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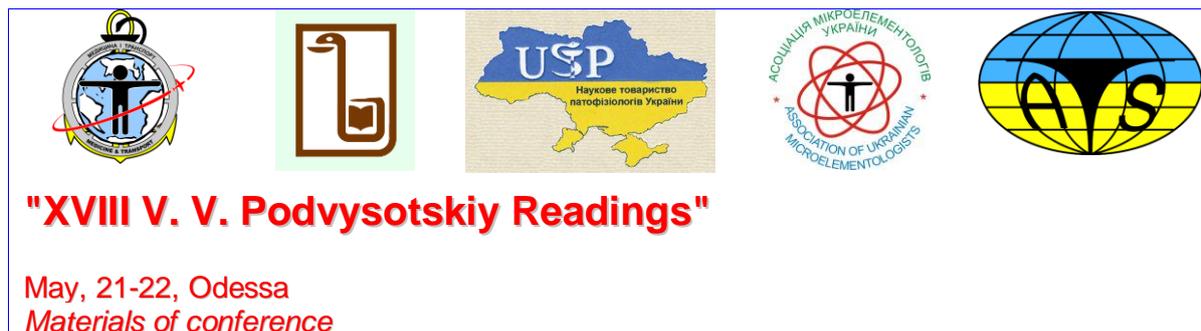
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## CORRECTION OF DESTRUCTIVE CHANGES IN CONNECTIVE TISSUES OF DIFFERENT ORGANS DURING CHRONIC NITRATE AND FLUORIDE INTOXICATION BY NANOSIZED SILICA OXIDE

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### Abstract

This experiment carried on 35 white Wistar rats aimed at studying the markers of connective tissue destruction during chronic nitrate-fluoride intoxication and its correction by nanosized silica oxide. It has been found out that co-administration of sodium fluoride (10 mg/kg body weight) and sodium nitrate (500 mg/kg of body weight) for 30 days increases the total content of glycosaminoglycans (GAG), heparin/heparan and keratan/dermatan fractions, as well as content of chondroitin fraction and concentration of L-oxyproline in the gastric mucosa. The content of N-acetylneuraminic acid (NANA), free L-oxyproline and gexuronic acids are increased in femoral bone and lumbar vertebrae. These changes point to depolymerization of componets of organic connective tissues matrix (collagen, glycoproteins, proteoglycans). Application of nanosized silica oxide suspension under conditions of chronic

nitrate-fluoride intoxication reduces the total GAG content, keratan / dermatane fraction ratio, chondroitin fraction and L-oxyproline concentration in the gastric mucosa. We also observed a decrease in free L-oxyproline concentration, gexuronic acids and NANA content in femoral bone and vertebrae. The results allowed us to conclude that chronic nitrate-fluoride intoxication leads to the destruction of connective tissue components in different organs (gastric mucous membrane, femoral bones and vertebrae). Usage of nanosized silica oxide under conditions of chronic nitrate-fluoride intoxication reduces the destruction of connective tissue in these structures.

**Key words: nitrates, fluorides, nanosized silica oxide, chronic intoxication, gastric mucosa, bones**

### **Introduction**

The problem of co-impact produced by environmentally dangerous factors of chemical nature as inorganic nitro compounds and fluorides on public health is a long-standing challenge in Ukraine. In the Poltava region, over 300 thousand population are exposed to higher nitrate loads. In some districts drinking water obtained from wells has nitrate level, which exceeds the average index in 5 – 15 times, and, moreover, sometimes is 30- 40-fold higher than normal. The main aquifer of the Poltava region, Ukraine (Buchaksky water-bearing stratum) is formed by sand with significant content of phosphorite layers and inclusions that conditions the ingress of fluorides into water in up to 8-9 mg/l concentration [12]. The impact of man-made load (fluoride content in the waters of the mining industry regions reaches 5 mg/l) also contributes into the drinking water pollution.

In the European Union there is a problem of heightened concentration of fluoride in drinking water in Central Poland [6]. The same problem is relevant to United Kingdom since there is increased concentration of fluoride in commercial drinks and food [20].

Another dangerous ecological pollutant which causes adverse effects on human and animal health are the nitrate containing compounds. Nitrates as soluble compounds are capable of ground and drinking water pollution. This is observed in Catalonia (Spain) and other regions of Europe [13]. Due to the free economic zone in Europe food from nitrate polluted regions can freely move all over the Europe [19]. Therefore possibility of excessive combined intake of nitrates and fluorides is not excluded.

It is the nature of nitrates and fluorides impact on the connective tissue in mammals that is one of the most widely discussed issues. On the one hand, there are some works reporting nitrates as NO (nitrogen monoxide) donators that promote bone mass growth in

experimental studies on animals and human subjects with osteoporosis [21], improve gastric mucosal blood supply and mucus production, promote mucus thickness [14], thereby supporting osteo- and gastroprotective mechanisms. On the other hand, surplus intake of sodium nitrate results in the rupture of gastric mucosa integrity [11], metabolic and biomechanical disorders in the bones of rats, limits their regenerative capability [7, 15].

