Contributions to Studies Concerning the Behaviour of Al (III) Ion in Some Biological Systems

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The aim of this study is to bring personal contributions referring to the influence of aluminum ion (Al3+) on some biological systems. For this research the biological system was represented by an experimental design on domestic rabbits, working with three groups (one control group – C and two experimental groups – E1 and E2). The procedure was realized by aluminum solutions administration, with and without association of citrate to E1 and E2 groups, compared to control group. We followed the variation of some lipids in blood serum (total lipids, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol), the iron and total capacity for binding iron from blood serum, but also the modification of the concentration of trace and macroelements from liver of experimental animals. In our study are also presented chemical interactions specific for oxidative stress, process owed to the presence of aluminum ion (Al3+), which cause variations in lipidic metabolism.

Key words: aluminum ion (Al3+), microelements, macroelements, oxidative stress, biological systems

Aluminum is present in environment in large amounts, but also in small amounts in living organisms, and generally does not participate in biological and metabolically processes, because of his chemical nature. However, the aluminum intake from various sources (soil, food, antiperspirants, drinking water, vaccines etc.), leaded to discovery of many implications of Al3+ ion in physiological processes and thus to endeavour to establish the interactions of this ion with other compounds in biological systems [1]. Aluminum prefers oxygen donor groups for complexation. The stability of complexes in biological systems depends on pH, which in blood plasma is 7.4, when aluminum solubility is low because of the presence of Al(OH)3, insoluble form of Al3+ ion. But in the presence of citrate, the hydroxide form is solubilised, and Al3+ ions are linked by the small citrate molecule, then is fast absorbed in blood stream, after crossing the intestinal wall. In the blood, the main transportation molecule of Fe3+ is the transferrine, but in this molecule, only 30% of available sites are occupied by the Fe3+. A lot of studies shown that among iron, the transferrine binds Al3+, but not so strong, because the ion ray is lower that in the case of Fe3+, causing the decreasing of the coordination capacity with transferrine donors atoms [2]. There are techniques for aluminum evaluation in tissues by atomic absorption in graphite furnace, without offering informations about aluminum speciation involved in biochemical processes [3].

Aluminum salts themselves do not stimulate phospholipid lysosomes peroxidation, but greatly enhance peroxidation induced by Fe2+ at acid pH [4]. Lipid peroxidation appears during the oxidative processes in cell (cell respiration), and there are chemical reactions forming free radicals, which normally are counteracted by the antioxidant system of the body (antioxidant enzymes). If their quantity outruns the antioxidant capacity of the body, as in our case, due to an excess of metallic ions catalyzing oxidative reactions in cells, then an oxidative stress can appear [5, 6].

The most important modification of lipids produced by reactive oxigen species (ROS) are registered in cell or extracell membrane lipids, when peroxides are the final product of oxydation [6-8].

In the presence of Fe2+ in reaction with H2O2 (Fenton reaction), the hydroxyl radicals are formed:

\[ M^{n+} + H_2O_2 \rightarrow M^{(n+1)} + HO^\cdot + HO^\cdot \]

or

\[ Fe^{n+} + H_2O_2 \rightarrow Fe^{(n+1)} + HO^\cdot + HO^\cdot \]

They can react with lipids from cellular membrane and can form lipid radicals. Lipid radicals can also react with oxygen and form lipid peroxides in an auto propagation reaction chain [8, 9]. The peroxides can also initiate Fenton reactions forming peroxide radicals, which are very reactive.

\[ RH + HO^\cdot \rightarrow R^\cdot + H_2O \]
\[ R^\cdot + Fe^{n+} \rightarrow R^+ + Fe^{2+} \]

The Fenton reaction is the first step of the Haber-Weiss reaction, which results in the formation of hydroxyl radical, and other ROS, as follow:

\[ O_2^\cdot + H_2O_2 \rightarrow HO^\cdot + HO^\cdot + O_2 \]

These reactions are catalyzed by Fe, Cu, Cr, and also other transitional metals.

There are food products or pharmaceutical products with antioxidant role, acting as inhibitors of free radicals or ROS [10, 11].

Experimental part
The experiment was made on three groups of domestic rabbits – Oryctolagus Cuniculus species (one control group
and two experimental groups). The experiment was performed during 10 days. Animals were maintained in optimal physiological conditions, according with UE Law 305/2006, concerning animal protection in scientific researches. We made subcutaneous injections in cervical zone, for solutions administration.

The control group (C) and two experimental groups (E1 and E2) had 8 animals each. The solutions were administered in the 6th day and the 8th day, and animals were killed in the 10th day of the experiment, after anaesthesia with chloroform. Animals from control group were injected subcutaneous with 2 mL of physiological solution of NaCl 0.9%. The aluminium chloride solution was administered by subcutaneous injection to all experimental groups as follow: to E1 group, 50 mg AlCl3/kg b.w. (b.w. – body weight), and to E2 group 50 mg/kg b.w. associated with 2% m/v citrate from sodium citrate.

After anaesthesia with chloroform, animals were killed. Blood samples were taken in standard sampling tubes, containing EDTA followed by TL (total lipids), TG (triglycerides), CHOL (cholesterol), HDL-chol (High-density lipoprotein-cholesterol), LDL-col (Low-density lipoprotein-cholesterol) determination with a Roche-Hitachi automatic analyzer. All reagents for assays are delivered in kits by Roche.

Liver tissue was prepared for analyse as follow: before samples digestion the liver tissues were weighted on a Mettler Toledo AG204 analytical balance. Digestion of hepatic tissue 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 5 mL of 65% nitric acid (Merck) and 1 mL of 30% H2O2, the sample solution was transferred into a 50 mL volumetric flask and dilute to volume with double deionised water (< 5 µS/cm).

