International Journal of Occupational Medicine and Environmental Health, Vol. 14, No. 4, 369—373, 2001

THE EFFECT OF SODIUM FLUORIDE ON THE ADENINE NUCLEOTIDE POOL IN ERYTHROCYTES OF WISTAR RATS

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Abstract. The effect of sodium fluoride on the content of adenine nucleotides, adenine nucleotide pool and energy potential of erythrocytes was studied in male Wistar rats, depending on the dose and time of exposure. Sodium fluoride was administered for 4 and 8 weeks at 4 or 16 ppm through a gastric tube. The concentration of fluorine in serum, ATP, ADP and AMP content in blood and erythrocytes, adenine nucleotide pool and energy potential of erythrocytes were calculated. The results were expressed in SI units and compared statistically with Student's t-test (Statgraphics v. 5.0 software). A significant reduction in the content of ATP and ADP and an increase in the content of AMP in erythrocytes was found after 4 weeks of exposure to 4 or 16 ppm NaF. The adenine nucleotide pool and energy potential were reduced with the smaller dose. After 8 weeks, the ADP content remained significantly reduced with the smaller dose, while the greater dose was associated with a higher energy potential of the cells. Correlations between serum concentration of fluorine, content of adenine nucleotides and adenine nucleotide pool in erythrocytes were noted in all study groups.

Key words:
Erythrocytes, Adenine nucleotides, Nucleotide pool, Energy potential, Fluorine

INTRODUCTION

The toxicity of fluorine compounds is usually discussed in the context of industrial contamination of the environment which exposes organisms living in contaminated areas to chronic effects of fluorine. Important sources of fluorine in the environment of large agglomerations are glass-works, metallurgy and fertilizer plants, with contamination in their vicinity exceeding several-times the permissible levels [1,2]. Exposure to these sources is aggravated by uptake of fluorine in food. Plant and animal products such as meat, milk, fruits and vegetables, and additionally, preparations used to fight caries in children and osteoporosis in adults contribute to accumulation of the element during the whole lifespan [1,3]. A comprehensive understanding of the dynamics of fluorine interactions in organisms inhabiting the contaminated environment is relatively difficult. We are unable to determine the level of imission of fluorine through the respiratory and gastrointestinal systems or excretion with urine, feces and sweat. It is usually accepted that the mechanisms of fluorine toxicity at the cellular level depend on the dose and time of exposure. Such interactions have been the subject of many experimental studies, both *in vivo* and *in vitro* [4–7].

Fluorine is bestowed with the highest electro-negativity and biological activity among all elements [8]. It enters the bloodstream through the lungs and gastrointestinal system. Intestinal absorption, tissue distribution and excretion by the kidneys depend on the value and direction of transmembrane pH gradient [9].

The distribution of fluorine between plasma and erythrocytes is unbalanced. Gumińska [10] has reported that

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75% of fluorine is found in plasma. Erythrocytes seem to have a special mechanism protecting against entry of fluorine or facilitating its removal [7]. Metabolic disorders in erythrocytes caused by fluorine involve competition with calcium and magnesium ions [1,11,12]. It has been demonstrated that fluorine ions inhibit specific (magnesium-dependent) regulatory enzymes of glycolysis, among them hexokinase (EC.2.7.1.1), phosphofructokinase (EC.2.7.1.11), phosphopyruvate hydratase (EC.4.2.1.11), and pyruvate kinase (EC.2.7.1.40) [11,13]. Fluorine may act directly by disrupting hydrogen bonds in the polypeptide chains and alter the enzyme tertiary structure, or indirectly by displacing cations necessary for catalytic activity [13].

Adenine nucleotides (ATP, ADP and AMP) undergo reversible transformations during glycolysis and the pentose cycle and thus can serve as indicators of energy metabolism in red blood cells [14]. The aim of the investigation was to determine their concentration in rat erythrocytes exposed to sodium fluoride for 4 or 8 weeks. Moreover, the pool of nucleotides and energetic potential in erythrocytes were estimated. The dose and time-dependent correlation between fluorine concentration in serum and adenine nucleotides and the remaining parameters was also analysed.

MATERIALS AND METHODS

The study was performed in autumn in male Wistar rats, weighing about 200 g. All rats had free access to water and were fed *ad libitum* with granulated chow (Murigran, Bacutil, Warsaw). Four study groups of 10 rats each were formed and exposed for 4 or 8 weeks to sodium fluoride administered through a gastric tube as an *ex tempore* aqueous solution at a dose of 4.42 mg/kg/24h (approx. 4 ppm) or 17.68 mg/kg/24h (approx. 16 ppm). The study rats were sacrificed by decapitation (without anaesthesia) together with the same number of control animals. Blood was collected into heparinized (250 IU Heparinum, Polfa, Poland) plastic test tubes and analyzed forthwith.

