R.I. Ibragimov, N.I. Ibragimova
Azerbaijan Medical University, Baku, Azerbaijan

INFLUENCE OF COMBINED USE OF PROBIOTIC AND ANTITOXICANT ON LIPID AND HEMATOLOGICAL STATUS AFTER NICKEL INTOXICATION IN THE BACKGROUND OF EXPERIMENTAL ATHEROSCLEROSIS

e-mail: iri-71@mail.ru

Metal exposure is a recently recognized risk factor for cardiovascular disease and is associated with the progression of atherosclerosis. There are data on the biotoxic effect of heavy metals on the hematological status and intestinal microbiota. In this study was studied the effectiveness of the combined use of a probiotic and a plant antitoxicant on the state of lipid and hematological status in conditions of chronic nickel poisoning against the background of experimental atherosclerosis. The results of the study showed that the experimental therapy reduced the severity of disturbances in the lipid and hematological status: the content of high-density lipoproteins increased and the atherogenicity index significantly decreased, as well as a tendency to normalize the level of reduced hemoglobin and mid-cell hemoglobin in erythrocytes. The data obtained show the feasibility of combined use of a probiotic and a plant antitoxicant in the complex therapy of chronic nickel poisoning through drinking water, which is especially important for patients with atherosclerotic vascular lesions.

Key words: lipid and hematological status, nickel, experimental atherosclerosis, probiotic, antitoxicant.

Р.І. Ібрагімов, Н.І. Ібрагімова

ВПЛИВ ОДНОЧАСНОГО ВИКОРИСТАННЯ ПРОБІОТИКА ТА АНТИТОКСИКАНТУ НА ЛІПІДНИЙ І ГЕМАТОЛОГІЧНИЙ СТАТУС ПІСЛЯ ІНТОКСИКАЦІЇ НІКЕЛЕМ НА ФОНІ ЕКСПЕРИМЕНТАЛЬНОГО АТЕРОСКЛЕРОЗУ

Вплив металів – нещодавно визнаний фактор ризику серцево-судинних захворювань та пов'язаний з прогресуванням атеросклерозу. Є дані про біотоксичну дію важких металів на гематологічний статус та мікробіоту кишечника. У цьому дослідженні вивчено ефективність спільного застосування пробіотика та рослинного антитоксиканту на стан ліпідного та гематологічного статусу в умовах хронічного отруєння нікелем на фоні експериментального атеросклерозу. Результати дослідження показали, що експериментальна терапія зменшила вираженість порушень у ліпідному та гематологічному статусі: збільшився вміст ліпопротеїдів високої щільності та відбулося значне зниження індексу атерогенності, а також проявилася тенденція до нормалізації у рівні зниженої концентрації гемоглобіну та середньоклітинного гемоглобіну в еритроцитах. Отримані дані показують доцільність спільного використання пробіотика та рослинного антитоксиканту у комплексній терапії хронічних отруєнь нікелем через питну воду, що особливо важливо для пацієнтів з атеросклеротичними ушкодженнями судин. Ймовірно, додавання пробіотика через регуляцію функціональної активності та різноманітності мікробіоти кишечника на тлі рослинного антитоксиканту при хронічному отруєнні нікелем на тлі атеросклерозу може бути іншим напрямком регулювання ліпідного обміну та гематологічних порушень.

Ключові слова: ліпідний та гематологічний статус, нікель, експериментальний атеросклероз, пробіотик, антитоксикант.

Studies have noted that exposure to heavy metals, a recently recognized risk factor for cardiovascular disease, is associated with the progression of atherosclerosis [11, 13]. Clinical studies have shown that body levels of lead and cadmium are associated with differential circulating lipid profiles [7], positive correlations were found between lead and cadmium levels and total cholesterol levels and low-density lipoproteins in e-waste recycling workers [5].

In the literature of recent years, there are separate works on the toxic effect of such a metal as nickel, long-term exposure to which has a genotoxic, hematotoxic, teratogenic, immunotoxic and carcinogenic effect [4]. However, it was not possible to identify works on its effect on the course of atherosclerotic processes and on hematological disorders in atherosclerosis.

In recent years, scientific research has also included separate works on the biotoxic effect of various heavy metals on hematological status. The influence of lead and cadmium has been analyzed mainly, and there is also separate information on the influence of copper, mercury, and arsenic. A decrease in hemoglobin and erythrocytes has been shown among both children [3] and adults [10, 15] in certain regions of various countries where there is environmental pollution. Experimental studies have shown that when exposed to heavy metals (arsenic, lead, cadmium, copper), hematological changes mainly demonstrate a decrease in the number of certain formed elements of the blood, as well as hemoglobin [12].

