EFFECT OF ACUTE EXPERIMENTAL ALUMINUM POISONING ON HEMATOLOGIC PARAMETERS

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Having in mind the presence of aluminum in industry, as well as in households, we wanted to define changes in the number of erythrocytes and aluminum content in the plasma and erythrocytes following acute aluminum poisoning under experimental conditions. The experiment involved gerbils which received intraperitoneally aluminum chloride solution at a dose of 3.7g per kg of body mass. The experimental group was sacrificed after 24, 48, 72 and 96 hours from the beginning of the experiment by cardiac punction or by abdominal artery punction. The control group was treated with saline, only. The number of erythrocytes and hemoglobin concentration were considerably reduced with respect to the control group, most likely as the result of cell membrane changes and reduced life cycle of erythrocytes. During the experiment, aluminum content in the plasma was increased in the first and second day of the experiment, it started to decline on the third day, while on the fourth day it returned to its original value, which proves again the existence of a special mechanism of "clearance" of aluminum in plasma.

Key words: aluminum poisoning, erythrocytes, plasma

INTRODUCTION

The primary function of erythrocytes is to carry oxygen bound to hemoglobin from the lungs to tissues. In some animals there is no hemoglobin in the erythrocytes, but it circulates as free plasma protein. However, when it is free in human blood plasma, about 3% of free hemoglobin leaks through the capillary membrane into the interstitial spaces and through the glomerular kidney membrane into the glomerular filtrate.

Apart from oxygen transport, erythrocytes have other functions, as well. For example, they contain a great quantity of carboanhydrase that geatly accelerates the reaction between carbon-dioxide and water. The speed of this reaction enables water to react in the blood with a large quantity of carbon-dioxide and that

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quantity is also transported from the tissues to the lungs in the form of bicarbonate ions (HCO_3^-). Furthermore, hemoglobin in erythrocytes is an excellent acid-base buffer (for the majority of proteins), thus erythrocytes are mostly responsible for the buffer capacity of whole blood (Ersley and Gabuzda, 1985).

Normal erythrocytes are in the form of biconcave discs. Approximate diameter is about 7.5 μm, thickness is 1.9 μm at the thickest spot. The approximate volume of erythrocytes is 83 cubic micrometers. When erythrocytes pass through the capillaries, their shape is considerably changed. In fact, erythrocytes are like a bag that can be easily deformed getting nearly every shape. Furthermore, as normal erythrocytes have considerable excess of cell membrane in relation to the quantity of substance within that cell, the deformity does not stretch the membrane and therefore does not lead to cell rupture (Clark, 1988).

The approximate number of erythrocytes in the blood in a healthy male amounts to 5.2±0.3 x 10/L, and in a normal female amounts to 4.7±0.3 x 10/L. The number of erythrocytes can be also affected by altitude.

Erythrocytes have the ability to concentrate hemoglobin up to approximately 34 g/dL of a cell. The concentration never increases above that value since there is a metabolic limit in the cell metabolism for hemoglobin synthesis. In normal individuals the hemoglobin percentage in each erythrocyte is almost always near the maximum. However, when hemoglobin synthesis in the bone marrow is not sufficient, hemoglobin concentration in the erythrocytes may fall considerably below the limit, and the volume of an average erythrocyte may be decreased due to the decreased quantity of hemoglobin which fills the cell (Nayak, 2002).

When the hematocrit (the volume percentage of erythrocytes in whole blood – normally amounts to 40-45%) and hemoglobin average erythrocyte number are normal, in 100 mL of blood there is an average of 16 g of hemoglobin in men and 14 g in women. One gram of pure hemoglobin can bind approximately 1.39 mL of oxygen. Accordingly, 100 mL of blood can absorb more than 21 mL of oxygen in healthy men and 19 mL of oxygen in women. Up to the fifth year of age erythrocytes in man are produced in the marrow of all bones. However, marrow of long bones, except the proximal parts of humerus and tibia, are gradually substituted by fat tissue, so after the age of twenty erythrocytes are not produced anymore. After that age the greatest part of erythrocytes is produced in the marrow of membranous bones, such as vertebrae, sternum, ribs and innominate bones. Even in those bones, with time the bone marrow becomes less productive with time (Koraćević *et al.*, 1996).

