CONSEQUENCES OF AD LIBITUM ALUMINIUM ADMINISTRATION DURING THREE GENERATIONS ON RATS BLOOD GSH DINAMICS

CONSECINȚELE APORTULUI DE ALUMINIU AD LIBITUM, TIMP DE TREI GENERAȚII, ASUPRA DINAMICII GSH-ULUI SANGUIN LA ȘOBOLANI

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The influence of ad libitum aluminium sulphate, Al$_2$(SO$_4$)$_3$, administration during three generations on the blood glutathione (GSH) values in rats was studied. This study was carried out on 34 Wistar rats divided in 9 experimental batches (L$_I$, L$_II$, L$_III$, L$_I^F_1$, L$_II^F_1$, L$_III^F_1$, L$_I^F_2$, L$_II^F_2$, L$_III^F_2$) and one control batch (C). Aluminium sulphate has been administrated in drinking water, ad libitum. GSH was measured quantitatively at Perkin-Elmer spectrophotometer through Beutler et. al. method (4), at 412nm. Hemoglobin (Hb) was measured through Drabkin method (9) at the automatic analyzer Bekman Coulter. GSH is considered to be the most powerful and most important of body’s self-generated antioxidants. It is a powerful antioxidant, it neutralizes free radicals and prevents their formation. Because aluminium (Al) can cause oxidative damage on cellular biological processes by inhibiting GSH regeneration through the inhibition of NADPH supply in mitochondria, but only a little inhibitory effect on the GSH generation in cytosol (17), the consequences of ad libitum Al$_2$(SO$_4$)$_3$ administration was a limited decrease of GSH values.

Key words: GSH, aluminium, rats.

Introduction

Aluminum is the third most abundant element of the earth's crust constituting 8.2 percent. It is a major component of a large number of minerals such as alumina-silicates, feldspars, bauxite and clays (8). At neutral pH, Al minerals are insoluble, but solubility increases at lower pH. Cronan and Schofield (6) have shown that the acidification of lakes and streams by acid rain mobilized Al from the soil to the aquatic environment. Domestic tap water may contain aluminum either naturally or because Al has been added as a flocculant in the
treatment process. Al\(_2\) (SO\(_4\))\(_3\) is the most widely used coagulant for clarifying turbid drinking water (15). The levels of dissolved Al in waters are strongly influenced by pH and the presence of other substances in the water.

Aluminum is found in the tissues of all plants and animals. The concentration in foods varies widely, depending upon the product, the type of processing, and the geographical origin (18).

The total consumption of Al in a normal diet is believed to be between 1 and 20 mg/day (2,14,21). The richest natural dietary sources of Al are herbs and tea leaves. Aluminum salts are used as emulsifiers in some processed cheese (700 mg/g), in some baking powders (20-26 mg/g), cake mixes and pickled vegetables (14).

Aluminum salts are common buffers in drugs. Buffered aspirin contains up to 50 mg Al per tablet (5).

Aluminum may comprise 25 percent by weight of antiperspirants, either powders or solutions.

**Materials and Methods**

This study was carried out on 34 male, white Wistar rats divided in 9 experimental batches (L\(_I\), L\(_II\), L\(_III\), L\(_I\)F\(_1n\), L\(_II\)F\(_1n\), L\(_III\)F\(_1n\), L\(_I\)F\(_2n\), L\(_II\)F\(_2n\), L\(_III\)F\(_2n\)) and one control batch (C) with 7 rats. The experimental batches are divided in 3 groups:

- **G\(_I\) = L\(_I\), L\(_II\), L\(_III\) - male rats – maintained for 6 month 200ppb, 400ppb and 1000ppb aluminum sulphate, in drinking water, ad libitum.
- **G\(_2n\) = L\(_I\)F\(_1n\), L\(_II\)F\(_1n\), L\(_III\)F\(_1n\) - male young rats, obtained from the mating of male rats belonging to the groups L\(_I\), L\(_II\), L\(_III\) with females that were exposed to aluminum intoxication only during gestation.
- **G\(_3n\) = L\(_I\)F\(_2n\), L\(_II\)F\(_2n\), L\(_III\)F\(_2n\) - male young rats, obtained from the mating of male rats belonging to the groups G\(_2n\) (L\(_I\)F\(_1n\), L\(_II\)F\(_1n\), L\(_III\)F\(_1n\)) mating with females that were exposed to aluminum intoxication during gestation.
- **Control batch (C) – male rats – maintained tape water, ~50ppb, the maximum limits of Romanian standard 1342/1991.

Aluminum sulphate was administered to the male young rats in drinking water, ad libitum, for 3 month after the wean.

200ppb is the maximum allowed aluminum sulphate concentration according to 1342/1991 STAS.

400ppb and 1000ppb are aluminum sulphate levels of drinking water for animals that can be encountered in the Slatina region.

After 6, respectively 3 month, the rats were euthanatized, blood samples being used for determinations.
GSH was determined through Beutler et al. method (4) at a Perkin-Elmer spectrophotometer, $\lambda = 412$nm, and the hemoglobin (Hb) through Drabkin method (9) at the automatic analyzer.

**Results and Discussions**

The blood test results are presented in the following tables (1,2 and 3) and fig.(1).