Literature data indicate that metal intake can also affect the intestinal microbiota, changing its diversity and composition, which in turn leads to changes in gene expression, changes in metabolism, immunity, neurological dysfunction and causes various other disorders and contributes to the development or progression of various diseases [6], including atherosclerosis [9].

The above data convincingly indicate a link between atherosclerotic diseases and exposure to heavy metals, which in turn cause changes in hematological status and intestinal microbiota. It should also be noted that data on the effectiveness of chelation therapy, which is currently the main method for removing heavy metals, in reducing cardiovascular events are contradictory [8].

Based on the above, it seemed interesting to study the effect of chronic nickel poisoning on the course of atherosclerotic processes, as well as to develop correction methods to reduce the risk of developing complications of atherosclerosis, which is of great importance for older age groups of the population. The study of the effect of safe drugs, namely probiotics and herbal remedies for the prevention and treatment of chronic poisoning with heavy metals seems to be a very important and promising direction in medicine.

The purpose of the study was to investigate the combined use of a probiotic and an antitoxicant on the state of lipid metabolism and hematological parameters in conditions of chronic nickel poisoning against the background of experimental atherosclerosis.

Materials and methods. The experiments were carried out on 60 non-linear white male rats weighing 200–250g and were conducted in accordance with the recommendations set out in the "European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes" (Strasbourg, March 18, 1986) and approved by the Ethics Committee of the Azerbaijan Medical University.

The studies were conducted in 4 series of 10 animals each. Group 1 – intact animals; Group 2 – animals with experimental atherosclerosis; Groups 3 and 4 – animals with experimental atherosclerosis exposed to nickel nitrate intoxication, respectively, for 15 and 30 days; Group 5 – where rats after a month of nickel nitrate intoxication against the background of experimental atherosclerosis received a probiotic and a plant antitoxicant.

Experimental atherosclerosis (EA) was created according to I.V. Savitsky et al. (2016) [14] and is based on the multifactorial theory of atherosclerosis development and its consequences. Rats received mercazolil – 25 mg/kg, methylprednisolone – 0.17 mg/kg and 15 % aqueous ethyl alcohol solution in free access instead of water against the background of an atherogenic diet (1 g cholesterol, 20 g corn and 20 g cow oil) for 2 weeks. After 15 days were studied lipid metabolism for confirming atherosclerotic changes.

After modeling atherosclerosis, the animals were exposure to nickel intoxication at a dose of 2 mg/kg through drinking water. 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 mean daily water consumption of the animals) and was added to the drinking water of the drinkers.

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

The study of hematological parameters (complete blood count) was performed on an automatic hematological analyzer RT-7600 (Germany). The hematological status was monitored using the following parameters: white blood cell count (WBC); granulocyte count (GR); lymphocyte count (LYM); red blood cell count (RBC); hemoglobin concentration (HGB) and mean cell hemoglobin (MCH).

Considering that the use of probiotics is considered a very promising direction for the removal of heavy metals, through bioaccumulation, binding or transformation of heavy metals through various enzymatic reactions [1, 2], in our studies was used the probiotic 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 symbiotic microorganisms (Lactobacillus acidophilus (2.0x10 CFU), Lactobacillus casei (2.0x10 CFU), Bidobacterium bidum (1.0x10 CFU), Bidobacterium lactis (1.0x10 CFU), Lactococcus lactis (2.0x10 COE), Lactobacillus salivarius (2 x 107)

A complex plant antitoxicant consisting of a mixture of licorice, rosehip, grape seed, oat and burdock bran in a ratio of 3:2:1:1:2 (Eurasian patent 201600043 dated 06/25/2018) was also added to drinking water in drinkers at a dose of 8 mg/kg for a month.

The processing of the obtained digital results was carried out using the programs "Microsoft Excel 2010", "Statistica 10.0". Comparison of mean values in samples was performed using the nonparametric Mann-Whitney U-test. The critical level of significance was accepted in cases of at least $p \le 0.05$.

Results of the study and their discussion. The results of the studies of the lipid profile and hematological indices after intoxication with nickel nitrate against the background of experimental atherosclerosis and, accordingly, after complex therapy with the combined use of a probiotic and a plant antitoxicant showed the following changes. Digital data on changes in lipid metabolism during intoxication with nickel nitrate and, accordingly, after the said complex therapy are presented in Table 1.

