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INFLUENCE OF ALUMINIUM ON THE CONCENTRATION OF PHOSPHORUS AND ADENYL NUCLEOTIDES IN WHITE RAT ERYTHROCYTES

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The experiment covered 126 white male rats divided into two groups: 70 poisoned rats (group one) and 56 control ones (group two). Animals from the first group were perorally introduced 1 per cent of water solution of $AlCl_3$ in a dosis of 3 mg Al^{3+}/kg body mass daily for 40 days. The concentrations of inorganic phosphorus and adenyl nucleotides such as AMP, ADP and ATP in rat erythrocytes were determined on the 5th, 10th, 15th, 20th, 30th, and 40th day of trial. An elevation of ATP concentration tending towards a shift to the left of ATP/ADP balance during the second half of the experiment was established. Inorganic phosphorus reduced significantly during the whole trial. These alterations testify to disturbed processes of erythrocytic energy metabolism

Key-words: Aluminium, phosphorus, AMP, ADP, ATP, erythrocytes, rats

INTRODUCTION

Some toxic effects of aluminium are related to disturbances of phosphorus metabolism (4, 7, 9). It has been proved that peroral application of aluminium in high doses reduces intestinal absorption and retention of phosphorus (11, 13) as well as both serum and erythrocytic concentrations of some adenyl nucleotides (9, 10). However, the changes of these parameters influenced by alluminium applied in moderate and low doses are insufficiently clarified yet.

Phosphorus and its organic compounds such as adenosine mono- (AMP), di- (ADP) and tri- (ATP) phosphates in the cell are important indicators of the status of energy metabolism. Revealing the changes in energetic and metabolic processes of erythrocytes under the influence of toxic substances in low doses would be of essential importance for clarifying the compensatory mechanisms of the organism towards the toxic action of these substances.

That is why we decided to investigate the effect of aluminium in a relatively low dose on the concentration of inorganic phosphorus and its organic compounds in white rat erythrocytes.

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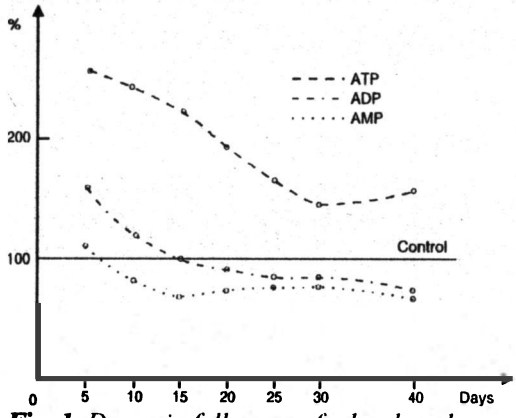

Fig. 1. Dynamic follow-up of adenyl nucleotide level in erythrocytes

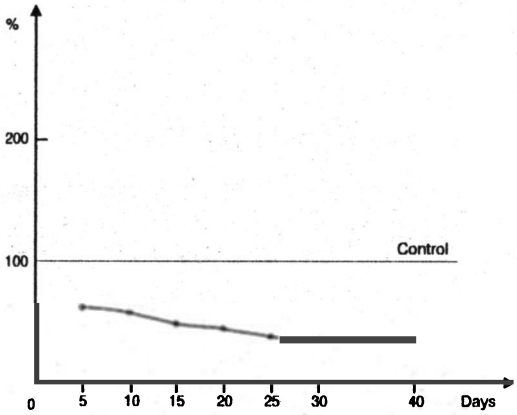

Fig. 2. Dynamic follow-up of inorganic phosphorus level in erythrocytes

MATERIAL AND METHODS

The experiment was carried out on 126 white male rats of Wistar breed (group one consisted of 70 treated animals but group two - of 56 control ones) with initial mean body mass of 125,0 ± 10,0 g. The animals of the first group were perorally introduced 1 per cent solution of aluminium chloride in a dose of 3 mg Al^{3+}/ kg b.m. by using a soft probe every day for 40 days. The animals of the second group were administered distilled water by an analogous way. All the experimental animals were nourished common vivarium food. Ten treated and eight control animals each were killed by decapitation on the 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, 30^{th}, and 40^{th} day of experiment.

The concentrations of AMP, ADP, and ATP in erythrocytes were determined by Boehringer's test kits while those of inorganic phosphorus (P_i) were estimated after Glick's method (6). Erythrocyte mass was obtained by threefold washing-up the blood with 0,9 per cent solution of sodium chloride and centrifugating at 4 °C and 3000 g/min for 10 min (1). Erythrocytic hemolysate was prepared with 0,1 per cent solution of EDTA at pH 7,0 in an erythrocyte : EDTA ratio of 1 : 10.

The results were statistically processed by the methods of variation analysis and of least squares.

RESULTS

ATP concentration increased already on the 5^{th} day while the mean value of the control group was 1366,78 mmol/10^{12} Erys. There was certain reduction later on, however, the concentration remained even if insignificantly higher than that of the control group until the end of experiment (Fig. 1).

ADP concentration was on the increase to a significant extent (by 85,5 per cent towards the second group, $p < 0,02$) already on the 5^{th} day after which it decreased. At the end of experiment ADP level was by 23,4 per cent lower than that of the control group ($p < 0,05$) (Fig. 1).

The content of AMP for the first group was of 52,13 ± 6,3 mmol/10^{12} Erys on the

5th day after which it began to decline. However, because of considerable individual aberrations the difference towards the control values was statistically significant on the 20th day only ($p < 0,01$) (Fig. 1).

The concentration of P_i changed most sensitively and unidirectionally. It decreased proportionally to the treatment duration and P_i level was by approximately 68 per cent lower than that of the control group at the end of experiment while the mean control value was near to 0,31 mmol/10^{12} Erys ($p < 0,001$) (Fig. 2).

