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The state of oxidative-antioxidant homeostasis under the influence of subtoxic doses of sodium fluoride

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ABSTRACT
The objective of the study was to study the state of oxidative-antioxidant homeostasis under the conditions of the formation of experimental fluoride intoxication.24 white rats of the Wistar population were daily injected with sodium fluoride solution (20 mg/kg of animal weight). The continuation of intragastric xenobiotic intake was 1.5 months. The control group was monitored for 2 weeks toanalyse their condition. After 2 weeks, and The study program was conducted after 2 weeks when healthy mature (3 months) males weighing 180-200 grams were selected . At the end of the experiment, the control and experimental groups were clogged by decapitation. Evaluation of oxidative-antioxidant homeostasis was doneon the basis of the following indicators: the intensity of biochemiluminescence (BCL) of homogenates of internal organs and blood serum, the blood levels of SH-groups, glutathione, haptoglobin, diene conjugates (DC), malondialdehyde (MDA), cysteine, catalase activity, peroxidase, ceruloplasmine, glutathione peroxidase, superoxide dismutase. Vitamin C was determined in the adrenal glands. Analyzing the effect of fluoride intoxication on the state of oxidative and antioxidant system, it was shown that stimulation of free radical lipid peroxidation, the accumulation of peroxides, hydroperoxides, free radicals and inhibition of the antioxidant system are characteristic in this process. The obtained results confirm that all these mechanisms underlie the formation of structural and metabolic mechanisms of the pathogenesis of fluoride intoxication.
Keywords: *biochemiluminescence; diene conjugates; malondialdehyde; Wistar white rats.*

1. INTRODUCTION

The problem of correlation and interaction of oxidative and reduction processes in biological systems is the leading one in the list of substantiation of pathogenetic mechanisms of the formation of structural and metabolic disorders from organs, systems and body functions [1, 18, 33]. The structure and function of the oxidative and antioxidant systems of the body is an actual topic in recent years [13, 19]. The role of the latter in metabolic processes is aimed at limiting the activation of lipid peroxidation and the formation of reactive oxygen species, oxidative radicals O_2, OH, HO_2, singlet oxygen O_2, hydrogen peroxide, hydroperoxides, organic peroxides, epoxides, aldehydes, ketones, and others, i.e. compounds that have a damaging effect on the cell and its components [2, 20, 32]. Bioantioxidants, or quenchers of excited electronic states, are opposed to these compounds in the body [9, 21, 31]. It should be noted that the free radical oxidation process exists normally in all organs and tissues and this is the important link in metabolism [15]. The acceleration or inhibition of free radical oxidation leads to pathology as low concentrations of peroxides, hydroperoxides are simply necessary for the body [3, 25]. It is believed that prolonged activation of free radical oxidation leads to changes in the composition of membrane lipids, their permeability, which is manifested in a violation of the function of structural units [4, 26-29]. One of the reasons contributing to the activation of oxidation in the tissues of the body is the intake of toxic chemicals, including fluorides [5, 11].

2. MATERIALS AND METHODS

The objective of the study was to study the state of oxidative-antioxidant homeostasis under the conditions of the formation of experimental fluoride intoxication.

This work is a fragment of KhMAPO study "Pathochemical mechanisms of action of radioiodine on the organism and principles of their early diagnosis and correction", state registration number 0117U000589 of Ukrainian Scientific and Technical Information Center.

The experimental part of the work was performed on 24 white rats of the Wistar population (18 experimental and 15 control), which were daily injected with sodium fluoride solution at the rate of 20 mg/kg of animal weight. The duration of intragastric xenobiotic intake was 1.5 months. The control group was monitored for 2 weeks to monitor their condition. Healthy mature (3 months) males weighing 180-200 grams were selected in a period of 2 weeks, and the study program was conducted. At the end of the experiment, the control and experimental groups were clogged by decapitation.

Evaluation of oxidative-antioxidant homeostasis was carried out on the basis of the following indicators: the intensity of biochemiluminescence (BCL) of homogenates of internal organs and blood serum, the blood levels of SH-groups, glutathione, haptoglobin, diene conjugates (DC), malondialdehyde (MDA), cysteine, catalase activity, peroxidase, ceruloplasmine, glutathione peroxidase, superoxide dismutase. In the adrenal glands, vitamin C was determined. The basis of the assessment of oxidative-antioxidant homeostasis was based on generally accepted methods. Prolonged intake of sodium fluoride in subtoxic doses (20 mg/kg weight) led to the development of intoxication.

Biochemiluminescent studies were carried out with the HLMNC 1-01 device. The object of study was blood serum, homogenates of internal organs. Spontaneous BCL was recorded. Evaluation of the results was carried out by the intensity and kinetics of the reaction of the glow.

3. RESULTS

In our research we found the increasing degree of oxygen uptake by hepatocyte mitochondria The intensification of lipid peroxidation during induced fluoride intoxicationis confirmed by studies of the accumulation of diene conjugates and MDA in blood serum, as well as by an increase in the intensity of serum BCL and homogenates of some internal organs (Table 1).