Table 1

Shown lipid metabolism in the blood serum of rats with the combined use
of a probiotic and an antitoxicant after nickel intoxication against the background of EA (M±m, n=10)

Indices	Intact state	Modeling of EA	15 days of intoxication against the background of EA	30 days intoxication against the background of EA	30 days after treatment
OX mmol /l	1.86±0.16	2.4±0.34 P=0.000	2.42±0.21 P=0.000	2.58±0.16 P=0.000	2.1±0.15 P=0.006
LDL mmol /l	0.17±0.03	0.27±0.03 P=0.000	0.3±0.02 P=0.000	0.35±0.03 P=0.000	0.19±0.02 P=0.048
HDL mmol /l	1.33±0.13	1.11±0.09 P=0.000	0.95±0.11 P=0.000	0.88±0.05 P=0.000	1.19±0.08 P=0.037
TG mmol / 1	0.43±0.06	0.47±066 P=0.183	0.49±0.062 P=0.069	0.54±0.06 P=0.000	0.409±0.06 P=0.762
CAI	0.4±0.11	1.17±0.29 P=0.000	1.57±0.27 P=0.000	1.95±0.27 P=0.000	0.75±0.21 P=0.000

Note: P – compared to data from intact animals.

As can be seen from the table, modeling of atherosclerosis led to significant changes in the biochemical parameters of lipid metabolism. The content of TC statistically significantly increased by 29 %, TG by 10 %, and LDL by 60 % compared to the data of intact rats. In parallel, a statistically significant decrease in the content of HDL by 16 % and an increase in CAI by almost 3 times compared to the parameters of animals in the intact state were noted.

Subsequent chronic intoxication with nickel nitrate for a month contributed to a gradual worsening of the intensity of lipid metabolism disorders. Thus, on the 15th day of intoxication against the background of EA, the level of LDL increased quite significantly – already by 81 %, and this was already 21 % more than after modeling atherosclerosis. The content of HDL continued to decrease and was less than in intact animals by 28 %, which was 11 % less in comparison with the data with EA. Accordingly, CAI increased almost 4 times in comparison with the data of intact animals, and this was more than with EA by 99 %.

One month after nickel intoxication with nickel against the background of EA, the TC content was already 39 % higher than in intact animals, and the LDL level increased more than 2 times relative to the data of intact rats, which, compared with the values after modeling atherosclerosis, was 10 % and 48 % higher, respectively. The TG level was 27 % higher than in intact animals, which was 17 % higher than in EA. The HDL content 30 days after nickel nitrate intoxication against the background of EA was already 34.1 % lower, which was 18 % lower than after modeling atherosclerosis. CAI in the specified period increased 4.8 times compared with the data of intact animals, and was 194 % higher than in EA. The obtained data indicate that exposure to nickel nitrate aggravated lipid metabolism disorders and contributed to the further progression of atherosclerotic processes.

Results of hematological studies after intoxication with nickel nitrate against the background of EA and, accordingly, after complex therapy with the combined use of a probiotic and a plant antitoxicant showed the following changes. Digital data of hematological changes during intoxication with nickel nitrate and, accordingly, after the said complex therapy are shown in Table 2.

Table 2

Hematological parameters of rats with combined use of a probiotic
and an antitoxicant after nickel intoxication against the background of EA (M±m, n=10)

Indices	Intact state	Modeling of EA	15 days of intoxication against the background of EA	30 days of intoxication on the background of EA	30 days after treatment
WBC (10^9/L)	6.67±0.94	11.74±0.91 P=0.000	7.55±0.65 P=0.037	6.15±0.6 P=0.211	6.12±0.6 P=0.130
LYM (10^9/L)	4.33±0.66	5.55±0.22 P=0.001	4.69±0.34 P=0.049	4.36±0.48 P=0.970	4.47±0.35 P=0.595
GRA (10^9/L)	6.16±0.69	6.83±0.69 P=0.064	5.46±0.95 P=0.054	4.87±0.52 P=0.00 0	5.651±0.56 P=0.130
RBC (10^ 12 /L	5.63±0.67	6.376±0.48 P=0.031	6.1±0.59 P=0.173	5.77±0.41 P=0.650	5.19±0.53 P=0.120
HGB (g/L)	135.5±10.74	115.4±7.92 P=0.000	110.4±5.19 P=0.000	104.3±6.63 P=0.000	117.8±13.4 P=0.008
MCH (pg)	29.18±1.69	29.18±2.37 P=1.000	24.8±2.17 P=0.000	22.3±2.05 P=0.000	26.1±2.13 P=0.007

Note: * – p≤ 0.05 when compared to data from intact animals.

Thus, after modeling atherosclerosis, the most significant and reliable increase was in the number of WBC – by 76 %, LYM by 28 % compared to the data of intact animals. The number of GRA, RBC increased insignificantly, but the average content of HGB was lower by 15 % compared to the data of intact rats. Such an imbalance between the number of RBC and the content of HGB after modeling atherosclerosis indicated relative erythrocytosis associated most likely with an increase in blood viscosity.

