in reduced glutathione content in erythrocytes of desert mice after aluminum application*

The effect of aluminum application to reduced glutathione content in erythrocytes is indicated in figure 2.

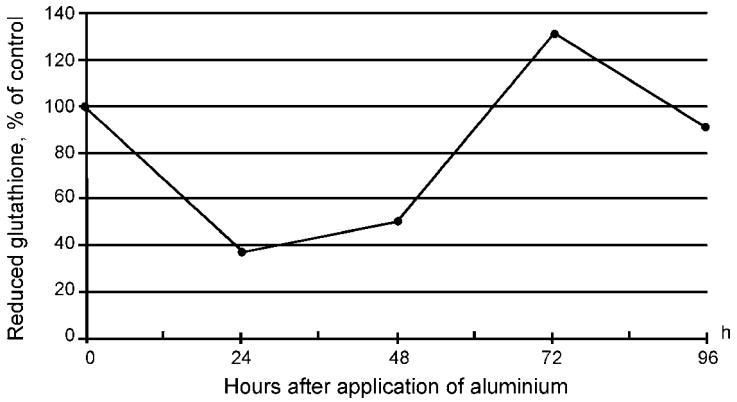


Figure 2. Changes in quantity of reduced glutathione in desert mouse erythrocytes after aluminium application
*Significant change in relation to the control group ($p<0.01$)

One day after aluminium application a significant decrease of 30% of control values in reduced glutathione content was noticed. Two days later the reduced glutathione content was still significantly decreased (50%), while three and four days later these values were not significantly changed in relation to the control (130% and 80% respectively).

*Changes in GR enzyme activity in erythrocytes of desert mice after aluminum application*

Figure 3 represents the activity of glutathione reductase enzyme in erythrocytes after aluminum application. A significant decrease of enzyme activity has been noticed after the first and second day from the beginning of the experiment. There of the activity decreased to 50% of the control value which represents a considerable decrease of enzyme activity. On the third day the enzyme activity slightly rose, but it was still less than the control values for about 50%. On the fourth day of the trial, enzyme activity was at the same level as in the control group.

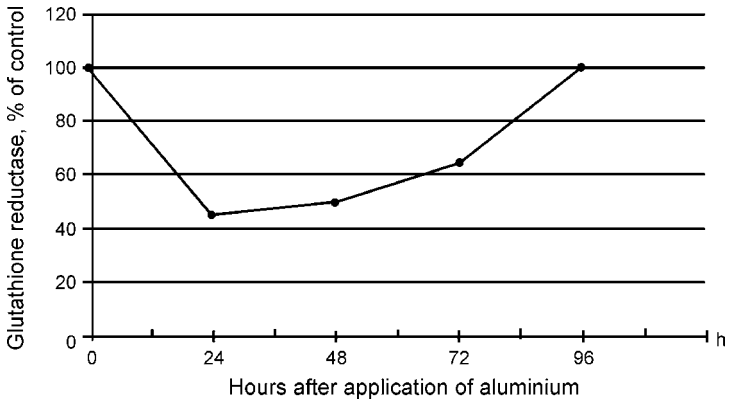


Figure 3. Activity of enzyme glutathione reductase in desert mouse erythrocytes after aluminium application
*Significant change in relation to the control group ($p<0.01$)

Redox status of erythrocytes is very important for the maintenance of erythrocytes membrane integrity which enables the change of erythrocyte shape, and at the same time enduring great deformities to which erythrocytes are exposed (Koraćević *et al*., 1996). The glutathione reductase system has been mostly affected. A drastic decrease in reduced glutathione and decrease of glutathione reductase activity have been noticed (Figures 2 and 3). As it can be observed in Figures 2 and 3, the redox status of erythrocytes is changed and even more in the case of glutathione reductase, where the decrease of enzymes activity in relation to the control, but as well to aluminum quantity in erythrocytes, is often greater than in the case of reduced glutathione.

Namely, after the first day there is a considerabe decrease in glutathione reductase activity as well as in reduced glutathione. This trend is maintained for three days for glutathione, and two days in the case of reduced glutathione. Activities of both enzymes increase on the fourth day, but are still far from total recovery. This phenomenon has a temporary aspect, because recovery appears in both enzymes on the fourth day. This effect is surely dependent on the quantity of applied aluminum. The degree of damage of the glutathione system, in the first place is correlated with aluminum concentration in the erythrocytes themselves, which further indicates that aluminum, when present in erythrocytes, is the cause of toxic effects, i.e. aluminum ions are responsible for the effects on glutathione reductase and glutathione itself.

Glutathione reduction is expressed through the hexosomonophosphate shunt which produces NADPH, then glutathione reductase transfers those reductive equivalents to glutathione. It appears that reduced glutathione, serves as the basic antioxidant source of reduction equivalents that prevent oxidation in the erythrocytes themselves. Dramatic decrease of glutathione reductase activity is after 24 and 48 hours when it falls to about 40% of the control values. This, of course, must reflect in the function of erythrocytes and one of the possibilities is the damage of erythrocyte membranes to such an extent that they break during erythrocyte passage through narrow spleen canals. Consequently, decrease of oxidative status, decrease of cell ability to defend from oxidative stress, lead to membrane damage, and in turn are not able to endure deformities with which erythrocytes are already familiar in the normal circulation. This is a still hypothetic question and requires further research.

