EXPERIMENTAL MODELS AND INSTRUMENTAL SURVEYS FOR RISK ASSESSMENT IN HYGIENE AND EPIDEMIOLOGY

UDC 577.352.3
DOI: 10.21668/health.risk/2016.3.07

MECHANISMS OF ENDOTHELIUM FUNCTIONS AND INTERNAL ORGANS DISORDERS UNDER EXPOSURE TO COBALT CHLORIDE (EXPERIMENTAL RESERACH)

Gigolaeva L.V.[1,2], Tibilov A.G.[2]

[1]Severo-Ossetinskaya State Medical Academy RF Ministry of Public Health, 40, Pushkinskaya str., Vladikavkaz, Severnaya Ossetia-Alania Republic, 362019, Russian Federation
[2] Federal Service for Surveillance over Consumer Rights Protection and Human Well-being Severnaya Ossetia-Alania Republic regional office, 17a, Telmana str., Vladikavkaz, Severnaya Ossetia-Alania Republic, 362021 Russian Federation

When cobalt gets into a human body it can become a risk factor causing pulmonary and cardiovascular health disorders. In this work we present the results of our research which was dedicated to functional and biochemical mechanisms of endothelial dysfunction and internal pathologies evolvement under cobalt intoxication during the experiment.

We detected system-organ character of oxidation processes activation as per data showing increase in secondary product malonic dialdehyde in erythrocytes and internal organs homogenates, as well as contribution of anti-oxidation system imbalance into lipid peroxidation development. We also studied disorders of NO-forming endothelium function and participation of L-arginine and -L–N^G- arginine being an analogue of eNOS expression endogenic inhibitor, methyl ether (L-NAME or L-nitro-arginine-methyl-ester) in this process under cobalt intoxication when oxidation processes are activated. When rats are under chronic cobalt intoxication, it leads to oxidation processes activation in them; here superoxide dismutase activity is depressed, and catalase and ceruloplasmin concentrations increase. We detected disorders in cholesterol metabolism, nitrogen oxide production and its biological availability, and such disorders were combined with changes in micro-circulatory hemodynamics of visceral organs.

We assessed functional state of internal organs as per data related to Na^+,K^+-ATP-ase activity in homogenates, as well as to the activity of organic-specific and excretory enzymes in blood serum under cobalt intoxication. We were able to detect the role of changes in cholesterol metabolism, namely atherogenesis, being a risk factor causing poor nitrogen oxide biological availability. To correct the detected disorders pathogenetically, we used coenzyme Q_{10}as endogenic antioxidant and regulators of eNOS expression, namely L-arginine, L-NAME, as well as their combinations with coenzyme Q_{10}.

Keywords: *atherogenesis, nitrogen oxide, lipid peroxidation, anti-oxidation system, micro-circulatory hemodynamics, cobalt chloride, cholesterol.*

Introduction. Systematic monitoring of environmental factors carried out by Rospotrebnadzor regional office in Severnaya Ossetia-Alania republic, detected that heavy non-ferrous metals salts were contained in soils in Vladikavkaz in quantities significantly higher than hygienic standards. [5, 8]. A share of soil samples not conforming to hygienic standards as per heavy metals salts content amounts to 70%, taken in dynamics over many years of monitoring. According to the data of specific production emissions analysis in the republic, cobalt compounds weight being emitted into the atmosphere occupies the 3rd place after lead and cadmium. Non-ferrous metallurgy enterprises are the main pollutants that emit this toxicant into environment.

Toxicity of high cobalt concentration can be explained through its hypoxic effect, lipid peroxidation activation and antioxidant protection depletion (APD) [1,2,7,8]. Excessive introduction of

Gigolaeva Larisa Vladimirovna – Candidate of Medical Sciences, Head of the social and hygienic monitoring department (e-mail: gigolaevalarisa@mail.ru, tel.: +7 (867) 258-14-34).

