

Effect of Long-Term Aluminium Chloride Intoxication on Selected Biochemical Parameters and Oxidative-Antioxidative Balance in Experimental Animals

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Abstract

Aluminium is numbered among trace elements with a moderate toxic effect on living organisms. We have attempted to assess the influence of long-term aluminium chloride intake on the biochemical parameters and oxidative stress biomarkers in experimental animals. Three months' administration of aluminium chloride in drinking water at a dose of 80 mg/l significantly elevated blood serum urea and creatinine concentration, as well as oxidative stress biomarkers such as: TBARS content in erythrocytes hemolysate, protein carbonyl groups concentration and 8-hydroxy-2'-deoxyguanosine excretion with urine.

Keywords: aluminium, blood serum biochemical parameters, oxidative stress biomarkers

Introduction

Aluminium is one of the trace elements with a moderate toxic effect on living organism [1]. The main food sources of aluminium are: hard cheese, grain products (flour), herbs and tea leaves. Chronic exposition to this trace element can cause alterations in skeletal, nervous, hematopoietic and respiratory systems [2, 3, 4, 5]. Aluminium ions alter properties and structure of cellular membranes, inhibit many enzymes like alkaline phosphatase, acetylcholinesterase, and adenylyl cyclase [6, 7, 8, 9]. Antagonistic interactions between aluminium ions and other elements such as: calcium, magnesium, iron, silicon, phosphorus, copper, and zinc were observed in biological systems [1, 10]. In our own investigation an attempt has been undertaken to assess the effect of long-term aluminium intoxication on biochemical parameters and oxidative biomarkers in experimental animals.

Material and Methods

In 1989 the FAO/WHO Expert Committee on Food Additives reported that the daily intake of aluminium in children ranges between 2 and 6 mg, while in adults 6-14 mg [11, 12, 13].

According to Drugs Institute guidelines [14] in investigations carried out on animals, a dose of drug applied to the rodents should be 4- to 10-fold higher than a human dose.

In our investigation rats received 6.4 ± 2.1 mg/kg body mass/day of aluminium chloride [$\text{AlCl}_3 \times 6 \text{H}_2\text{O}$], which makes 0.57 mg/kg b.m./day of aluminium. Assuming that a daily intake of aluminium in adults (with mean body weight 70 kg) ranges between 0.0857 mg and 0.2 mg/kg body weight, the doses applied to the rats exceeded 6-fold the minimal dose and 2.8-fold the maximal dose, on average 4-fold of human dose.

The study comprised 20 male Wistar rats divided into two groups of ten. The control group received pure drinking water for 3 months, while the investigated group received

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Table 1. Effect of aluminium chloride on select biochemical parameters in rat blood serum.

| | Urea [mg%] | Creatinine [mg%] | AspAT [U/l] | AlAT [U/l] | Glucose [mg%] |
|---------------------------|------------|------------------|-------------|------------|---------------|
| Control group | 33.0±4.31 | 0.43±0.05 | 110.4±15.5 | 42±5.49 | 140±15.85 |
| Aluminium receiving group | 42.6±5.28 | 0.65±0.03 | 114.0±16.7 | 43.1±6.1 | 134±14.2 |
| Statistical significance | p<0.01 | p<0.05 | | | |

Table 2. Effect of long-term aluminium chloride intoxication on selected oxidative stress biomarkers.

| | Serum TBARS [μ M/l] | TBARS in erythrocyte hemolysates [μ M/gHb] | Protein carbonyl groups [nM/mg of protein] | Urine 8-hydroxy-2'-deoxyguanosine [nM] |
|---------------------------|--------------------------|---|--|--|
| Control group | 3.02±0.4 | 16.4±4.35 | 4.02±0.94 | 0.45±0.17 |
| Aluminium receiving group | 3.33±0.7 | 22.9±3.1 | 6.2±1.1 | 11.5±4.8 |
| Statistical significance | | p<0.05 | p<0.05 | p<0.01 |

drinking water with 80 mg/l of aluminium chloride [$\text{AlCl}_3 \times 6\text{H}_2\text{O}$], also for 3 months. The animals were kept in animal quarters at constant temperature and humidity with free access to "Murigran" chow for small laboratory animals. After 3 months the animals were placed in metabolic cages where urine was collected and then in general narcosis they were terminated and blood was collected for determination.