Thus, in comparison with the control, the content of DC during fluoride-induced intoxication increases 1.98 times, respectively, and the MDA level increases 2.26 times. The calculations, in this case, suggest a specific effect of fluorides on the accumulation of oxidation products.

As shown by the experimental results (Table 1), the intensity of chemiluminescence (CL) of blood serum and homogenates of internal organs during model fluoride intoxication increased compared with the control. So, for blood, this increase was 1.85 times. Due to the high sensitivity and high accuracy of CL, the method captures the specific effect of fluoride intoxication, in contrast to conventional biochemical studies. In this case, the difference in the glow is 25.3%, which allows us to judge the specific effect of fluoride intoxication.

In animals in which experimental fluoride intoxication developed we analyses the state of white and red blood, and thenwecompared with similar indixes of the control group.

The following tests were used in the work: the number of blood leukocytes (g/l), the leukocyte formula (%), the number of red blood cells (T/l), the content of hemoglobin and methemoglobin (mmol/l). The indicators of white and red blood were determined according to generally accepted methods.

Subtoxic oral intake of sodium fluoride in the body of white rats within 1.5 months led to a decrease in the content of red blood cells, hemoglobin, leukocytes and an increase in the level of methemoglobin in the blood (Table 2).

Changes in the leukocyte formula were statistically characterized by an increase in the percentage of segmented and stab neutrophils, basophils against the background of a general decrease in the number of lymphocytes (Table 3).

We found the following shifts in the state of the leukocyte formula (Table 3): the number of segmented neutrophils increases compared to the control value by 2.7 times during intoxication, and stab numbers by 2.1 times. The content of lymphocytes, on the contrary, is significantly reduced - 2 times in the conditions of fluoride intoxication.

The major role of sulfur-containing compounds in the destruction of lipid peroxides has been shown. Important in these transformations belong to SH-groups, glutathione, vitamin C, haptoglobin. Our changes in the dynamics of the content of these antioxidants (Table 4) towards a statistically significant decrease can be interpreted as a disruption of the protective-compensatory reaction of the body, especially caused by fluoride intoxication.

In subsequent experiments of Bharti V. and others, a number of biochemical parameters were studied reflecting the state of the antioxidant system and the redox processes represented by the enzyme system [10]. In addition to the enzyme activity (in organs and tissues) of catalase, peroxidase, ceruloplasmin, lactate and malate dehydrogenases, cholinesterase, glucose-6-phosphate dehydrogenase, alanine and aspartic aminotransferases, alkaline phosphatase (Table 5), studied in a subacute experiment. From the Table 5 it follows that with model fluoride intoxication, a decrease in the activity of adenylate cyclase (blood), glutathioperoxidase, leucine aminopeptidase (serum), and all biochemical parameters of the kidneys and liver is observed. However, increased levels of alkaline phosphatase, α- hydroxybutyrate dehydrogenase, γ-GT in serum were measured. The content of total serum lipids was also increased.

Studies have shown that under the influence of chemical toxins in the liver, an accumulation of DC and MDA occurs [13-15]. These compounds appear at the stage of free radical formation and indicate the accumulation of peroxides and hydroperoxides in the tissues of the body, which have a damaging effect on the cell. In this case, DC - fatty acid molecules containing double bonds, are considered as intermediate, and MDA as the final product of lipid peroxidation [22].

A direct relationship was noted between the level of free radical oxidation of lipid systems in the body and the intensity of CL of biological systems [6, 21]. In this case, the change in the intensity of blood BCL can be considered as an integral reaction of the body, which makes it possible to judge the nature of molecular and electronic disorders of biological structures under various physiological conditions and pathological processes.

Considering BCL as a reflection of the intensity of lipid peroxidation in the body, it can be assumed that under the influence of fluorides, the stable ratio of the products of free radical oxidation of lipids and the antioxidant system changes, fluoride intoxication stimulated the free processes of lipid peroxidation [9, 13].

Considering that the condition of the hematopoietic system is an integral indicator of the homeostatic function of the body, an indicator of many diseases and intercurrent poisoning, we studied the effect of subtoxic doses of sodium fluoride on the state of white and red blood in animal experiments under conditions of intoxication [1, 14].

According to some authors, such dynamics in terms of the content of indicators of white and red blood is characteristic of toxic oxidative chronic stress. In this regard, it should be assumed that the prolonged intake of excessive amounts of sodium fluoride activates free radical processes that serve as a source of generation of reactive oxygen species, free radicals, peroxides, and hydroperoxides, which have an inhibitory effect on the state of the antioxidant system [18]. Being strong oxidizing agents, these molecules can lead to the accumulation of methemoglobin in the blood and to the violation of the physicochemical properties of the membranes, which we discovered in the conditions of the formation of chronic fluoride intoxication [7, 23].

