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Influence of Drinking Water-Administered Aluminium on Morphology and Respiratory Function of Blood in Rats

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Abstract

Our study examined changes in the red blood system of rats under the influence of aluminium chloride administered in a physiological way with drinking water in the following doses : 0.5 g/l, 1 g/l and 2 g/l. The significant decrease of erythrocyte numbers, haemoglobin levels and haematocrit values which accompanied the highly significant statistical elevation in reticulocyte contents were observed in intoxicated animals. Also, the decrease of mean cell volume (MCV), average diameter of erythrocyte (D) and its surface area (S) were noted. Respiratory surface of blood volume unit ($S_{resp.}$) and coefficient F in group received $AlCl_3$ at doses of 0.5 g/l were slightly higher then in control group. Groups that received 1 g/l and 2 g/l decreased in S_{resp} and coeficcient F. Our data showed that aluminium intoxication impairs respiratory function of the blood.

Keywords: aluminium toxicity, blood morphology, respiratory function of blood

Introduction

The first works indicating aluminium toxicity appeared at the end of the 19th and beginning of the 20th centuries. These studies were based on observations of relationships between the presence of these elements in water and fish vitality and reproduction. In the 1960s harmful influence of aluminium was connected with acid rains and its toxicity for plants, animals and humans was proven. Works concerning toxicity of different aluminium species, conditions of its rise, environmental migration and influence on living organisms gathered momentum in the 1980s [13].

It is well known that aluminium toxicity is difficult to confirm in short time intervals. The reason for this is the very slow ligands exchange in Al complexes [2, 12]. Only long-term effects of Al^{3+} ions on an organism causes changes in skeletal, digestive, nervous and haemopoietic systems [15, 36]. Significant influence on the activity of numerous enzymes is a basis of these pathological disturbances. This element forms tight complexes with ATP, therefore inhibiting numerous enzymes that use ATP as a substrate. This restraining effect of aluminium ions manifests in case of neurotransmitters – γ –aminobutiric acid, noradrenaline, serotonine [24], and enzymes that take part in heme biosynthesis [5, 6, 10, 31, 32, 33].

The most frequently described blood problem is anaemia. It is important to note, that this disorder was observed in patients after dialyses when aluminium ions were present in dialysis fluid [4]; also in the case of laboratory animals that were administered solutions of aluminium salts intraperitonally or intravenously [18,

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Table 1. Changes in red blood cell system of rats after intoxication with aluminium.

Parameters	Control X ± SD	Doses of aluminium					
		0.5 g/l		1 g/l		2 g/l	
		X ± SD	Δ %	X ± SD	Δ %	X ± SD	Δ %
Erythrocytes [mln/mm^3]	7.88 ± 0.360	7.16 ± 0.603*	-9.14	6.67 ± 0.241*	-15.36	6.62 ± 0.302*	-15.99
Haemoglobin [g %]	15.14 ± 1.573	14.53 ± 0.936	-4.03	13.45 ± 1.627*	-11.16	13.74 ± 1.060*	-9.25
Haematocrit [%]	46.15 ± 2.135	45.85 ± 1.732	-0.65	44.05 ± 1.442*	-4.55	47.50 ± 1.154*	+2.92
MCV [μm^3]	58.57 ± 1.281	64.06 ± 1.363*	+9.37	65.99 ± 1.221*	+12.67	71.73 ± 1.932*	+22.47
MCH [pg]	19.22 ± 2.644	20.30 ± 2.732	+5.62	20.15 ± 2.423	+4.84	20.75 ± 2.106	+7.96
MCHC [%]	32.81 ± 2.451	31.69 ± 1.962	-3.41	30.53 ± 2.136*	-6.95	28.93 ± 2.013*	-11.83
Reticulocytes [‰]	26.47 ± 5.158	42.60 ± 8.980*	+60.94	81.28 ± 22.79*	+207.06	90.60 ± 18.66*	+242.27
Average diameter of erythrocyte (D) [μm]	6.39 ± 0.796	6.51 ± 0.667	+1.88	6.65 ± 0.707	+4.07	6.85 ± 0.722	+7.20
Surface area of erythrocyte (S) [μm^2]	64.15 ± 1.165	66.59 ± 1.623*	+3.80	69.40 ± 1.452*	+8.18	69.89 ± 1.745*	+8.95
Respiratory surface of blood volume unit (S_{resp}) [mm^2/mm^3]	486.2 ± 7.04	496.6 ± 7.36*	+2.14	463.2 ± 5.21*	-4.73	462.8 ± 5.42*	-4.81
Coefficient F	30.06 ± 1.782	30.83 ± 1.261	+2.56	25.49 ± 1.959*	-15.20	24.36 ± 1.142*	-18.96

* - differences statistically significant

33]. Thus, the aim of our studies was to examine changes in peripherial blood after physiologically administering aluminium chloride, through drinking water.