Tibilov Alan Germanovich – Director (e-mail: ses@osetia.ru; tel.: +7 (867) 251-90-30).

cobalt into a body induces lipid peroxidation and causes oxidizing stress evolvement, combined with endothelium dysfunction, damage to biological macromolecules, cells membrane structures and enzymes [8,17]. As endothelium is exposed to heavy non-ferrous metal salts, it becomes the primary target of their impact, and endothelium dysfunction plays pathogenetic role in damage done to internal organs cells membranes under oxidizing stress. Lower production and biological availability of nitrogen oxide, which is the basic vasodilatating factor, makes the greatest contribution into the mechanism of endothelium dysfunction evolvement under oxidation processes activation [2,6,8,12–16]. Changes in cholesterol metabolism as atherogenesis risk factor can make for disorders in NO transportation into unstriped muscle cell of a vessel (Metelskaya, 2005) [6].

Peters K. et al [16] showed in their research that cobalt divalent ions (Co2 +) induce expression of genes reacting with hypoxia. Due to this fact cobalt compounds can be even used to imitate hypoxia [15]. Then, imbalance in oxidation-anti-oxidation system is a significant risk factor causing pulmonary structures pathologies, bronchopulmonary dysplasia, for example (Goryunov, Gioev, 2009) [2].

High cobalt dozes penetrating a body via air passages at hazardous productions lead to higher content of cholesterol, beta-lipoproteins and crude lipids in blood, but lecithin-cholesterol coefficient reduces [17].

But still we haven't been able to find sufficient data on the influence exerted by oxidation processes on nitrogen oxide metabolism as risk factor causing vascular complications evolvement under cobalt intoxication, where endothelium function disorder becomes a pathogenetic component. Changes in metabolism of nitrogen oxide as a significant vasodilatating factor undoubtedly contribute into mechanisms of endothelium dysfunction evolvement. And we consider studying cause-and-effect relations of oxidation processes activation in nitrogen oxide metabolism at vascular complications and working out pathogenetic correction mechanisms to be a vital scientific task.

Taking all the above-stated into account, we defined our research goal as examining impact exerted by cobalt chloride on biochemical parameters of endothelium dysfunction in rats in the course of experiment with cobalt intoxication.

Data and methods. We accomplished our research on 275 "Vistar" rat bucks from the same age group (10-14 months), with body weight equal to 175-220 grams. Experimental rats were divided into 10 groups, 15-20 rats in each. Animal maintenance and experiments were carried out in full conformity with "Rules of work execution with the use of experimental animals" (USSR Ministry of Education, 1984), as well as Helsinki declaration principles (2000) and international recommendations (Ethic code) on accomplishing medical and biological research with the use of experimental animals (International scientific organizations council, 1985). The focus group N1 was made up of intact animals (n=22, comparable with the basic group as per age and body weight), 55 rats were included into the reserve group. We modeled cobalt intoxication of rats from experimental groups via cobalt chloride introduction in a dose equal to 6mgr/kg of an animal body weight (assuming DL_{50}). That dose was optimal (selected as per decrease rate) as it didn't cause animal death but still could lead to relevant changes in examined processes. We observed toxic effects development for 30 days. During the experiment rats received standard nutrition, had free access to drinking water and were in natural light conditions.

We used the following parameters in our experiment: lipid peroxidation intensity in erythrocytes membranes as per data related to changes in malonic dialdehyde concentration obtained via colorimeter with the use of tiobarbituric acid [1]; we assessed anti-oxidation system state as per catalase activity [4] and superoxide dismutase activity according to adrenaline auto-oxidation technique; and we applied Ravin technique to assess ceruloplasmin concentration in blood serum [3]. We used the value of crude cholesterol concentration in blood serum and its content in low-density lipoproteins and high-density lipoproteins to detect disorders in cholesterol metabolism. We determined total NO_X metabolites concentration via Griess diazotization reaction [2]. .