Aluminum causes, as well, drastic disturbances of glutathione reductase in the central nervous system (Milovanović, 1996). Acute application of aluminum decreases the level of glutathione reductase and reduced glutathione, so it should be pointed out that aluminum is highly effective on the glutathione system. However, the mechanism of this effect is not clear and needs further study.

*Changes of MDA concentration in erythrocytes of desert mice after aluminum application*

The effect of aluminum application on the quantity of malonil dialdehyde in erythrocytes of desert mice has been presented in Figure 4. On the first and second day after aluminium application, there was an increase of MDA quantity for about 20% in relation to the previous day. On the third and fourth day of the experiment, MDA quantity is again near the level of the control value.

It should be emphasized that there are slight or no changes in the content of malonil dialdehyde. In theory, there are two explanations for malonil dialdehyde quantities to remain unchanged. Firstly, superoxide dismutase activity is to such an extent effective that it had prevented membrane breaking, but on the other hand, there is a possibility for same erythrocytes to be destroyed. As all other parameters, malonil dialdehyde is also measured in isolated erythrocytes, that means in erythrocytes that are intact. It is clear that in the case of erythrocyte destruction malonil dialdehyde in them can not be measured. In other words, there is not a critical quantity of malonil dialdehyde in terminally damaged

erythrocytes, this will not be measured, at least not by methods used for surviving erythrocytes. This could be the possible explanation for anemia (Ganchev *et al*., 1998; Zatta *et al*., 1997). Namely, due to decreased of activity of glutathione reduction system, free radicals appear, which lead to cell membrane destruction.

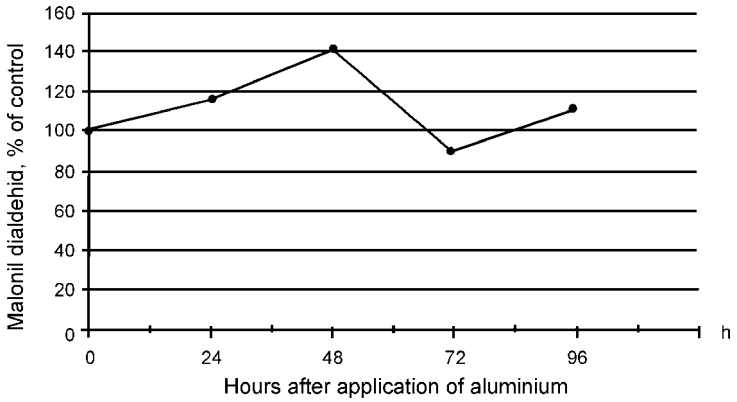


Figure 4. Quantity of malon dialdehyde in desert mouse erythrocytes after application of aluminium
*Significant change in relation to the control group ($p<0.05$)

## CONCLUSION

The presented study results and discussion, can conclude that aluminum considerably decreases the capacity of the glutathione reduced system, affecting glutathione reductase, thus leading to a decrease in reduced glutathione.

As far as superoxide dismutase activity is concerned, it has been noticed that it is increased, possibly as an attempt of cell defense from the increased quantity of free radicals.

It contrast to these enzymes, lipid peroxidation index, expressed through malonil dialdehyde concentration remains unchanged, this could mean that malonil dialdehyde is measured in erythrocytes which are not structurally damaged yet (Mahieu *et al*., 2000).

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## PROMENE AKTIVNOSTI ENZIMA SUPEROKSID DIZMUTAZE, glutathione REDUKTAZE, REDUKOVANOG GLUTATIONA I KOLIČINE MALONIL DIALDEHIDA NAKON AKUTNOG TROVANJA ALUMINIJUMOM

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SADRŽAJ

Značajna i sve šira primena aluminijuma u modernom dobu motivisala nas je da ispitivanjem metabolizma superoksid dizmutaze (SOD), glutation reduktaze (GR), redukovanog glutationa GSH) i malonil dialdehida (MDA) utvrdimo da li se i koliko menja antioksidativni status eritrocita životinja akutno trovanih aluminijumom. U eksperimentu su korišćeni pustinjski miševi, kojima je aplikovan rastvor aluminijum hlorida intraperitonealno u dozi 3,7 gr po kilogramu telesne mase, a žrtvovanje eksperimentalne grupe životinja je vršeno 24, 48, 72 i 96 časova od početka eksperimenta kardijalnom punkcijom i punkcijom abdominalne aorte.

Kontrolna grupa je tretirana fiziološkim rastvorom. Uočeno je da u toku eksperimenta aktivnost SOD raste, verovatno kao pokušaj odbrane od povećane količine slobodnih radikala. Osim ovoga, aluminijum značajno smanjuje kapacitet glutation reduktaznog sistema, pogađajući njegovu enzimsku komponentu, glutation reduktazu što se iskazuje smanjenjem redukovanog glutationa. Za razliku od ovih enzimskih sistema, indeks lipidne peroksidacije izražen preko MDA kao indikatora stanja membrana ostaje neizmenjen, ali to može da znači da se malonil dialdehid merio u još uvek strukturalno neoštećenim eritrocitima.