Over recent years there has been demonstrated the efficacy of correcting metabolic disturbances in the blood of rats exposed to excessive combined intake of nitrates and fluorides by nanosized silica oxide [2]. Nevertheless, the effect of this enterosorbent on depolymerization of organic connective tissues matrix is still unclear.

The aim of this study is to investigate markers of connective tissue destruction during chronic nitrate-fluoride intoxication and its correction by nanosized silica oxide.

### **Materials and methods**

The study was conducted on 35 mature rats of the Wistar line. Rats were randomly assigned to 3 groups. The control group consisted of 10 animals, which were given physiological solution (0.9% solution of sodium chloride) daily for 30 days intragastrically. The group of chronic nitrate-fluoride intoxication consisted of 15 animals, which were given sodium nitrate at a dose of 500 mg / kg and sodium fluoride daily at a dose of 10 mg/kg for 30 days intragastrically [1]. Correction group consisted of 10 animals, which were given, during modelling of chronic nitrate-fluoride intoxication, suspension of nanosized silica oxide at a dose 100 mg/kg [3].

The research was conducted in compliance with the standards of the Convention on Bioethics of the Council of Europe's 'European convention for the protection of vertebrate animals used for experimental and other scientific purposes' (Strasbourg, 18.III.1986). Animals were sacrificed under thiopental anesthesia by blood aspiration from heart.

Gastric mucous membrane, femoral bone and lumbar vertebrae were washed with physiological solution and separated from adjacent tissues with a scalpel. Next, they were homogenized with a tris buffer solution (pH = 7.4) to obtain 10% tissue homogenate.

The total content of glycosaminoglycans (GAG) was determined by the content of uronic acids formed by their hydrolytic cleavage with concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). The content of uronic acids was determined by the special reaction of these acids with carbazole according to the guidelines [8]. The concentration of free L-xyproline was determined in the reaction of pyrrole-2-carboxylic acid, which is formed by oxidation of L-xyproline, with paradimethylaminobenzaldehyde according to the methodological recommendations [8]. N-acetylneuraminic acid (NANA) concentration was measured by the Hess method [8].

The content of different GAG fractions was determined by the content of uronic acids after preliminary fractionation of GAG by the Volpi method [17].

The data was processed using the Microsoft Excel and Realstatistics extension for this program. After establishing the normality or abnormality of the distribution of obtained data by the Shapiro-Wilk method, the statistical significance of the difference between the groups was assessed using the Mann-Whitney U-criterion. The difference was considered statistically significant if  $P < 0.05$ .

### Results and discussion

Chronic nitrate-fluoride intoxication increases the total content of GAG in the gastric mucosa (Table 1) by 62.6%, when compared with control group data.

Table 1. Markers of gastric mucosa connective tissue destruction during chronic nitrate-fluoride intoxication and its correction by nanosized silica oxide suspension. (M±m)

Parameters	Groups		
	Control group, n=10	Chronic nitrate and fluoride intoxication, n=15	Usage of nanosized silica oxide under conditions of chronic nitrate and fluoride intoxication, n=10
Total GAG content, $\mu\text{mol}$ of Uronic acids / L of tissue homohenate	0.99 $\pm 0.07$	1.61 $\pm 0.03^*$	1.32 $\pm 0.06^{**}$
Heparin/Heparan fraction content, $\mu\text{mol}$ of Uronic acids / L of tissue homohenate	0.39 $\pm 0.04$	0.54 $\pm 0.01^*$	0.69 $\pm 0.05^{**}$
Keratan/Dermatan fraction content, $\mu\text{mol}$ of Uronic acids / L of tissue homohenate	0.30 $\pm 0.03$	0.66 $\pm 0.02^*$	0.32 $\pm 0.02^{**}$
Chondroitin fraction content, $\mu\text{mol}$ of Uronic acids / L of tissue homohenate	0.30 $\pm 0.03$	0.42 $\pm 0.01^*$	0.34 $\pm 0.02^{**}$
L-oxypoline concentration, $\mu\text{mol}$ /g of tissue	0.116 $\pm 0.003$	0.865 $\pm 0.006^*$	0.132 $\pm 0.008^{**}$

Note (in table 1-2): \* –  $P < 0.05$  compared with values of intact rats, \*\* –  $P < 0.05$  compared with values of the second group (group of chronic nitrate-fluoride intoxication).

The content of heparin/heparan fraction increases by 38.5%. The content of the keratan/dermatan fraction increases by 120%. The content of chondroitin fraction increases by 40%. The concentration of L-xyproline elevates by 7.5 times.

Application of nanosized silica oxide suspension under conditions of chronic nitrate-fluoride intoxication reduces the total GAG content in the gastric mucosa by 18%, when compared with the second group data. The content of heparin / heparan fraction increases by 27.8%. The content of the keratan / dermatane fraction drops by 51.5%. The content of chondroitin fraction decreases by 19%. The concentration of L-xyproline decreases by 6.6 times.