Materials and Methods

Blood samples were received from the Department of Animal Physiology Institute of Veterinary Medicine, Agricultural University of Lublin. Samples were collected from animals subjected to the following procedure.

Male Wistar rats age 2-3 months were maintained under standard laboratory conditions. The animals had permanent access to water and food. Animals were adapted to laboratory conditions (temperature 22±2°C, 12h/12h day/night cycle, moisture 55 ± 5%) for 14 days. After this period rats were divided randomly into three experimental groups and a control group of 15 animals each. Control animals were given pure water and experimental rats received water with aluminium chloride solution added ($AlCl_3$ x 6 H_2O) in three concentration levels – 0.5 g/l, 1 g/l, 2 g/l. Water was administered in 1.25 l graduated glass watering apparatus and was changed every day, while taking note of daily usage. It was about 30 ml, so animals were taken with contaminated water following about 15, 30 and 60 mg $AlCl_3$ per day. Because the Al bioavaiability from water is estimated at about 0.3% used doses, it may be recognised as not large in comparison those taken by humans, e.g. with drugs including this element [26, 36].

After 6 weeks of intoxication the animals were next anaesthetized with Nembutal (Abbott, England) at a dose of 50 mg/kg b.w. i.p. Then the left heart ventricle was punctured with a heparinized needle. Blood samples were collected and the following parameters were determined: erythrocyte count by chamber method, level of haemoglobin (Hb) with the cyanmethemoglobin method and hematocrit (Hct) with micro-method. Values of red blood indices (MCV, MCH, MCHC) were calculated in accordance with the models given by Wintrobe [29]. Reticulocyte count (Rt) per 1000 erythrocytes was determined on smears stained with brillant cresyl blue [21]. Erythrocyte measurements were performed on Pappenheim stained smears using a graded lens. 400 blood cells were measured for each subject. The values obtained were used to calculate mean erythrocyte diameter (D), blood cell surface (S), respiratory surface area per 1 mm^3 of blood ($S_{resp.}$) and respiratory coefficient (F) according to Gill's model [11].

The results thus obtained were analyzed statistically using ANOVA followed by Duncan's test. Data were considered significant when $p< 0.05$.

Results

In all intoxicated groups a significant drop in the number of erythrocyte, haemoglobin level and hematocrit value with an accompanying a statistically significant increase of reticulocyte content were observed. Also, increases of mean cell volume (MCV), average cell diameter (D) and

average surface of erythrocyte (S) was observed. Respiratory surface area of blood volume unit (S_{resp}) and coefficient F value were slightly higher at group administered aluminium chloride solution in 0.5 g/l dose compared to control. In groups that received aluminium chloride at doses of 1 g/l and 2 g/l, a decrease of $S_{resp.}$ and coefficient F were observed (Tab.1).

The course of anisocytosis curves was dependent on aluminium doses. In the case of rats receiving $AlCl_3$ solution in concentrations 0.5 g/l and 1 g/l were observed greater percentage of red blood cells measurements in range of 6.1-6.5 μm, then in control rats and the displacement of anisocytosis curve to the right. This displacement was somewhat stronger marked in groups receiving greater doses of aluminium. Percentage of red blood cells in range 6.1-6.5 μm in animals receiving aluminium at dose 2 g/l was almost the same as in control group. However, displacement of curve into right was very visible. It is important to notice the lower percentage of erythrocyte in range of 7.1-7.5 μm in all experimental groups than in control (Fig.1).

Discussion

The reduction in erythrocyte count, haemoglobin levels and hematocrit values in animals intoxicated with aluminium administered in drinking water observed in our studies is in agreement with Vittori et al. [27] and other authors, which applied salts of this element by different ways [1, 7, 8, 18, 19, 32, 34]. The above changes and accompanying high reticulocytosis indicate to haemolytic effect of aluminium. Hemolytic activity of this element is connected with changes in cell membrane of red blood cells. Rabbit erythrocytes in hydrolytically stable lipophilic aqueous $Al(acac)_3$ solution assume the form of echinoacanthocytes [9]. In the presence of Al ions human erythrocytes lost their typical biconcave shape, turning into acanthocytes and stomatocytes [28]. This element, like other xenobiotics, generates free radicals and reactive oxygen species in cells, which cause fatty acid superoxides and oxidation of SH – groups in cellular membrane proteins, and this leads to reduction of membrane fluidity and in consequence to substantial damage of cellular membrane [16, 35]. It also leads to a decrease in activity of membrane ATPases and in effect to cellular accumulation of adenylates and to reduce rate of ATP : ADP converting. This carry to restriction of energy essential to maintenance of membrane integrity and to blood cell dysfunction and haemolysis, especially after longer period of aluminium effect [10, 14, 16, 22]. Although animal organisms do posses a large poole of anti-oxidants, which protects them from free radicals, but in the specific case of aluminium intoxication, the activity of many of these anti-oxidative constituents is inhibited. The activity of superoxide dismutase (SOD) [25], peroxidase (PX), catalase (CAT) and glutatione peroxidase (GPX) [23] as well as L-ascorbic acid [34]. Reduction of membrane fluidity causes a decrease of cell blood ability to change shape. Cells possesing a membrane with reduced ability to change shape are trapped in reticulo – endothelial system, and thus are eliminated from circulation. Decrease in the number of erythrocyte can be a result not only of the haemolytic influence of aluminium, but also of reduced erythrocyte duration.