One aliquot of heparinized blood was used to determine packed cell volume (PCV) with a microhematocrit. Another was deproteinized with perchloric acid for measurement of ATP, ADP and AMP content in the acid-soluble fraction of erythrocytes. The method was essentially according to Schmit [15] using the Biochemical Test Combination (Boehringer, Mannheim, Germany) and readings were taken at 340 nm. The value of the nucleotide pool (TAN) and the energy potential (AEC) was calculated according to the formulas:

$$TAN = (ATP) + (ADP) + (AMP)$$

$$AEC = \frac{1}{2} \bullet \frac{(ADP) + 2(ATP)}{(ATP) + (ADP) + (AMP)}$$

Blood in plastic tubes was centrifuged at 2000 RPM for 15 min, whereupon the serum was withdrawn and used to measure fluorine concentration according to Marut [16].

The results were expressed in SI units and compared statistically with Student's t-test (Statgraphics v. 5.0 software). The correlation coefficient (r) was determined for fluorine levels in serum vs erythrocyte parameters in each study group.

RESULTS

Mean content of ATP in erythrocytes and blood of rats exposed to 4 or 16 ppm NaF for 4 weeks was significantly lower than in controls. The same result was obtained for ADP, while the content of AMP was significantly higher (Table 1). When exposure was extended to 8 weeks, the differences ceased to be statistically significant, saved for ADP content in erythrocytes and blood which was significantly reduced by the lower dose of NaF only (Table 2).

The energy potential and adenine nucleotide pool in erythrocytes after 4 weeks of exposure to 4 or 16 ppm NaF were reduced, but the difference was not significant for the energy potential and higher dose of NaF. The energy potential was significantly higher after 8 weeks of exposure to 16 ppm NaF. The adenine nucleotide pool remained reduced, but the difference was not significant (Table 3).

Mean concentration of fluorine in serum of rats exposed to 4 ppm NaF for 4 weeks was 71.05 μmol/l, insignificantly

Table 1. Concentration (µmol/l) of adenine nucleotides in erythrocytes and blood of rats after 4 weeks of exposure to NaF

Group	ATP		ADP		AMP	
	Erythrocytes	Blood	Erythrocytes	Blood	Erythrocytes	Blood
4 ppm	332.57*	139.68*	289.73**	121.68**	111.05**	46.64*
(n = 17)	+45.861	+36.525	+25.057	+15.075	+15.677	+5.033
16 ppm	390.77*	164.12*	285.33*	119.84**	100.88*	42.37*
(n = 20)	+45.592	+36.524	+36.637	+26.006	+21.169	+16.069
Control	44.46	180.88	384.85	157.79	62.27	25.53
(n = 10)	+52.183	+25.041	+36.588	+22.622	+17.707	+4.217

* $p \leq 0.05$, ** $p \leq 0.01$.

Table 2. Concentration (µmol/l) of adenine nucleotides in erythrocytes and blood of rats after 8 weeks of exposure to NaF

Group	ATP		ADP		AMP	
	Erythrocytes	Blood	Erythrocytes	Blood	Erythrocytes	Blood
4 ppm	447.12	201.20	245.08*	110.28*	69.00	31.05
(n = 15)	+40.433	+19.744	+11.973	+5.674	+13.203	+6.132
16 ppm	428.24	192.71	291.92	131.36	54.99	24.75
(n = 19)	+35.535	+17.187	+37.362	+17.484	+12.798	+5.209
Control	417.39	183.65	326.38	143.61	57.00	25.08
(n = 10)	+24.529	+11.321	+11.253	+5.908	+11.535	+4.617

* $p \leq 0.05$.

Table 3. Energy potential and nucleotide pool (µmol/l) in erythrocytes of rats exposed to NaF

Group	Dose	Energy potential	Nucleotide pool
4 weeks	4 ppm	0.65 + 0.063*	733.35 + 15.496**
	16 ppm	0.71 + 0.078	776.98 + 25.95*
Control		0.72 + 0.029	888.54 + 15.493
8 weeks	4 ppm	0.75 + 0.034	761.20 + 25.960
	16 ppm	0.78 + 0.069*	755.16 + 30.359
Control		0.72 + 0.019	800.77 + 14.671

* $p \leq 0.05$, ** $p \leq 0.01$.

more than the control value (54.08 µmol/l). After 8 weeks, the content reached 82.59 µmol/l, a statistically significant difference. Exposure to 16 ppm NaF for 4 weeks resulted in a significantly higher concentration of 83.62 µmol/l, but after 8 weeks the level was 57.89 µmol/l and did not differ significantly from the control value.