The apparatus used for metal determination from solutions was an atomic absorption spectrometer, produced by Perkin-Elmer, with Zeeman effect for background correction and transversal heating of graphite tube. Fe, Zn, Mn, Mg, Ca were determined in air-acetylene flame, Na and K by the atomic emission, and Al and Cu by electro-thermal atomisation. We used appropriate ionisation control substances for flame and matrix modifiers in graphite tube. For calibration of the apparatus, we used (Fe and Al) standard single element solutions of 1000 mg/L, produced by Merck, making dilutions for calibration standards, to obtain a calibration curve in linear range. The calibration curve control was made with a multielement standard solution (Merck). The obtained results were expressed in µg/L solution, and reported after calculations to ig/g w.t. (w.t. - wet tissue), considering the volume of volumetric flask used (50 mL) and the tissue sample initial weight.

Experimental data were processed with Descriptive Statistic Program (EXCELL) and T test (f x function) [12]. The results were reported as mean ± standard deviation (X ± S.D.), and asterisk symbol (*) for significant differences between results for \( p<0.05 \).

In calculations, information about uncertainty of calibration curve, but also uncertainty of volume of flasks, pipettes and mass measurement of hepatic tissue from experimental animals, were not considered.

**Results and discussions**

The main modifications in experimental conditions caused by the aluminum chloride administration to studied animals at the serum lipid level, are presented in figure 1. The liver is the main organ for detoxification of organism, but in case of overdoses hepatic injuries appears. This effect was presented in our research, revealed by the modifications of lipid parameters in blood after aluminum chloride administration.

In a research study was shown the effect of aluminum (Al3+) on lipid peroxidation and the result showed that Al3+ increases lipid peroxidation of HDL-col, because of the lipid hydro peroxides enhance in blood samples of aluminum treated animals compared to control animals [4, 15]. The effect is higher at acidic pH values. In our experiment was registered only a small variation of HDL-col value for experimental groups E1 and E2. Most interesting is the behavior of LDL-col, because the association of aluminum chloride with citrate seems to increase LDL-col levels despite of the decrease of LDL-col in aluminum chloride administration without citrate. Observing the CHOL variation in figure 1, this CHOL is modified in the same way with LDL-col, which is typical in atherosclerotic disease [5].

Iron concentration from blood serum shows an anemia, confirmed by variation of total iron bond capacity (TIBC) which increases after iron elimination but, without changing very much the hemoglobin concentration (table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>UM</th>
<th>Group C</th>
<th>Group E1</th>
<th>Group E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>( \bar{X} \pm S.D. )</td>
<td>( \bar{X} \pm S.D. )</td>
<td>( \bar{X} \pm S.D. )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.75±1.28</td>
<td>12.47±2.30</td>
<td>11.22±2.54</td>
</tr>
</tbody>
</table>

Fig. 1. The status of lipidic parameters in blood serum in control and experimental groups.
Iron from deposits (transferrin), may be the cause of this phenomenon and it is possible that if we would have sacrificed the animals after a longer period of time we could discovered a much smaller quantities of iron in liver.

Transferrine is the most important transporter of iron and other ions in the blood circulation [14]. The mineral balance can be changed because of various factors e.g. environmental factors, nutritional factors, or health problems. Transferrine can transport these ions and deposit them replacing the iron or in addition with iron. But, any modification in Fe – transferrin can enhance the retention of these metals in liver or other organs. The increase of copper concentration is obvious in case of E1 and E2 groups, but more to E1 group where the increase is 438%.

In his experimental work, Dejica, said that hepatic overload with copper cause progressive hepatic lesions [5]. As in case of overload iron, the lipoperoxidation in hepatic mitochondria is associated with mitochondrial metabolism perturbation. It is well known that zinc has a good ability to delay the oxidative processes in cell. Zinc does not directly interact with oxidant species, but has an indirect effect in relation with iron and copper from biochemical reactions forming ROS leading to oxidative stress appearance [16].

**Conclusions**

After aluminum chloride administration, aluminum accumulates in liver at levels of 175% (for E1) compared to control group. At the same time, the increase of copper concentration is obvious in case of E1 and E2 groups, but more to E1 group where the increase is 438%.

In table 3 we present the distribution of some metals (Na, K, Ca, Mg) in liver, to experimental animals from control group compared to experimental group.

In figure 3 we show that aluminum is accumulated in liver, and the increase is of about 175%. The citrate excess administrated to E2 group does not allow aluminum accumulation in liver. Iron, copper, and zinc had the same variation as aluminum. Iron concentration increased in liver’s content in respect to control group. The removal of iron from deposits (transferrin), may be the cause of this phenomenon and it is possible that if we would have sacrificed the animals after a longer period of time we could discovered a much smaller quantities of iron in liver.

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involved in some enzymatic reactions, with a protective role against the oxidative stress. On the other hand, these trace metals are catalysts in cell oxidative reactions, and in large amounts (overdose) accelerate these reactions, affecting the membrane lipids. This situation is revealed by the modifications in lipid status in blood serum.

It is again confirmed that aluminum ions (Al\(^{3+}\)) cause anemia. This can be seen by the increase in iron status, and the total iron binding capacity of transferrin in blood. In conditions of serum iron removal, the transferrin can bind aluminum and deposit it in the liver.

It is obvious that in presence of citrate some trace elements can be removed from liver. Variation of macroelement concentration in liver is important for calcium concentration. Calcium is accumulated in hepatic cells in oxidative stress because of the membrane lipid injuries and this affect calcium transport in and out of the cell.

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