After 15 days from the start of nickel intoxication against the background of EA, the following picture was observed. The increased number of formed elements of the blood compared to the data after modeling atherosclerosis began to decrease. The number of WBC decreased by 53 %, LYM and GRA decreased by 20 % and 21 %, respectively. The number of RBC on the 15th day of intoxication changed insignificantly, but MCH decreased more significantly and was 15 % less than after modeling atherosclerosis, which already indicated qualitative changes in erythrocytes.

The data of the hematological status on the 30th day of nickel intoxication against the background of EA indicated a progressive decrease in the number of formed elements. Thus, the number of WBC, LYM and GRA, compared with the data after modeling atherosclerosis, significantly decreased by 84 %, 27 % and 32 %, respectively. The content of HGB and MCH at this time of the study were lower than with EA by 8 % and 24 %, respectively.

Thus, the dynamics of hematological changes observed after chronic nickel nitrate intoxication for a month against the background of EA revealed an aggravation of hematological disorders that occurred after modeling atherosclerosis. The above data indicate a pathogenetic role of chronic nickel nitrate poisoning in aggravating disorders of both lipid and hematological status.

Summarizing the obtained data on the toxic effect of nickel on the lipid and hematological status indicators against the background of experimental atherosclerosis, it can be said that our results are consistent with the data of other researchers who have shown a pathogenetic relationship between intoxication with various other heavy metals and the development of hematological changes [12]. It is known that heavy metals entering the body through the gastrointestinal tract, changing its microbiota, contribute to the aggravation of the course of various pathologies [6], including atherosclerosis [9], which we also observed in our studies. It can be concluded that chronic intoxication with heavy metals, including nickel, has an aggravating effect on the course of pathological processes, that is, the toxic effect on the lipid and hematological profile are important pathogenetic mechanisms of action of heavy metals.

Analysis of the effect on lipid metabolism and hematological parameters of the combined use of the probiotic Symbiolact compositum and plant antitoxicant after intoxication with nickel nitrate against the background of EA showed the following. Under the influence of the treatment, the HDL content statistically significantly increased by 24 % relative to the value before its use. The values of TC, LDL and TG relative to the values before the use of the combined treatment decreased by 26 %, 86 % and 31 %, respectively. CAI after the treatment decreased almost 3 times compared to the data for 30-day intoxication with nickel nitrate.

The results of the studies on hematological changes after complex experimental therapy showed that further decrease in the number of formed elements of the blood was prevented. Thus, the number of GRA in comparison with 30 days after the challenge increased on average by 12 %, HGB – by 10 % and MCH – by 13 %, respectively.

Summarizing the results of the conducted studies, it can be said that nickel nitrate intoxication in rats with EA has an aggravating effect on the course of atherosclerotic processes: the level of TC, LDL, TG and CAI increases with a parallel decrease in HDL. The progressive increase in lipid metabolism disorders is accompanied by an increase in hematological disorders. The number of WBC and RBC in relation to the data after modeling atherosclerosis significantly decreased, but a more significant and reliable decrease was observed in the amount of GRA, as well as the content of HGB and MCH.

Combined use of the probiotic Symbiolact compositum and complex plant antitoxicant showed a positive effect. The applied experimental therapy reduced the severity of disturbances in lipid and hematological status. An increase in HDL content and a significant decrease in HIA were observed, as well as a tendency toward normalization in the level of HGB and MHC.

The obtained data show the feasibility of the combined use of a probiotic and a plant antitoxicant in the complex therapy of chronic heavy metal poisoning through drinking water, which is especially important for patients with atherosclerotic vascular lesions. Probably, the addition of a probiotic through the regulation of the functional activity and diversity of the intestinal microbiota, against the background of a plant antitoxicant in chronic nickel poisoning against the background of EA may be another direction for regulating lipid metabolism and hematological disorders. The effectiveness of probiotics, which is a

promising direction in medicine for the removal of heavy metals [1, 2], can be increased by adding herbal remedies, the action of which can be explained by prebiotic and antioxidant action, enhancing the effect of probiotics.

Conclusions

1. After intoxication rats with EA nickel nitrate, a significant worsening of disturbances in lipid and hematological status is observed.

2. The combined use of the probiotic Symbiolact compositum and plant antitoxicant after intoxication with nickel nitrate against the background of EA had a pronounced corrective effect on disturbances in lipid and hematological status.

3. The combined use of a probiotic and a plant antitoxicant may be another direction for regulating lipid metabolism and hematological disorders in chronic heavy metal poisoning, especially in patients with atherosclerotic vascular lesions.

