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EFFECT OF THE COMPLEX PROBIOTIC ON LIPID METABOLISM AND OXIDATIVE STRESS AFTER POISONING WITH NICKEL AGAINST THE BACKGROUND OF EXPERIMENTAL ATHEROSCLEROSIS

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The work studied the effect of the complex probiotic on the state of lipid metabolism and oxidative stress in white male rats after poisoning with nickel nitrate against the background of experimental atherosclerosis. After modeling atherosclerosis, rats were exposed to nickel nitrate for 30 days. In the experimental group, a month after poisoning, the animals received the complex probiotic, which was added to the drinking water of the drinkers for a month. It was found that when male rats were chronically poisoned with nickel nitrate after modeling atherosclerosis, there was a progressive increase in lipid metabolism disorders and oxidative stress processes. The use of a complex probiotic after poisoning with nickel nitrate improved lipid metabolism and oxidative stress. The obtained data show the important practical significance of probiotics in the treatment of nickel poisoning, especially in patients with atherosclerotic vascular damage.

Key words: atherosclerosis, lipid metabolism, oxidative stress, nickel, complex probiotic.

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ВПЛИВ КОМПЛЕКСНОГО ПРОБІОТИКА НА ЛІПІДНИЙ ОБМІН І ОКСИДАТИВНИЙ СТРЕС ПІСЛЯ ОТРУЄННЯ НІКЕЛЕМ НА ФОНІ ЕКСПЕРИМЕНТАЛЬНОГО АТЕРОСКЛЕРОЗУ

У роботі досліджено вплив комлексного пробіотика на стан ліпідного обміну та оксидативний стрес у білих щурів-самців після отруєння нітратом нікелю на фоні експериментального атеросклерозу. Після моделювання атеросклерозу щурів піддавали дії нітрату нікелю протягом 30 днів. У дослідній групі через місяць після отруєння тварини отримували комплексний пробіотик, який протягом місяця додавали до питної води. Встановлено, що при хронічному отруєнні самців щурів нітратом нікелю після моделювання атеросклерозу спостерігається прогресуюче наростання порушень ліпідного обміну та оксидативного стресу. Застосування пробіотика після отруєння нітратом нікелю покращувало ліпідний обмін та оксидативний стрес. Отримані дані свідчать про важливе практичне значення пробіотиків у лікуванні отруєнь важкими металами, особливо у пацієнтів з атеросклеротичним ураженням судин.

Ключові слова: щури, атеросклероз, ліпідний обмін, оксидативний стрес, нікель, пробіотик.

The modern environmental situation is characterized by an oversaturation of pollutants of various natures, the most common and dangerous of which are supertoxicants – heavy metals [5]. It has been shown that exposure to heavy metals is an important and underestimated risk factor for the development of atherosclerosis and its consequences [1]. Moreover, heavy metals also damage the intestinal microflora, which plays an important role in the elimination of heavy metals entering through the gastrointestinal tract.

Experimental studies have shown that exposure to low doses of the heavy metal cadmium through the food chain affects the intestinal microbiota, modifies it and disrupts the intestinal barrier, resulting in disruption of the metabolism of the intestinal microbiota [9].

At the turn of the 21st century, an idea was formed of the human intestinal microbiota as an important metabolic organ of the body, the state of which determines the health of not only the intestines, but also the entire body. There are a number of studies showing the role of gut microbiota in cardiovascular diseases. It has been established that when the microbiota is disrupted, trimethylamine-N-oxide, which is a proatherogenic compound, is produced from choline and phosphatidylcholine [2].

For the treatment of hyperlipidemia, statins are the first-line drugs, however, they given the many side effects (liver damage, muscle pain, digestive dysfunction, etc.), so currently underway search for non-statin, safer treatment options [11]. The scientific literature also shows that conventional chelating agents used for acute heavy metal toxicity have serious side effects and are unsuitable for subchronic and chronic heavy metal toxicity [8].

The use of probiotics is considered as a new strategy to prevent the toxicity of mercury [3], lead [7] and atherosclerosis [13].

In the literature of recent years, have appeared separate works showing the role of nickel, long-term exposure to which has genotoxic, hematotoxic, teratogenic, immunotoxic, carcinogenic and other pathogenic consequences [6]. However, there are no studies examining the effect of this toxicant on the course of atherosclerotic processes and the development of methods for their correction.

The purpose of the study was to establish the influence of nickel on atherosclerosis indicators of lipid metabolism and oxidative stress, and the possibility of correcting these disorders with the drugs containing symbiont microorganisms (probiotics).

Materials and methods. The studies were carried out on 40 nonlinear white male rats weighing 200–230 g. All experiments were carried out in accordance with the recommendations set out in the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986).