MATERIALS AND METHODS

Hematologic effects of aluminum poisoning

Aluminum oversaturation is noticed in people only in isolated clinic environments. This kind of oversaturation occurs in patients with renal insufficiency under dialysis or in patients with uremia that receive compounds which include aluminum, having the property to bind phosphate. The majority of

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patients with renal insufficiency are anyhow anemic, which is why defining the role of aluminum in the pathogeneses of renal disease anemia was difficult.

The following facts are to be emphasized:

1. there are many factors which are known to cause anemia, which again refer to patients on dialysis in the final phase of renal disease. That includes inadequate production of erythropoietin, blood loss during dialysis, as well as blood loss due to gastrointestinal bleeding (Altman *et al.*, 1987);

2. decrease of life span of red blood cells in combination with marrow defects, as well as loss of water soluble vitamins through dialysis (Laeming and Blair, 1979);

3. aluminum reactivity in water solutions, as well as insufficient knowledge regarding aluminum in biological solutions are the reasons for which it is not easy to be sure in the clinical relevance of analysis *in vitro* in which aluminum is used. This is particularly significant for tests in which aluminum concentrations above 25 ìm are applied (aluminum concentration that is rarely achieved even in patients intoxicated with aluminum) (Hamilton and Hardy, 1983).

*Mechanisms by which aluminum is able to weaken the production
of hemoglobin*

When production of heme and globin is decreased results in microcytic anemia. Decreased globin synthesis leads to microcytic anemia, as well as to the decrease of HEME due to defective iron utilization or subnormal protoporphirine production (Kaiser *et al.*, 1984). Anemia combined with aluminum oversaturation can hinder synthesis of heme and globin, although it is not sure that globin synthesis is disrupted. Aluminum can hinder heme synthesis affecting biosynthesis of protoporphirine or hinder normal iron metabolism (Berlyne and Yagil, 1973; Goldsman and Taylor, 1983).

The aim of the study

According to all stated facts, the aim of our research was to test the aluminum quantity in the plasma and erythrocytes, changes in the quantity of hemoglobin in the erythrocytes, as well as changes in the number of erythrocytes in gerbils after aluminum application.

General status of animals has been previously evaluated.

Clinical examination

The experiment involved desert mice (meriones inguiculatus) adult individuals of both genders of average body mass of 75 g, raised in standard laboratory conditions, at constant room temperature with free access to food and water.

Aluminum solution was applied to non-anesthetized animals intrapertitoneally in a dose of 3.7 g per kg of body mass for LD_{50}. Animals were sacrificed after 24, 48, 72 and 96 hours by cardiac punction or by abdominal artery punction. Standard blood smears stained according to Heim were made out of peripheral blood samples and red blood cells were analyzed. General status of animals at the end of the experiment was compared to animals' status before the

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beginning of the experiment. Apart from that, specific aluminum concentrations in the serum and erythrocytes were also tested by standard methods.

Experimental protocol included two groups of animals: the first, control group without therapy, treated only with saline, and the experimental groups treated with the above mentioned aluminum solution.

RESULTS

In the first and the second day upon the begining of the experiment aluminum quantity in the plasma has the tendency of increase by 50, i.e. 100% in relation to the control value. However, aluminum in plasma started to decline in the third and the fourth day to approximately the control level.