References

1. Arun KB, Madhavan A, Sindhu R, Emmanual S, Binod P, Pugazhendhi A et al. Probiotics and gut microbiome – Prospects and challenges in remediating heavy metal toxicity. J Hazard Mater. 2021; 420:126676. doi: 10.1016/j.jhazmat.2021.126676.

2. Bhattacharya S. An Insight into the Salutary Prospect of the Probiotic Microorganisms for the Remediation of Mercury Toxicity. J Environ Pathol Toxicol Oncol. 2023; 42(3):83–90. doi: 10.1615/JEnvironPatholToxicolOncol.2023047451.

3. Capitão C, Martins R, Santos O, Bicho M, Szigeti T, Katsonouri A et al. Exposure to heavy metals and red blood cell parameters in children: A systematic review of observational studies. Front Pediatr. 2022; 6(10):921239. doi: 10.3389/fped.2022.921239.

4. Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. Nickel: Human Health and Environmental Toxicology. Int J Environ Res Public Health. 2020;17(3):679. doi: 10.3390/ijerph17030679.

5. Igharo OG, Akinfenwa Y, Isara AR, Idomeh FA, Nwobi NL, Anetor JI, et al. Lipid profile and Atherogenic Indices in Nigerians Occupationally Exposed to e-waste: A Cardiovascular Risk Assessment Study. Maedica (Bucur). 2020;15(2):196–205. doi: 10.26574/maedica.2020.15.2.196.

6. Kaur R, Rawal R. Influence of heavy metal exposure on gut microbiota: recent advances. J Biochem Mol Toxicol. 2023;37(12): e 23485. doi: 10.1002/jbt.23485.

7. Kim DW, Ock J, Moon KW, Park CH. Association between heavy metal exposure and dyslipidemia among Korean adults: from the Korean National Environmental Health Survey, 2015–2017. Int J Environ Res Public Health. 2022;19: 3181.doi: 10.3390/ijerph19063181.

8. Lamas GA, Anstrom KJ, Navas-Acien A, Boineau R, Nemeth H, Huang Z et al. Edetate Disodium-Based Chelation for Patients with a Previous Myocardial Infarction and Diabetes: TACT2 Randomized Clinical Trial. JAMA. 2024;332(10):794–803. doi: 10.1001/ jama. 2024.11463.

9. Lindskog Jonsson A, Caesar R, Akrami R, Reinhardt C, Fåk Hållenius F, Borén J, et al. Impact of gut microbiota and diet on the development of atherosclerosis in Apoe −/− mice. Arterioscler Thromb Vasc Biol. 2018; 38(10): 2318–2326.doi: 10.1161/ATVBAHA.118.311233.

10. Manjarres-Suarez A, Olivero-Verbel J. Hematological parameters and hair mercury levels in adolescents from the Colombian Caribbean. Environ Sci Pollut Res Int. 2020;27(12):14216–14227. doi: 10.1007/s11356-020-07738-z.

11. McGraw K, Schilling K, Glabonjat R, Galvez-Fernandez M, Domingo- Relloso A, Martinez-Morata I et al. Urinary Metal Levels and Coronary Artery Calcification: Longitudinal Evidence in the Multi-Ethnic Study of Atherosclerosis. J Am Coll Cardiol. 2024 ;84(16):1545–1557. doi: 10.1016/j.jacc.2024.07.020.

12. Nikolić R, Krstić N, Jovanović J, Kocić G, Cvetković TP, Radosavljević-Stevanović N. Monitoring the toxic effects of Pb, Cd and Cu on hematological parameters of Wistar rats and potential protective role of lipoic acid and glutathione. Toxicol Ind Health. 2015; 31(3):239–246. doi.org/10.1177/0748233712469652.

13. Patwa J, Flora SJS. Heavy Metal-Induced Cerebral Small Vessel Disease: Insights into Molecular Mechanisms and Possible Reversal Strategies. Int J Mol Sci. 2020;21(11):3862. doi: 10.3390/ijms21113862.

14. Savitskiy IV, Sliusar AA, Miastkovskaja IV. Multifactorial modeling of atherosclerosis in rats. Journal of Education, Health and Sport. 2016; 6(3):233–240. doi: http://dx.doi.org/10.5281/zenodo.55402.

15. Soltani N, Sadeghi T, Mahmoodi MR, Saadloo M, Baneshi MR, Rajabi Z, et al. The biotoxic effects of heavy metals exposure in miners and non-miners Trace Elem Med. Biol. 2024; 84:127423. doi: 10.1016/j.jtemb.2024.127423.