Animals were kept under natural light conditions and free access to water and food. The studies were carried out on 4 groups of 10 rats each: 1st group – healthy intact animals (control); Group 2 – animals with modeling atherosclerosis; The 3rd group included animals with experimental atherosclerosis, which were poisoned with nickel nitrate for 30 days, and the 4th group of animals, which, after poisoning with nickel against the background of experimental atherosclerosis, received the complex probiotic Symbiolact compositum for a month in dosage 8mg/kg.

The model of atherosclerosis was created according to Savitsky I.V. et al. [2016], which is based on the polyetiological theory of the development of atherosclerosis. The animals received the antithyroid drug mercazolil at a dosage of 25 mg/kg, the immunosuppressive drug methylprednisolone at a dosage of 0.17 mg/kg and a 15 % aqueous solution of ethyl alcohol with free access instead of water against the background of an atherogenic diet (1 % cholesterol, 20 % unsaturated and 20 % saturated fat) for 2 weeks.

After modeling atherosclerosis, rats were chronically exposed to nickel nitrate at a dose of 2 mg/kg through drinking water for 30 days. The stock solution of nickel nitrate was calculated using the equation A=(X*B)*C: where X is the coefficient = 6.77, B is the average weight of the rat, C is the average daily water consumption of the animals.

Lipid metabolism was monitored by the level of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) in the blood serum using a standard set of reagents on a biochemical analyzer Bio Screen MS-2000 (USA). For the integral characteristics of the lipid spectrum, the cholesterol atherogenic coefficient (CAC) was also calculated using the formula CAC =TC – HDL / HDL.

The processes of free radical oxidation were studied based on the level of malondialdehyde (MDA) using the method of Gavrilov V.P. [1987], diene conjugates (DC) according to L. Placer modified by Gavrilov V.P. [1983] and catalase (CA) according to the method of Korolyuk M.A. [1988]. Changes in indicators were monitored before and after modeling atherosclerosis and 30 days after poisoning and, accordingly, the use of a complex probiotic.

In our studies as a complex probiotic was used Symbiolact compositum, which was added to drinking water at a dose of 8 mg/kg for a month. Symbiolact compositum (Symbiopharm, Germany) contains several types of symbiont microorganisms: Lactobacillus acidophilus (2.0 x 10 CFU), Lactobacillus casei (2.0 x 10 CFU), Bidobacterium bidum (1.0 x 10 CFU), Bidobacterium lactis (1 .0 x 10 CFU), Lactococcus lactis (2.0 x 10 CFU), Lactobacillus salivarius (2 x 107).

At the end of the experiment, the animals were removed from the experiment by decapitation under diethyl ether anesthesia, followed by collection of whole blood for biochemical analyses.

The obtained digital results were processed using the programs "Microsoft Excel 2010", "BioStat 6.0", "Statistica 10.0". Group indicators were arranged in a variation series and for each group the arithmetic mean value (M) and its standard error (m) were determined, and the width of the 95 % confidence interval was also indicated. Due to the prevalence of parameters that had a non-normal distribution, statistical analysis of the study results was carried out using non-parametric analysis criteria: two independent groups were compared using the Mann-Whitney U test. The level of statistical significance was accepted in cases of at least $p \leq 0.05$.

Results of the study and their discussion. The results of studies of lipid metabolism after modeling atherosclerosis and intoxication with nickel nitrate are presented in Table 1. As can be seen from the table, modeling atherosclerosis led to statistically significant changes in the biochemical parameters of lipid metabolism.

Table 1

Biochemical parameters of lipid metabolism in the blood serum of rats after nickel poisoning against the background of experimental atherosclerosis (M±m)

Indicators	Before modeling atherosclerosis (n=10)	After modeling atherosclerosis (n=10)	30 days after poisoning (n=10)
TC mmol/l	1.86±0.16	2.39±0.34*	2.58±0.16*
LDL mmol/l	0.17±0.03	0.27±0.03*	0.35±0.03*
HDL mmol/l	1.33±0.13	1.11±0.09*	0.88±0.05*
TG mmol/l	0.43±0.056	0.47±0.065	0.54±0.061*
CAC	0.404±0.113	1.17±0.29*	1.95±0.27*

Note: * – $p \leq 0.05$ relative to data from intact animals.

The content of TC increased by 29.1 %, TG – by 10.3 % compared to the data in intact rats. LDL levels increased very significantly (by 60 %). These changes were accompanied by a statistically significant decrease in HDL content and an increase in CAC by almost 3 times relative to the values in animals in an intact state.