Analysis of indicators indicates inhibition of sodium fluoride (at a dose of 20 mg/kg weight) of all functions associated with white and red blood. Studies show that the leading structural and metabolic mechanism for the formation of disorders from white and red blood can be free radical pathology, the pathological link of which is a violation of the physicochemical properties of biological membranes, which led to a change in the nuclear-cytoplasmic interaction, accompanied by inhibition of biosynthetic processes that lie in basis of subchronic fluoride intoxication [5, 19].

Studying the state of the rat antioxidant system in a subacute experiment with fluoride intoxication, we experimentally proved the effect of bioantioxidants on the activity of membrane lipid-dependent enzymes, which is similarly affected by both mild lipid removal and the accumulation of peroxides in the membranes.

Table 1. The accumulation of lipid peroxidation products in blood serum and the intensity of biochemiluminescence (BCL) under the conditions of fluoride intoxication in white rats.

Indexes	**Groups of animals, M±m**	
	Control (n=15)	Experimental (n=18)
Diene Conjugates (nmol/l), serum	2,30±0,15	4,55±0,43↑ P<0,05
Malondialdehyde (nmol/l), serum	0,96±0,03	2,17±0,25↑ P<0,05
Biochemiluminescence (imp/sec), serum	780,40±21,15	1447,95±86,13↑ P<0,05
Biochemiluminescence (imp/sec), liver	960,30±17,22	1565,42±37,81 P<0,05
Biochemiluminescence (imp/sec), kidney	1137,72±21,63	1620,83±44,51 P<0,05

Table 2. The effect of sodium fluoride on the state of white and red blood.

Group of animals, number	**Indexes, M±m**			
	Red Blood Cells (T/l)	Leukocytes (g/l)	Hemoglobin (mmol/l)	Methemoglobin (mmol/l)
Control n=15	5,4±0,48	8,7±0,40	11,4±0,30	1,63±0,07
Experimental n=18	3,7±0,26 P<0,05	5,1±0,32 P<0,05	8,6±0,27 P<0,05	2,95±0,04 P<0,05

Table 3. The effect of sodium fluoride on the leukocyte formula of white rats in a subacute experiment

Group of animals, number	**Leukocytes (%), M±m**					
	Segmented Neutrophils	Stab Neutrofils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control n=15	15,8±2,20	5,40±0,30	72,20±2,50	4,1±0,36	1,30±0,29	2,6±0,15
Experimental n=18	42,8±2,60 P<0,05	11,3±0,73 P<0,05	36,6±3,50 P<0,05	5,9±0,83 P>0,05	1,8±0,27 P>0,05	4,8±0,17 P<0,05

Table 4. The effect of fluoride intoxication on the state of the rat antioxidant system in a subacute experiment.

Indexes	**Groups of animals, M±m**	
	Control (n=15)	Experimental (n=18)
SH-groups, blood (mg%)	22,4±1,70	9,6±0,45↓ P<0,05
Glutathione, blood (mg%)	17,8±1,25	10,6±0,38↓ P<0,05
vitamin C, suprarenal gland (mg%)	26,2±1,53	14,8±0,95↓ P<0,05
Haptoglobin, Serum (g/l)	2,60±0,8	0,96±0,25↓ P<0,05

Table 5. The influence of fluoride intoxication in the conditions of subacute experience on the dynamics of biochemical parameters of white rats.

Indexes	Groups of animals, M±m	
	Control (n=15)	Experimental (n=18)
Catalase blood, (catal. number)	8,2±0,5	4,4±0,6↓ P<0.05
Peroxidase blood, (c.u.)	53,8±4,5	86,2±4,1↓ P<0.05

Indexes	Groups of animals, M±m	
	Control (n=15)	Experimental (n=18)
Ceruloplasmin blood, (u.extr.),	94,7±6,5	54,2±3,4↓ P<0,05
Lactate Dehydrogenase serum, (u.extr.)	20,2±1,65	44,6±1,78↑ P<0,05
Malate dehydrogenase serum, (u.Buchera)	16,7±0,83	8,4±0,36↓ P<0,05
Cholinesterase serum, (mcat/l)	160,40±2,70	98,20±2,50↓
Glucose-6-Phosphate Dehydrogenase blood, (mcat/l)	2,495±0,016	0,734±0,013 P<0,05
Aspartic Aminotransferase serum, (mcat/l)	0,630±0,007	1,67±0,009↑ P<0,05
Alanine Aminotransferase serum, (mcat/l)	0,820±0,005	1,53±0,006↑
General lipids serum, (g/l)	4,20±0,053	5,95±0,37↑

4. CONCLUSIONS

Analyzing the effect of fluoride intoxication on the state of the oxidative and antioxidant system, it was shown that stimulation of free radical lipid peroxidation, the accumulation of peroxides, hydroperoxides, free radicals and inhibition of the antioxidant system are characteristic in this process. The obtained results confirm that all these mechanisms underlie the formation of structural and metabolic mechanisms of the pathogenesis of fluoride intoxication.

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6. ACKNOWLEDGEMENTS

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