Morphological and Functional State of Major Salivary Glands under Conditions of Aluminum Chloride Excess in Drinking Water

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Morphology and function of the major salivary glands were studied in 50 albino rats drinking water supplemented with aluminum chloride for 2 weeks. Against the background of normal gland appearance, the salivation function and the composition of the saliva were changed: the concentrations of sodium and calcium ions and α-amylase activity were reduced. In parallel, cholesterol content was increased by 54%.

Key Words: *aluminum; major salivary glands*

Aluminum is a widespread mineral and living organisms appeared to be sufficiently adapted to this element during evolution. For instance, low absorption rate for aluminum salts in the gastrointestinal tract and high rate of aluminum excretion in the kidneys are typical of humans. This situation dramatically changed after appearance of artificial water-soluble aluminum compounds; moreover, these compounds can be easy delivered into blood flow during some medical procedures (hemodialysis). For instance, aluminum chloride enters the composition of antiperspirants, because it inhibits transpiration in the axillary region [13]. It was shown that long-term exposure to this compound for the treatment of hyperhidrosis can be associated with structural and functional degeneration of perspiratory gland acini [9]. However, little is known about the response of the major salivary gland to aluminum. Aluminum nitrate was previously found to distort the salivation function of the parotid gland [4].

Here we studied the structure and function of the major salivary glands (MSG) in rats drinking water with different concentrations of aluminum chloride for 2 weeks.

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MATERIALS AND METHODS

Experiments were carried out on 50 mongrel albino rats. The animals were placed in specially equipped room with limited access. Throughout the experiment, the rats were kept in standard cages under natural illumination and received pelleted balanced forage containing all important components *ad libitum* [3]. The animals received drinking water from 0.5-liter glass bottles through glass tips without restrictions for 2 weeks. In the experimental groups, the water contained 100, 300 and 500 mg/liter aluminum chloride. On the day of sacrifice, the salivary function was evaluated by collecting "oral fluid" after pilocarpine stimulation (5 mg/kg); the method was modified by us [3]. After decapitation, the submandibular gland (SMG) was isolated and fixed in 10% neutral formalin; mixed blood was collected. The experiments were performed under ketamine narcosis (5 mg/kg) according to euthanasia guidelines [1]. In the collected saliva, ion and organic composition and enzyme activities were analyzed; calcium ions (total) were detected by murexide method [2] and inorganic phosphorus was measured using molybdenum blue [6]. Potassium and sodium concentrations were determined using an EC-59-M ionometer (Kvertilab Ltd.). Ionometer accuracy for potassium ions was ±0.3 mmol/liter and for sodium

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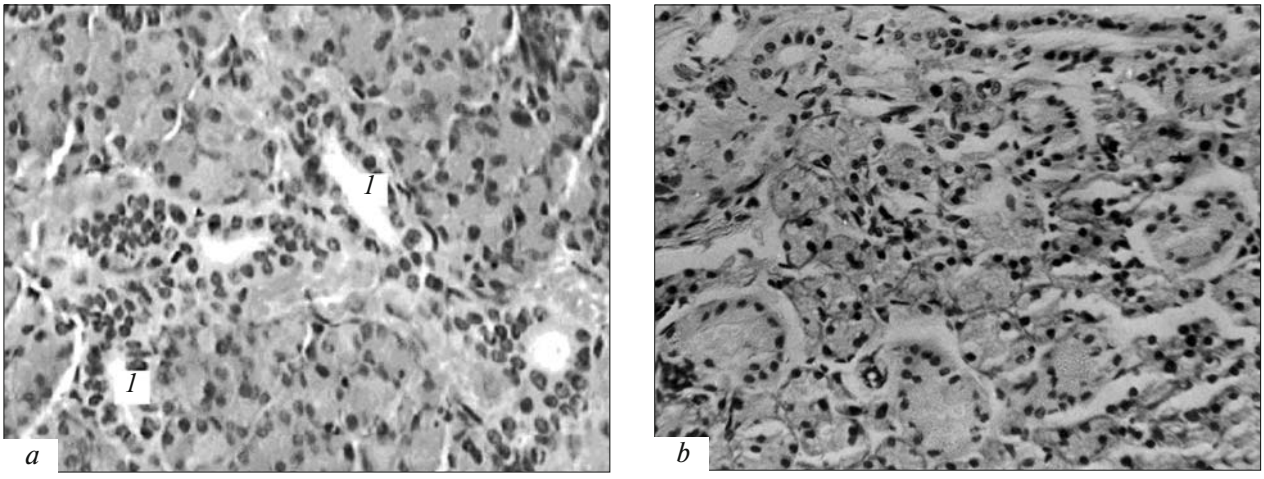

Fig. 1. Microscopy of rat SMG after exposure to 500 mg/liter aluminum chloride with drinking water for 2 weeks. Hematoxylin and eosin staining, ×250. *a*) control, *b)* experiment; *1*: duct.

ions ±4.0 mmol/liter. Cholesterol was measured using Ilca method [11], ALT [10] and α-amylase (AMY) activities [7] were measured using Lachema kits. Obtained data was processed using Statistica 5.0 software (Basic statistics). Reliability of differences ($p<0.05$) was assessed using Student's test (Biostat software).

RESULTS

The duration of the experiment corresponded to requirements for acute toxicity evaluation experiments [5].

Rats were chosen, because they are sensitive to general toxic effect of aluminum [12].

First, morphology of SMG was studied. Light microscopy revealed no visible structural changes in SMG during the experiment (Fig.1).

Then, functional state of the salivary glands was evaluated. Salivation function was studied and obtained biochemical values were standardized for 1 m^2 body surface area and salivation rate 10 ml/min/m^2 [3].