Subsequent chronic intoxication with nickel nitrate caused an increase in the intensity of lipid metabolism disorders. Thus, a month after priming, the TC content became higher than in intact animals by 38.7 %, and the LDL level increased more than 2 times compared to the data in intact rats. The TG content increased statistically significantly (by 27 %) compared to the data in control animals. The HDL level 30 days after poisoning with nickel nitrate against the background of experimental atherosclerosis was already 34.1 % lower, and CAC increased 4.8 times compared to data in intact animals.

These changes in lipid metabolism due to intoxication with nickel nitrate against the background of previous exposure indicate, in accordance with the above, the further development of atherosclerosis.

Data on the effect of modeling atherosclerosis and subsequent poisoning with nickel nitrate on indicators of oxidative stress are presented in Table 2.

Table 2

Biochemical indicators of oxidative stress in the blood serum of rats after nickel poisoning against the background of experimental atherosclerosis (M±m)

Indicators	Before modeling atherosclerosis (n=10)	After modeling atherosclerosis (n=10)	30 days after poisoning (n=10)
MDA mcmol/l	3.01±0.43	3.54±0.54*	4.8±0.22*
DC D232/ml	0.59±0.08	0.72±0.08*	0.8±0.04*
CA Mkt/l	12.8±1.39	11.3±1.42*	9.7±1.16*

Note: *– $p \leq 0.05$ when compared with data from intact animals.

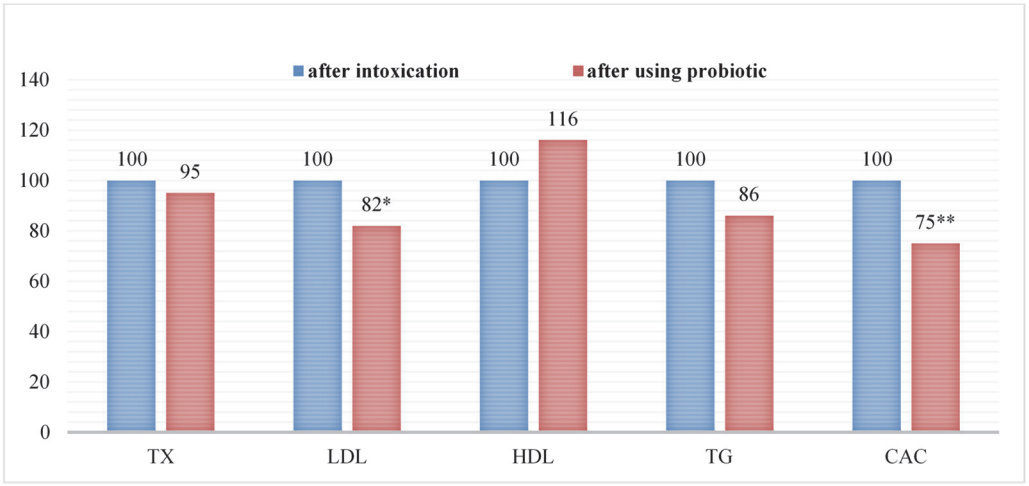
As can be seen from it, modeling of atherosclerosis caused a statistically significant increase in the levels of MDA and DC by 17.5 % and 19.7 %, respectively, compared with data from intact animals. At the same time, the CA level decreased statistically significantly by 12 %.

An increase in the levels of MDA and DC, as well as a decrease in the activity of CA, used as indicators of lipid peroxidation, indicate the development of oxidative stress due to the modeling of atherosclerosis. These results correlate with the data obtained on the same animals on the increase in the intensity of lipid metabolism, presented in the previous section. This serves as an additional argument in favor of the fact that the procedure used in the experiment caused a state of atherosclerosis in rats.