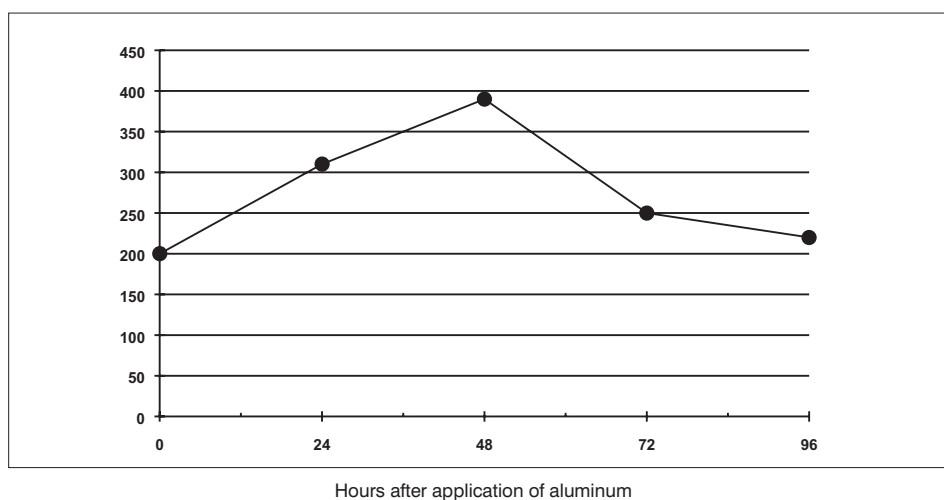Hours after application of aluminum

Figure 1. Quantity of aluminum in plasma of gerbils after application of aluminum

The change of aluminum level in the plasma after aluminum application in a dose of 3.7 g per kg body mass is presented in Figure 1. Twenty-four hours after aluminum application a considerable increase which amounted to about 50% of the control value of plasma aluminum occured. Forty-eight hours from the beginning of the experiment a further increase of aluminum quantity occured, which is now double the control value. However, after 72 hours aluminum quantity is decreased and the same trend continues, and after 96 hours it was almost at the level of the control value. Changes of aluminum in erythrocytes after aluminum application in a dose of 3.7 g per kg of body mass is presented in Figure 2. Twenty-four hours after the application values of aluminum in erythrocytes were 25% higher relative to the control group. This trend of increase in relation to the control still continues 48 hours after the application, as presented in Figure 2.

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Seventy-two hours after the application decrease of aluminum quantity occurs in erythrocytes in relation to the first and second day of the experiment, the value went slightly above the control, but the next day these values returned to the ones recorded for the first and second day of the experiment.
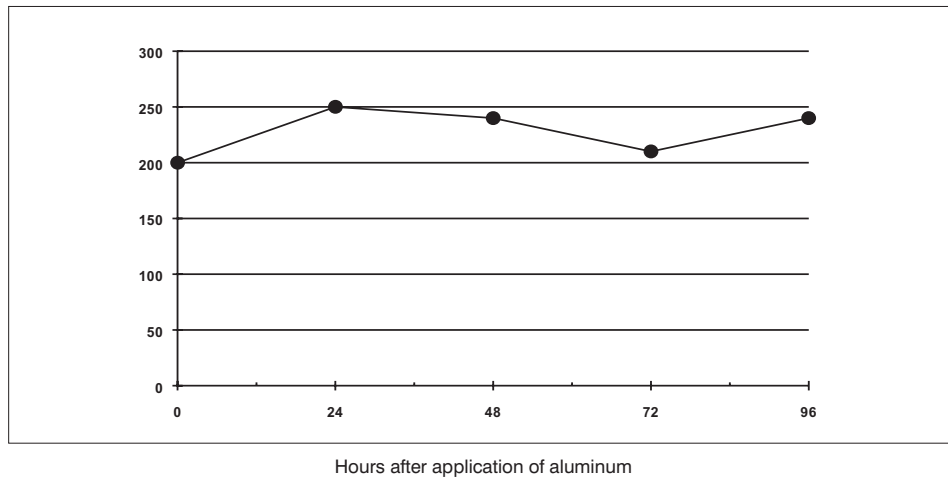