Table 1. GSH and hemoglobin mean values in the control batch (C) and experimental batches ($L_1, L_{1F_1n}, L_{1F_2n}$). Aluminum sulphate level – 200ppb

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>$L_1$</th>
<th>$L_{1F_1n}$</th>
<th>$L_{1F_2n}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH $\mu$mol/gHb</td>
<td>0.663 ± 0.092</td>
<td>0.623 ± 0.152</td>
<td>0.581 ± 0.078</td>
<td>0.512 ± 0.015</td>
</tr>
<tr>
<td>Hb g/100ml</td>
<td>15.25 ± 0.52</td>
<td>15.09 ± 0.35</td>
<td>14.05 ± 0.11</td>
<td>13.62 ± 0.21</td>
</tr>
</tbody>
</table>

Table 2. GSH and hemoglobin mean values in the control batch (M) and experimental batches ($L_{II}, L_{IIF_1n}, L_{IIF_2n}$). Aluminum sulphate level – 400ppb

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>$L_{II}$</th>
<th>$L_{IIF_1n}$</th>
<th>$L_{IIF_2n}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH $\mu$mol/gHb</td>
<td>0.663 ± 0.092</td>
<td>0.595 ± 0.098</td>
<td>0.532 ± 0.122</td>
<td>0.483 ± 0.117</td>
</tr>
<tr>
<td>Hb g/100ml</td>
<td>15.25 ± 0.52</td>
<td>14.35 ± 0.29</td>
<td>13.22 ± 0.22</td>
<td>12.14 ± 0.09</td>
</tr>
</tbody>
</table>

Table 3. GSH and hemoglobin mean values in the control batch (C) and experimental batches ($L_{III}, L_{IIIF_1n}, L_{IIIF_2n}$). Aluminum sulphate level – 1000ppb

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>$L_{III}$</th>
<th>$L_{IIIF_1n}$</th>
<th>$L_{IIIF_2n}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH $\mu$mol/gHb</td>
<td>0.663 ± 0.092</td>
<td>0.527 ± 0.088</td>
<td>0.491 ± 0.113</td>
<td>0.429 ± 0.157</td>
</tr>
<tr>
<td>Hb g/100ml</td>
<td>15.25 ± 0.52</td>
<td>12.42 ± 0.25</td>
<td>11.25 ± 0.41</td>
<td>9.95 ± 0.25</td>
</tr>
</tbody>
</table>

Gastrointestinal absorption was evaluated in Wistar rats using $^{26}$Al (12). These authors observed gastrointestinal absorption of 0.1 percent of administered dose. Concomitant intake of citrate led to more rapid, larger, and more variable absorption. There is no evidence that Al is absorbed through the skin and Al has not been found to penetrate the epidermis (19). Inhalation is another route of Al
exposure, but is probably a minor pathway. The lungs continually receive Al mostly as particles of Al, silicates and other poorly soluble compounds. The lungs have a higher concentration of Al than all other organs and the Al concentration increases with age (1).

The ingestion pathway is the most significant route of transfer of Al from the environment to animals and humans. Dunea et al. (7), Kaehny et al. (13) and Rozas et al. (20) report that the in which tap water was a significant source of human Al exposure when the water was misused in hemodialysis equipment. Based on those reports, the medical community has defined that the Al concentration in water used in hemodialysis solutions be no greater than 10 µg/L (0.01 ppm) (3,11).

The blood is responsible for the transport of Al throughout the body and approximately 80 percent of the Al in blood is bound to serum proteins. The remaining 20 percent is diffusible (10). Al is bound to serum transferrin, which may play a significant role in the distribution of Al. Minami et al. (16) have observed an age-dependent accumulation of aluminum in the aorta and cerebral arteries. Phosphorus and calcium appear to enhance Al accumulation.

Glutathione is the principal reducing agent in erythrocytes and the essential cofactor in the glutathione peroxidase reaction. In the course of reactions protecting hemoglobin from oxidation, prevents hemoglobin denaturation, GSH is oxidized, forming oxidized glutathione (GSSG).

Reduced glutathione acted as a principal scavenger of reactive oxygen species in mitochondria. Aluminum inhibited the regeneration of glutathione from the oxidized form, and the effect was due to the inhibition of NADP-isocitrate dehydrogenase the only enzyme supplying NADPH in mitochondria. In cytosol, aluminum inhibited the glutathione regeneration dependent on NADPH supply by malic enzyme and NADP-isocitrate dehydrogenase, but did not affect the glucose 6-phosphate dehydrogenase dependent glutathione formation. Aluminum can cause oxidative damage on cellular biological processes by inhibiting glutathione regeneration through the inhibition of NADPH supply in mitochondria, but only a little inhibitory effect on the glutathione generation in cytosol (17).
Because glutathione is the principal reducing agent in erythrocytes, the principal scavenger of reactive oxygen species in mitochondria, the consequences of ad libitum $\text{Al}_2(\text{SO}_4)_3$ administration at 200, 400 and 1000 ppb during three generation to Wistar rats, was the inhibition of the regeneration of glutathione from the oxidized form, a limited decrease of GSH and hemoglobin values in $G_1$, $G_{2n}$ and $G_{3n}$ groups.

Bibliography