Our results indicate that some changes in the concentrations of both Pi and adenyl nucleotides in erythrocytes originate after peroral administration of $AlCl_3$ in a dose of 3 mg/kg b.m. These changes are of different extent and nature. When manifested as a tendency P_i concentration is most involved remaining considerably reduced during the whole experiment. AMP level decreases progressively until the 20th day after which a slightly expressed elevation is observed. Equalized curves of both ADP and ATP concentrations demonstrate a progressive diminution during the whole experiment as ATP values are not lower than these of the control group.

DISCUSSION

According to Norseth (9) and Thurston (14), a syndrome of phosphorus exhaustion occurs under the influence of high doses of aluminium, particularly under conditions of a chronic experiment, and ATP content in tissues and erythrocytes reduces. Our results show the strongest increase of ATP at the early stages (by approximately 160 per cent) followed by that of ADP (by 62 per cent) and by that of AMP (by 12 per cent only) while P_i concentration decreases by some 35 per cent when compared with that of the control group.

ATP is the basic chemical compound that determines the energy potential of the biosystem. Having in mind existing data (4) that aluminium stimulates tissue formation and creates phosphatic and protein complexes when applied in low doses (12) our data allow the assumption that aluminium used in this dose stimulates the energic processes in erythrocytes during the initial stages of influence. This statement is confirmed by the enhanced G-6PDH activity in erythrocytes of the same animals (3). Probably, these discrepancies between literature and our own data are due to the different doses and duration of treatment. The fact that at the end of experiment one observes adenyl nucleotide content reduction indicates that a treatment of a longer duration even in the same dose could result in changes of opposite nature.

Intensive metabolic processes in erythrocytes require more energy which is provided mainly by both ATP and ADP. The considerably higher values of ATP/ADP ratio testify to an intensified metabolism in erythrocytes, too. The established increase of reticulocute count, particularly after the 15th day (8), is likely important in this respect, too, having in mind that energy accumulation in ATP macroergic links is more manifested in young cells (2). The strongly expressed diminution of both AMP and P_i during almost the whole experiment demonstrates a shift to the left of the balance

$$ATP \Leftrightarrow ADP + P_i \Leftrightarrow AMP + 2P_i$$

in contrast to the reported shift to the right under conditions of chronic application of aluminium in high doses (2). On the other hand, it is known that aluminium can bind both inorganic and organic phosphates already in the gastrointestinal tract and thus making them unusable for the organism. By this way a lower amount of phosphorus could enter the erythrocytes in order to be used as a cardinal source of ATP synthesis and its concentration decreases. Possibly, P_i content reduction until the end of experiment is due to exhaustion of phosphorus resources. In our opinion, a more definite answer to the question could be given when a more detailed investigation of phosphorus metabolism, including intestinal absorption, retention in different tissues, exchange between erythrocytes and plasma, as well as elimination by feces and urine under controlled dietary concentration is performed by a similar design.

CONCLUSION

Aluminium when applied perorally in a dose of 3 mg/kg b. m. induces significant changes in the concentrations of inorganic phosphorus and adenyl nucleotides in erythrocytes such as ATP increase with tendency towards shift to the left of ATP/ADP balance in the second half of experiment and strongly expressed reduction of inorganic phosphorus during the whole experiment testifying to disturbances in the processes of erythrocytic energy metabolism.

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Einwirkung des Aluminiums auf die Konzentration von Phosphor und Adenylnukleotiden in den Erythrozyten weißer Ratten

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Zusammenfassung: Ein Versuch wurde auf 126 männlichen weißen Ratten (70 vergiftet - die erste Gruppe und 56 Kontroltiere - die zweite Gruppe) durchgeführt. Man verabreichte den Tieren der ersten Gruppe täglich 40 Tage lang per os 1-prozentige Wasserlösung des $ALCl_3$ in einer Dosis von 3 mg Al^{3+}/kg Körpermasse. Am 5., 10., 15., 20., 25., 30. und 40. Tag wurden in den Erythrozyten der Tiere die Konzentrationen des anorganischen Phosphors und der Adenylnukleotiden - AMP, ADP und ADP bestimmt. Man stellte eine Erhöhung der Konzentration des ATP mit einer Tendenz zur Verschiebung des Gleichgewichtes zwischen ATP und ADP nach links in der zweiten Hälfte des Versuches fest. Der anorganische Phosphor sinkt wesentlich während des ganzen Versuches. Diese Veränderungen zeugen für Störungen in den Vorgängen des energetischen Stoffwechsels der Erythrozyten.

Influence de l'aluminium sur la concentration du phosphore et des adésines-phosphates dans les érythrocytes des rats blancs

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Résumé: On a expérimenté sur 126 rats mâles blancs (70 empoisonnés - Ier groupe et 56 - de contrôle - IIème groupe). Pendant 40 jours on introduisait quotidiennement et per os 1 % de solution aqueuse de $AlCl_3$ à dose de 3 mg Al^{3+} par kilo de masse corporelle dans les animaux du Ier groupe. Les concentrations du phosphore inorganique et des adésines-phosphates AMP, ADP, ATP dans les érythrocytes de ces animaux ont été déterminées les 5ème, 10ème, 15ème, 20ème, 25ème, 30ème et 40ème jours. On a établi pendant la deuxième moitié de l'expérience une augmentation de la concentration de l'ATP avec une tendence au deplacement de l'équilibre ATP/ADP à gauche. Le phosphore inorganique diminue fortement au cours de l'expérience. Ces changements témoignent des contreventions aux processus de l'échange énergique des érythrocytes