The use of heparin is an alternative approach to the treatment of stomach ulcers [9], since heparin prevents blood clots formation in expanded stomach vessels during nitric oxide hyperproduction, which is caused by chronic nitrate-fluoride intoxication [1]. Sulfated forms of heparin are involved in the attachment of growth factors [18], so an increase in concentration of heparin / heparan fraction during chronic intoxication can be considered as an compensatory response. During application of nanosized silica oxide suspension oxide increase in heparin / heparan fraction is a beneficial phenomenon for increasing regeneration rate of mucous membrane epithelium.

Co-administration of sodium fluoride and sodium nitrate is accompanied by probable increase in concentration of free L-xyproline in femoral bones and vertebrae by 14% та 18% respectively, NANA concentration by 70% in both structures, and elevation of gexuronic acids concentration by 51% та 47% respectively (Table 2).

These changes point to depolymerization of componets of organic connective tissues (bone) matrix (collagen, sialoglycoproteins, proteoglycans) under surplus intake of sodium nitrate and sodium fluoride.

Application of nanosized silica oxide suspension under conditions of chronic nitrate-fluoride intoxication reduces the free L-xyproline concentration in the femoral bone homohenate by 6%, gexuronic acid content - by 26.8%, NANA – by 37% compared with the relevant findings in the second group of the animals.

Administration of nanosized silica oxide suspension under experimental conditions decreases the free L-xyproline concentration in the vertebrae homohenate by 9.2%, gexuronic acid content - by 27%, NANA – by 37.9% compared with the relevant findings in the second group of the animals.

Table 2. Components of bone organic matrix during chronic nitrate-fluoride intoxication and its correction by nanosized silica oxide suspension. (M±m)

Parameters	Groups		
	Control group, n=10	Chronic nitrate and fluoride intoxication, n=10	Usage of nanosized silica oxide under conditions of chronic nitrate and fluoride intoxication, n=10
Femoral bone			
L-xyproline concentration, μmol/g of tissue	3.64 ±0.10	4.14 ±0.09 *	3.89 ±0.06 **
Gexuronic acid content, μmol/g of tissue	1.97 ±0.23	2.98 ±0.17 *	2.18 ±0.18 **
NANA, μmol/g of tissue	2.20 ±0.20	3.73 ±0.22 *	2.35 ±0.21 **
Vertebrae			
L-xyproline concentration, μmol/g of tissue	3.98 ±0.13	4.69 ±0.16 *	4.26 ±0.09 **
Gexuronic acid content, μmol/g of tissue	2.17 ±0.26	3.18 ±0.16 *	2.32 ±0.18 **
NANA, μmol/g of tissue	2.28 ±0.29	3.88 ±0.20 *	2.41 ±0.22 **

It is known that the extracellular matrix (ECM) of connective tissue plays one of the leading roles in the functioning of various organs. It provides stabilization and cementation of fibrous structures, inter- and intracellular interaction, regulation of water-salt metabolism, and is involved in antimicrobial tissue resistance [5].

The main structure-forming element of the stroma is fibroblasts, which most important function consists in the ECM production, and especially the synthesis of collagen, which is primarily responsible for the strength of biological structures. Proteoglycans are macromolecular compounds that consist of genetically different core proteins containing oligosaccharides, attached by the N- and O-glycoside bonds, and covalently bound side chains of glycosaminglycans [16]. The sulfation of the chain part of the latter provides the binding of many active biomolecules such as growth factors, for example.

Involment of connective tissue as a stage of the pathogenesis of chronic intoxication with nitrates and fluorides is largely due to the ECM destruction by histolytic enzymes (hyaluronidase, chondroitin sulfatase, protease, glucuronidase, collagenase), proinflammatory cytokines and effector compounds – matrix metalloproteinases, plasmin, serine proteinases of

polymorphonuclear leukocytes, reactive oxygen and nitrogen species [5]. Previously, we have shown that the combined effect of sodium nitrite and sodium fluoride leads to the activation of the transcriptional nuclear factor kappa B (NF- $\kappa$ B), which controls the biosynthesis of proinflammatory and prooxidant factors, including inducible NO synthase [4]. The influence of NF- $\kappa$ B on the development of oxidative and nitrosative stress in various organs [4], the process of bone tissue remodeling [10] has also been proven.

According to the data reported previously, nanosized silica oxide is the most effective compound compared with other enterosorbents (suspension of hydrolysed lignin and activated carbon) as well as the most effective means of correction for parameters of oxidative and nitrosative stress in the blood of rats under chronic combined intoxication by sodium nitrate and sodium fluoride [2].

### **Conclusions**

Chronic nitrate-fluoride intoxication leads to the destruction of connective tissue components in different organs (gastric mucous membrane, femoral bones and vertebrae). Usage of nanosized silica oxide under conditions of chronic nitrate-fluoride intoxication reduces the destruction of connective tissue in these structures.

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