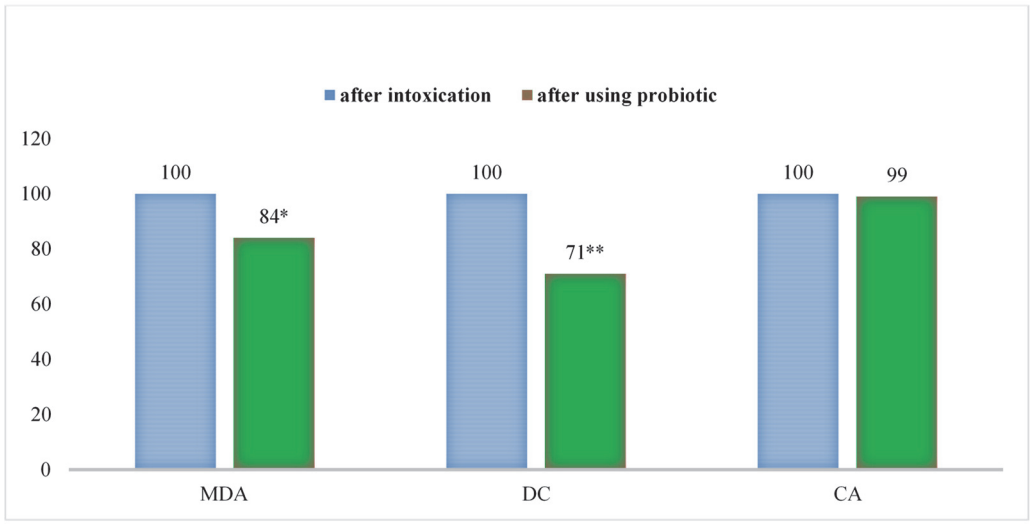
Subsequent poisoning with nickel nitrate after modeling atherosclerosis in experiments on animals of the third group caused an aggravation of disturbances in oxidative stress indicators that occurred after modeling atherosclerosis (Table 2). The level of MDA 30 days after poisoning significantly (by 59.6 %) increased compared to the data in intact animals, and the DC content increased by 33.4 % compared to the data in intact rats.

CA activity after a month of nickel poisoning against the background of experimental atherosclerosis was significantly reduced by 24.2 %. It is worth noting that similar changes occurred under these conditions and with the indicators of lipid metabolism in these same animals, as noted above.

Analysis of the effect on lipid metabolism and the state of oxidative stress of the complex probiotic Symbiolact compositum after intoxication with nickel nitrate against the background of experimental atherosclerosis showed the following (Fig. 1).



A



B

Fig. 1. Dynamics of changes in lipid metabolism indicators (A) and oxidative stress (B) after using the probiotic, *– $p \leq 0.05$ when compared with data before probiotic using.

Under the influence of the complex probiotic, the HDL content increased statistically significantly relative to the value both before its use and to the level in intact animals. CAC after application of Symbiolact compositum became less than after poisoning with nickel salt, although it remained higher than in intact rats. The values of TC, LDL and TG decreased relative to the values before the use of the probiotic, but did not reach a statistically significant level.

The levels of MDA and DC after the use of the complex probiotic compared to these indicators before its use decreased statistically significantly. CA activity was not significantly affected by the probiotic.

From the analysis of the obtained data, it follows that the use of the probiotic Symbiolact compositum counteracts lipid metabolism disorders and oxidative stress caused by the combined influence of experimental atherosclerosis and the heavy metal salt nickel.

Summarizing the obtained data, we can say that the revealed increase in the process of oxidative stress is obviously associated with the effect on the animals of the nickel salt used in the experiment. Many

studies have noted that heavy metals cause oxidative stress. In particular, it has been noted that oxidative stress plays a major and decisive role in nickel toxicity [6]. It is the oxidative stress caused by heavy metals that makes it a point of application for antioxidants to counteract their toxic effects, further supporting these effects. It should also be noted that our data are consistent with recent data, which show the important role of free radical lipid peroxidation in the etiology and progression of atherosclerosis [10]. Our data are consistent with other literature data on the lipid-lowering effect of certain strains of probiotic bacteria [4, 13] and can also increase the level of antioxidant properties [12]. It is likely that the use of the complex probiotic Symbiolact compositum, improving the diversity and taxonomic characteristics of the intestinal microbiota, plays an important role in preventing the synthesis of proatherogenic compounds in the intestine and restores the detoxifying role of the microbiota in the elimination of heavy metals with an increase in the body's antioxidant defense.

Conclusion

Summarizing the results of the studies, we can say that the creation of experimental atherosclerosis and subsequent intoxication with nickel nitrate causes a progressive increase in lipid metabolism disorders and oxidative stress processes in rats. This was evidenced by an increase in the level of lipid metabolism indicators such as total cholesterol, low-density lipoproteins, triglycerides, cholesterol atherogenicity coefficient and a decrease in the content of high-density lipoproteins. Levels of the oxidative stress indicators malondialdehyde and diene conjugates also continued to increase, with a parallel decrease in serum catalase activity.

The complex probiotic Symbiolact compositum had a positive effect under the combined influence of experimental atherosclerosis and nickel salt. The probiotic reduced the severity of changes in lipid metabolism and oxidative stress: against its background, the content of high-density lipoproteins increased and the cholesterol atherogenic coefficient decreased, as well as the content of malondialdehyde and diene conjugates.

The obtained data show the feasibility of using probiotics in the complex treatment of chronic nickel poisoning through drinking water, which is especially important for patients with atherosclerotic vascular damage.

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