The parameters of total stimulated salivation are presented (Fig. 2). The volume of the saliva decreased with increasing aluminum chloride concentration in drinking water: in animals receiving the concentration 500 mg/liter it significantly decreased by 76%. Other parameters of salivation (latency and total time of salivation) remained unchanged.

Taking into account antagonistic relationships between aluminum and other ions (calcium, phosphorus, *etc*. [8,14]), the content of Na^+, K^+, Ca^{2+} and inorganic phosphorus (P_i) was determined in the collected saliva. Addition of 300 and 500 mg/liter aluminum chloride to drinking water significantly decreased Na^+ content by 40% ($p<0.05$) and 50% ($p<0.01$), respectively. Blood concentration of Na^+ ions also decreased (Table 1). This result is not exclusively determined by changes in Na^+ ion excretion with saliva.

Changes in the content of K^+ ions in the blood and saliva were within measurement error.

Changes in Ca^{2+} levels were also noted. In the experimental groups, calcium levels in the saliva lineary decreased with increasing the dose of aluminum chloride. In animals receiving maximum doses (300-500 mg/liter), Ca^{2+} levels decreased to 43-53% of the control level. In parallel, blood concentration of Ca^{2+} ions significantly decreased by 22% in rats receiving maximum dose of aluminum chloride.

No significant changes in saliva and blood levels of ionized phosphorus were found.

Cholesterol, ALT and AMY levels were determined at the third stage.

Cholesterol levels in the saliva of experimental animals gradually increased and surpassed the normal by 54% in the group receiving water with maximum concentration of aluminum chloride ($p<0.01$). No sig-

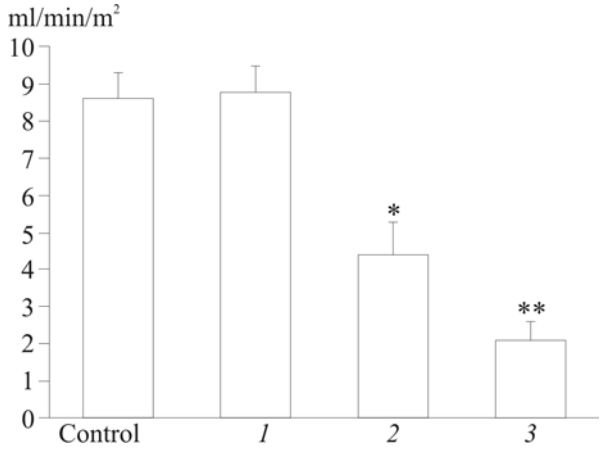

Fig. 2. Stimulated salivation in rats drinking water containing aluminum chloride in concentrations of 100 (*1*), 300 (*2*), and 500 (*3*) mg/liter. Ordinate: saliva volume. *$p<0.05$, **$p<0.01$ compared to the control.

TABLE 1. Ion and Biochemical Composition of the Blood and Pooled Saliva under Conditions of Excessive Aluminum Chloride Level in Drinking Water

Parameter			Control (*n*=14)	Aluminum chloride, mg/liter		
				100 (*n*=12)	300 (*n*=12)	500 (*n*=12)
Pooled saliva	ions	Na^+, mmol/liter	5.3±0.4	4.4±1.1	3.2±0.3**	2.7±1.0*
		K^+, mmol/liter	34±2	31±2	36±3	40±4
		Ca^{2+}, mmol/liter	3.20±0.25	2.90±0.26	1.45±0.35**	1.83±0.29**
		P_i^+, mmol/liter	1.58±0.16	1.56±0.14	1.49±0.24	1.45±0.15
	biochemistry	Cholesterol, mg%	33±2	35±6	42±5	51±3**
		ALT, U/liter	3.9±0.4	4.5±0.9	5.5±1.5	6.0±0.5*
		AMY, U/liter	900±250	810±100	710±48*	630±45**
Mixed (arteriovenous) blood	ions	Na^+, mmol/liter	134±5	125±3	127±4	119±4*
		K^+, mmol/liter	7±0.4	5±0.5	5±0.6	4±1.0
		Ca^{2+}, mmol/liter	2.7±0.2	2.9±0.3	2.4±0.3	2.1±0.1*
		P_i^+, mmol/liter	2.46±0.07	2.40±0.13	2.38±0.11	2.31±0.09
	biochemistry	Cholesterol, mg %	131±28	120±10	115±10	105±12
		ALT, U/liter	6.4±0.4	9.5±0.8	7.8±0.5	4.5±0.4
		AMY, U/liter	290±25	310±30	210±30*	155±35**

Note. *n*: number of measurements in the group. *p<0.05, **p<0.01 compared to the control.

nificant changes in blood cholesterol level were noted. ALT activity in the saliva and blood (marker of cell damage) did not differ form the control. Activity of gland-specific enzyme AMY decreased with increasing the dose of aluminum chloride. This trend appeared to be statistically significant by the end of the experiment. In pooled saliva this parameter significantly decreased by 30% (p<0.01). AMY activity in the blood significantly decreased by 47% at this term (2 weeks).

Comparing our findings with published data we noted that obtained results (except saliva content) were already described, but mainly for other organ systems, *e.g.* changes in lipid metabolism (cholesterol level) agree with the previously reported changes in physical properties of membrane lipids [15].

Thus, our study demonstrated that 2-week exposure to water-soluble salt aluminum chloride led to pronounced quantitative (reduced salivation function of MSG) and qualitative (ionic and biochemical composition of pooled saliva and blood) changes in the function of the salivary gland. However, no visible changes were revealed by light microscopy.

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