Hours after application of aluminum

Figure 2. Quantity of alluminum in erytrocytes of gerbils after application of aluminum
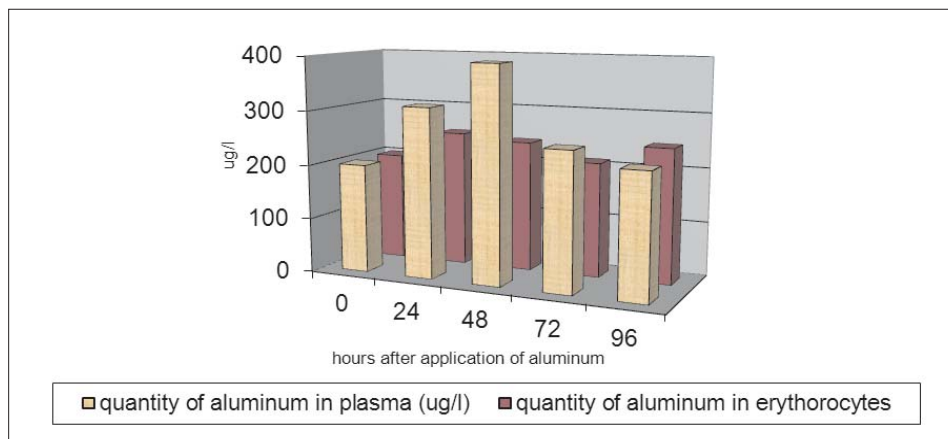

Figure 3. Quantity of aluminum in plasma and erythrocites of gerbils after application of aluminum

Figure 3 presents comparative analysis of the aluminum level in erythrocytes and plasma. On the first day after treatment a more significant aluminum increase in plasma than the increase of aluminum in the erythrocytes was measured. The rise was considerably greater in the plasma and amounts to

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50% of the control value. On the second day the aluminum quantity in the erythrocytes was approximately the same as after the first day, while aluminum quantity in the plasma is doubled in relation to the control value.

On the third day, the decrease of aluminum was noticed in the erythrocytes and in plasma in relation to the first and second day, while on the fourth day the level was approximately equalized, but still above the control level.

Changes in the number of erythrocytes in gerbils after aluminum application

As it is the case with hemoglobin, the number of erythrocytes decreases considerably on the second and third day from the beginning of the experiment, while it is almost the same on the first day as on the control day, and on the fourth, it increases in relation to the second and third day and it comes closer to the control value.
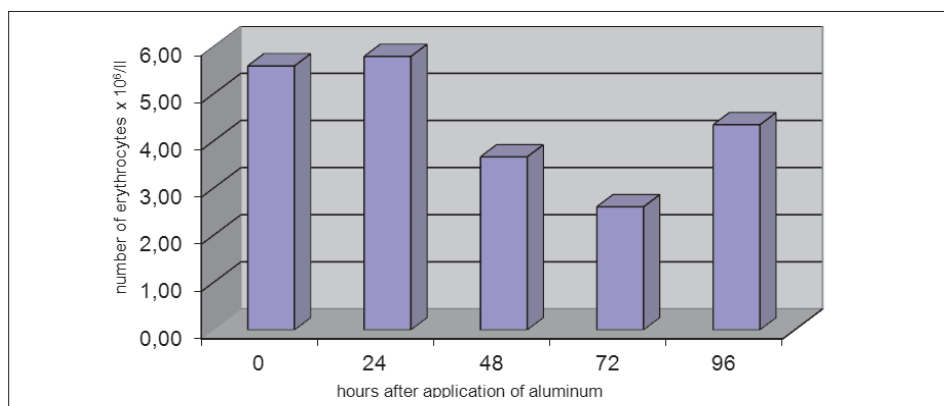

Figure 4. Number of erythrocites in gerbils after application of aluminum

The change in the number of erythrocytes in the gerbil after aluminum application in the dose of 3.7 g per kg of body mass is presented in Figure 4. On the first day after aluminum application the effects of aluminum action on the erythrocytes have not been noticed. However, on the second day upon the beginning of the experiment the quantity of erythrocytes decreased by one third in relation to the control group, and on the third day the number of erythrocytes still decreases to half of the control value. However, as in the case of hemoglobin, the recovery in erythrocytes is also relatively quick, and on the fourth day of administration the quantity of erythrocytes increases.