A large increase in reticulocyte content is a compensation reaction to haemolytic activity of aluminium, but it should be noted here that it does not compensate for the loss of erythrocytes. In the opinion of Zaman et al. [34] this is evidence of aluminium-induced disturbances of erythropoeisis and development of red blood cells. These suggestions have been confirmed by bone marrow examinations, which reveal a significant drop in the contents of proerythroblasts and all types of erythroblasts. Disturbance of erythrocyte development is associated with the creation of a very stable aluminium/ ATP complex. The absence of sufficient ATP impairs the system, which eliminates certain proteins during transformation of reticulocytes into mature erythrocytes [34].

The reduced level of haemoglobin can be associated with disturbances in heme biosynthesis as a result of inhibit linking of Fe with heme and drop in activity of enzymes taking part in heme biosynthesis, mainly dehydratase of delta-aminolevulonic acid (ALA-D) [3, 10, 30, 32].

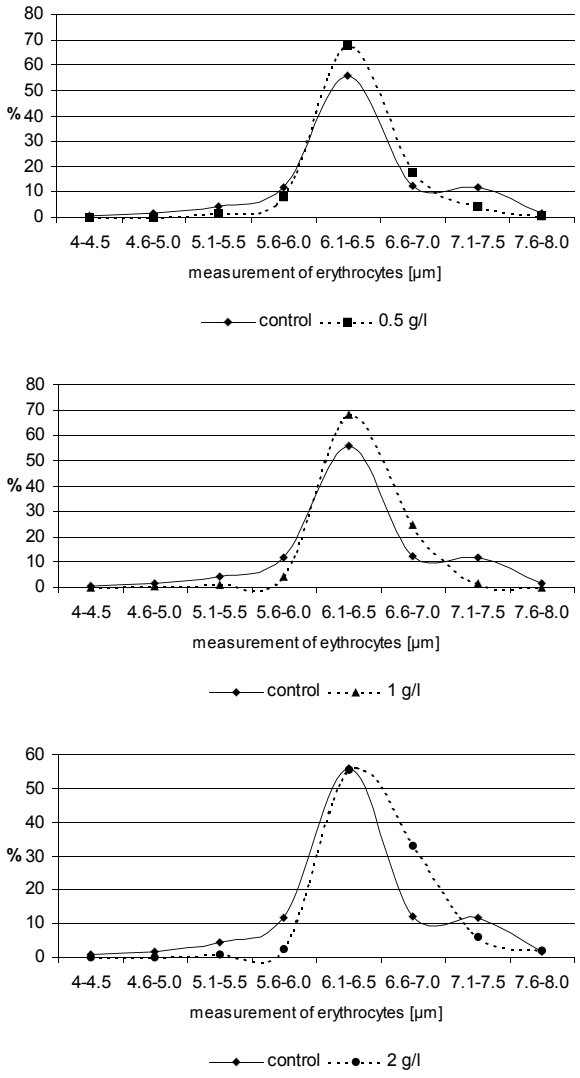

Fig. 1. Anisocytosis curves in dependence on aluminium dose.

The reason noted in our own research for the increase of average diameter of erythrocyte and erythrocyte surface area and also displacement of anisocytosis curves into right is probably much greater participation of reticulocytes as well as the swelling of the red blood cells. As has been mentioned, changes in cellular membrane under the influence of Al^{3+} ions provides an increase of level of dimalonic aldehyde, which inhibites Na^+K^+-ATPase activity [20]. A consequence of drop in activity of this enzyme is accumulation of Na^+ ions in the cell, which leads to an increase in volume [17]. In spite of increase of erythrocyte surface, total respiratory area and the value of coefficient F at experimental groups intoxicated by two greater aluminium doses were lower in comparison with control group, because of significant decreases in erythrocyte numbers and haemoglobin levels. It disturbs respiratory function of the blood by aluminium.

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