Correlations were found between fluorine level in serum and ATP, ADP, AMP content, and adenine nucleotide pool in erythrocytes of rats exposed to 4 or 16 ppm NaF for 4 or 8 weeks (Table 4).

DISCUSSION

Loss of ATP from erythrocytes *in vivo* has been observed both in workers and children chronically exposed to fluorine compounds [11,17,18]. A similar result *in vitro* was obtained in Ehrlich tumor cells cultured under conditions of aerobic glycolysis [19]. Metabolic disorders in erythrocytes have been attributed to interactions of fluorine with magnesium ions [12]. Fluorine inhibits specific magnesium-dependent enzymes of glycolysis in red blood [1,13], which are part of the erythrocyte metabolic control system

Table 4. Correlation coefficient (r) for serum concentration of fluorine vs content of adenine nucleotides (μmol/l) and energy potential in rat erythrocytes

Dependent variable (fluorine)		Independent variable				
		ATP	ADP	AMP	Nucleotide pool	Energy potential
Exposure 4 weeks	4 ppm	0.7861*	0.6268	0.2631	0.9017**	0.3658
	16 ppm	0.8285*	0.4676	0.8047*	0.9298**	-0.4393
	Control	0.4161	0.8188*	-0.0166	0.8362*	-0.0534
Exposure 8 weeks	4 ppm	0.8458*	0.4578	0.5912	0.8818*	0.1860
	16 ppm	0.5368	0.7715*	0.7364*	0.9425**	0.5990
	Control	0.5184	0.8755*	0.1767	0.7665*	0.1551

* $p \leq 0.05$, ** $p \leq 0.01$.

[20] and thus affects the reaction rates of glycolysis and indirectly alters the concentrations of ATP, ADP and AMP in erythrocytes [13,21,22].

The reports that chronic exposure to fluorine is accompanied by elevated levels of ATP and ADP in bovine [23] and human erythrocytes [17] have raised new questions concerning the mechanism of the action of fluorine. The present study addressed this issue by comparing two doses of NaF and two exposure times. After 4 weeks of exposure, either dose of NaF resulted in a significant reduction of ATP and ADP content, while the content of AMP rose significantly only at a smaller dose. ATP consumption by metabolic reactions leads to release of large quantities of inorganic phosphate (Pi) which inhibits AMP deaminase (EC.3.5.4.6). Thus, hydrolytic deamination of AMP [24] is inhibited and this nucleotide accumulates in the cell. Fluorine ions are also inhibitory for this enzyme [25]. Consequently, the present findings can be explained by a combined effect of Pi and F. Changes in the content of adenine nucleotides were accompanied by significantly higher levels of fluorine in serum of animals exposed to 16 ppm NaF. Furthermore, correlations between serum fluorine level, nucleotide content and nucleotide pool of erythrocytes were disclosed after 4 weeks of exposure (Table 4). These observations can be explained by disorders of carbohydrate metabolism directly affecting energy production by the cells. Such disorders could result from direct inhibition of magnesium-dependent enzymes [1,13], or partial inhibition of glucose transport through the cellular membrane [26]. The activity of cation pumps is reduced in parallel with ATP loss. Na^+, K^+-ATPase and Mg^{2+}-ATPase are further inhibited by fluorine ions [5,27]. These effects have been revealed *in vitro* and *in vivo* in erythrocyte ghosts from workers exposed to fluorine compounds [1]. Other authors [28] have reported that sodium fluoride inhibits potassium transport through the membrane of erythrocytes and affects its permeability to sodium ions [29].

The content of ATP and AMP rose after 8 weeks of exposure, which is in agreement with the results in environmentally exposed cattle and humans [17,23]. The adenine nucleotide pool was reduced only by the lower dose of fluorine. It thus appears that some compensatory mechanisms develop during longer exposure, resulting in a significant rise of the energy potential. This result may otherwise be attributed to significantly lower levels of fluorine in serum as compared with 4 weeks of exposure. Karlisz and Szymaniak [30] have chronically exposed rats to several doses of fluorine (0, 10, 30, 50 ppm) and found that the level of fluorine in serum remained constant after 3 months, irrespective of the dose. The present results suggest that serum levels of fluorine are reduced after 8 weeks of exposure to values that the organism is able to tolerate. The underlying compensatory mechanism might involve the increased uptake of fluorine by soft tissues [9,11], hard tissues [4], or greater excretion in urine [1].

The data presented here showed that after a four-week exposure of rats to 4 and 16ppm NaF the reduction in the content of ATP and ADP, as well as the increase in the content of AMP in erythrocytes were observed. The influence of NaF after 8 weeks was not significant.

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Received for publication: May 15, 2001
Approved for publication: October 30, 2001