Blood serum urea, creatinine and glucose levels, and AspAT and AlAT activity were determined with Kone-Pro device and Bio-Merieux reagents

Oxidative-antioxidative balance was assessed by measuring:

- content of the compounds reacting with thiobarbituric acid (TBARS) in blood serum and erythrocytes hemolysate acc. to Rice-Evans [15]
- content of carbonyl groups acc. to Levine et al. [16] and Reznik et al. [17]
- urine 8-hydroxy-2'-deoxyguanosine concentration with HPLC method.

The obtained results were statistically analyzed with Mann-Whitney nonparametric test using Statistica program No. SP7105488009G51.

The protocol of the study was approved by the Local Bioethics Committee (consent No 11/00).

Results

Effects of aluminium chloride on the selected biochemical parameters in rats blood serum is presented in Table 1.

Administration of aluminium chloride solution in drinking water (80 mg/l) to rats for three months resulted in statistically significant increases in blood serum urea and creatinine concentration.

The effects of long-term aluminium chloride intoxication on selected oxidative stress biomarkers are presented in Table 2.

Aluminium chloride increased statistically significantly serum TBARS concentration in erythrocyte hemolysates, plasma carbonyl groups content and urine 8-hydroxy-2'-deoxyguanosine concentration.

Discussion

In this study, during three months observation of rats receiving aluminium chloride, decreases in water and food intake and transient diarrhoea occurred, which resulted in lowering of final body mass of animals in comparison to the controls (differences statistically significant). Rats' body mass after three months of the study was 324±22g in the control group and 270±25g in the investigated group. In the course of the experiment no changes were observed in the behaviour of animals. In humans, chronic exposure to aluminium ions may result in mood changes, dysmnnesia, convulsions, muscular weakness, pathological fractures of bones. Aluminium accumulates mainly in bones, spleen, liver and lungs [3,4,7,8]. In our study the content of aluminium was not investigated in the mentioned organs. Assessment of harmful effect of aluminium ions was based on the analysis of selected biochemical parameters. Statistically significant increase of serum urea and creatinine concentration in animals receiving aluminium chloride is of interest. The increase of serum urea and creatinine concentration can be a consequence of critical accumulation of this metal in kidneys and following renal failure development. Aluminium is excreted mainly by kidneys [1]. Chronic exposure to aluminium also results disruptions in mineral balance disturbances. In the biological systems aluminium ions replace iron and magnesium ions [18]. They also alter cellular membrane structures and activity of many enzymatic processes [9], reduce Fe^{2+} binding to ferritin, and disturb hem synthesis [1]. Free iron ions released from biological complexes by

aluminium can catalyze hydrogen peroxide decomposition to hydroxyl radical- according to Fenton's reaction. This high hydroxyl radical reactivity is able to initiate cellular damage [19, 20, 21, 22, 23]. In our investigations the increase in oxidative stress biomarker concentrations in animals intoxicated with aluminium chloride was observed. Significant increase of lipid peroxidation products in erythrocytes hemolysate and only moderate (not significant statistically) in serum were noted.

Aluminium chloride also increases protein carbonyl group concentrations. These protein modifications were determined by Stadtman et al. [12] as reactions of amino acid residue oxidation - mainly lysine, arginine and proline. The carbonyl group content assays, according to Popadiuk et al., is a sensitive marker of free radical processes in vivo [22].

Deoxyribonucleic acid hydroxylation products, i.e. 8-hydroxy-2'-deoxyguanosine [21, 24] are, together with lipid peroxidation and protein hydroxylation products, biomarkers of oxidative processes. In our investigations 3 months' aluminium chloride intoxication of rats resulted in statistically significant increases of 8-hydroxy-2'-deoxyguanosine concentration in 24-hour urine collection. Already in 1978 Matsumoto [25] and Morimura [26] reported the destructive effect of aluminium ions on deoxyribonucleic acid resulting from their reactions with phosphate residues and bases of nucleic acid chains.

Conclusions

The long-term aluminium intoxication of rats besides other harmful effects causes an increase in oxidative stress.

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