Changes in the quantity of hemoglobin in the erythrocytes of gerbils after
*   aluminum application*

On the first day after aluminum application the quantity of hemoglobin in erythrocytes was slightly increased in relation to the control group. However, already on the second day, and even more on the third day, hemoglobin quantity

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decreased by about 50 and 60% in relation to the control value, while on the last day of the experiment hemoglobin quantity returns to the control value level.
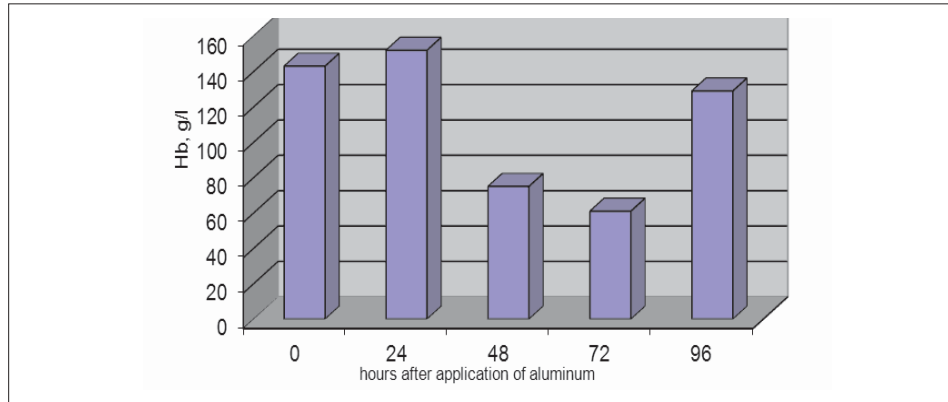

Figure 5. Quanity of hemoglobin in erythrocytes of gerbils after application of aluminum

Changes of hemoglobin quantity in the erythrocytes after aluminum application in the dose of 3.7 g per kg of body mass is presented in Figure 5. Twenty-four hours after aluminum application no effects on this parameter have been noticed. However, 48 hours after administration hemoglobin quantity in the erythrocytes is decreased to one half of the control values (Figure 5) and this situation is seen also after 72 hours. The recovery was relatively quick, and after 96 hours the quantity of hemoglobin in the erythrocytes returned to normal.

DISCUSSION

Erythrocytes are cells for which the correct functioning of antioxidative mechanisms is of vital importance due to a significant production of free radicals (Dean, 1987).

Aluminum is of great interest due to its possible toxic effects. The aim of our study was to find out whether acute aluminum poisoning leads to disturbance in the function of erythrocytes and what are the hematological effects of this poisoning.

The role of aluminum in renal diseases is of particular importance since the use of mineral water creates considerable quantities of aluminum in the organism.

The results of our study show that the number of erythrocytes and hemoglobin concentration considerably decrease in relation to the control group. The recorded decrease of the number of erythrocytes and hemoglobin quantity may speak in favor of the changes in the cell membrane and possibly a shorter life of erythrocytes. In similar papers (Milačić, 1989) the decreased number of erythrocytes together with increased serum iron and decreased value of the factors of energetic potential of the cell membrane (ATP, ADP, AMP) have been explained by the mechanism of hemolysis. This could be as well the explanation

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for our results: fragile erythrocytes passing through narrow spleen canals due to decreased membrane elasticity prematurely break, so, their number is decreased thus anemia occurs.

During the experiment, the increase of aluminum content was noticed in the plasma already after the first day upon the beginning of aluminum application, while after the second day this increase reached the peak and began slowly to clear from the plasma and decreased after the third day, returning back to normal on the fourth day. This increase and decrease of aluminum values show that there are clear and effective mechanisms for aluminum "clearance". We point out once more that aluminum is to a great extent eliminated through the kidneys. There is also elimination through the biliar tract and mammary glands, but it is minimal. It is of interest to note that the increase of aluminum concentration in the erythrocytes is somehow "late" in relation to the increase of aluminum concentration in plasma. This is understandable as it takes more time for aluminum to penetrate the very cells. However, aluminum concentration in the erythrocytes is mantained at the increased level even at the time when aluminum concentration in the plasma starts to fall. Intraperitoneal aluminum application could be the possible cause of the delayed increase of aluminum concentration in the erythrocytes.

Apart from acute toxic effects of aluminum there are also chronic toxic effects since metabolism functions and the speed of excretion are not known. It is possible that slight aluminum increase in serum represents the equilibrium between aluminum in the erythrocytes and aluminum in the serum. We can conclude that our experimental model is appropriate within the scope of the study as aluminum ions have demonstrated specific effects due to their presence. While the aluminum concentration in the plasma has normalized after four days, the increased concentration still existed in the erythrocytes.

Monitoring of plasma aluminum concentration does not reflect the exact aluminum concentration in tissues that might be eventually affected as aluminum concentration in the plasma does not correspond with aluminum concentration in the later phases. In the initial phases of exposition, the more aluminum concentration in plasma, the more aluminum concentration in the erythrocytes and in other tissues was observed, while the opposite is not applicable. If there is no exposition, it is impossible to make conclusion that aluminum in plasma really reflects aluminum concentration in tissues that are being tested (Colomina *et al.*, 2002; Pratico *et al.*, 2002; Sun *et al.*, 1999).

Aluminum action on hematologic parameters presents distinct effects in acute poisoning. There is considerable reduction in the number of erythrocytes and hemoglobin quantity after the second day of the experiment. However, these effects are not constant, therefore after the fourth day hemoglobin concentration and the number of erythrocytes is back to the control value. That is one of the indicators of acute poisoning. Again, we can conclude that the mechanism of aluminum action on the erythrocytes is not clear, nor are mechanisms of microcytic anemia (Becaria *et al.*, 2003). The interference of aluminum with iron metabolism has almost been excluded because anemia is microcytic. However, it has been presented that feritin is in the normal concentration, i.e. there have been no disturbances in the iron concentration. It is possible that the results of our study

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point to some other possibility, i.e. that the decrease of the number of erythrocytes is caused by their metabolic damage. The result is that damages related to erythrocytes are not systemic. Namely, they are not the results of some systemic damage, but they affect the cells directly (Kim and Clesceri, 2001).

CONCLUSION

Based on the results of our research, we can conclude that after acute poisoning aluminum concentration in the plasma is increased, while it would be completely normalized after four days, but increased aluminum quantity in the erythrocytes persists even after the fourth day. Aluminum in the applied dose leads to a decrease in the number of erythrocytes and hemoglobin quantity proportionally to its concentration in the blood plasma.

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UTICAJ AKUTNOG TROVANJA ALUMINIJUMOM U EKSPERIMENTALNIM USLOVIMA NA HEMATOLOŠKE PARAMETRE

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SADRŽAJ

Imajući u vidu široku zastupljenost aluminijuma u industriji i domaćinstvu, u eksperimentalnim uslovima su utvrđivane funkcionalne karakteristike eritrocita i količina aluminijuma u plazmi i eritrocitima posle akutnog trovanja aluminijumom. U eksperimentu su korišćeni pustinjski miševi, kojima je rastvor aluminijumhlorida aplikovan intraperitonealno u dozi 3,7 g po kilogramu telesne mase, a žrtvovanje (eksperimentalnih grupa životinja) je vršeno 24, 48, 72 i 96 časova od početka eksperimenta kardijalnom punkcijom i punkcijom abdominalne aorte. Kontrolna grupa je tretirana fiziološkim rastvorom. Broj eritrocita i količina hemoglobina su su bili značajno sniženi u odnosu na kontrolnu grupu, najverovatnije zbog promena na ćelijskim membranama i kraćeg veka eritrocita. U toku eksperimenta dolazilo je i do rasta sadržaja aluminijuma u plazmi i to prvog i drugog dana eksperimenta, da bi trećeg dana on počeo da pada i četvrtog dana se vratio na bazalni nivo. To još jednom potvrđuje postojanje posebnog mehanizma „klirensa" aluminijuma iz plazme.