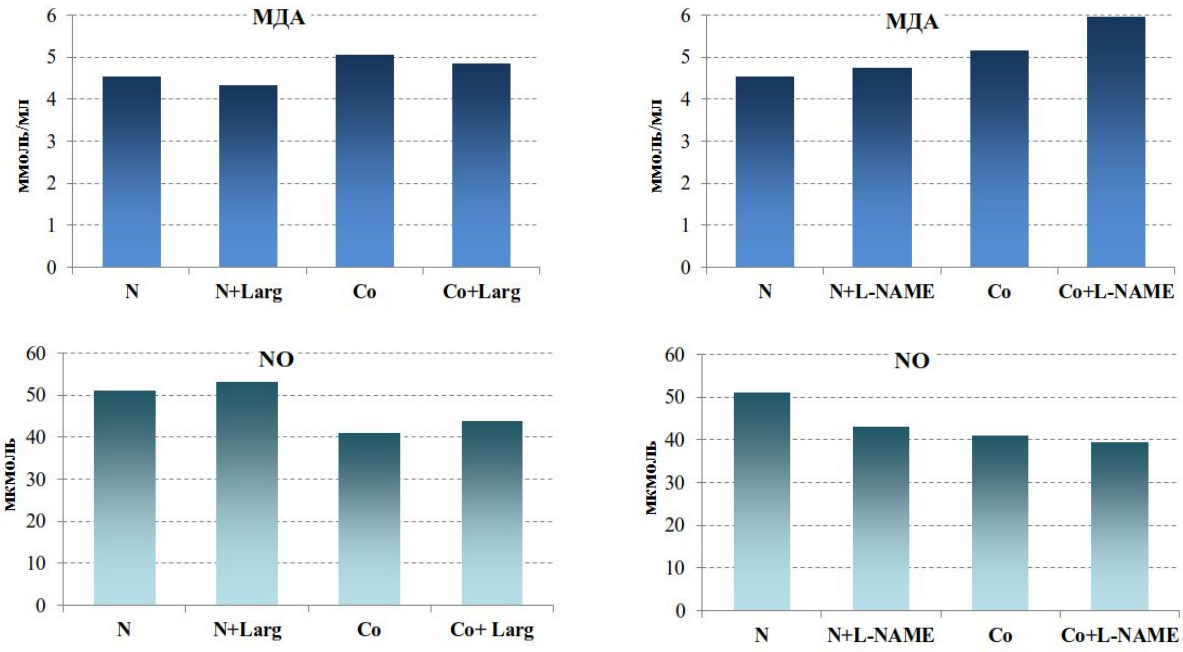
Results and discussion. System oxidation processes became apparent in experimental rats under long-term cobalt intoxication induced by parenteral introduction of cobalt chloride. We detected increased malonic dialdehyde concentration not only in erythrocytes, but also in renal tissue homogenates: 212% increase in cortical layer and 92% increase in medulla ($p<0.001$); 48,3% increase in hepatic tissue and 52.1% increase in myocardial tissue ($p<0.001$). As we studied correlations between cobalt content in blood serum and lipid peroxidation activity in erythrocytes, we determined direct corre-

lation between these two parameters at cobalt poisoning lasting for a month. Analysis of antioxidation system enzymes activity showed authentically ($p<0.001$) lower superoxide dismutase activity in blood serum and erythrocytes (by 27.7%), higher catalase activity (by 64.1%) and ceruloplasmin concentration (by 11.9%), which should be considered as a possible occurrence of cellular compensatory reaction.

As oxidation processes were activated under cobalt intoxication of the rats total NO metabolites concentration decreased in them by 19.7% ($p<0.001$) but malonic dialdehyde (a lipid peroxidation product) concentration increased by 10.9% (figure 1). To confirm these chemical factors' contribution, we accomplished correlation analysis which detected negative strong relation between increase in malonic dialdehyde concentration in blood and decrease in total NO metabolites concentration ($r = - 0.72$).

L-arginine concentration is the key component in the chain of mechanisms leading to NO deficiency evolvement and endothelium dysfunction (BogerR.H., Bode-BogerS.M., 2001) [9, 10] . To reveal the role of L-arginine substrate

availability for NO-synthase enzyme, we accomplished a specific experimental series introducing L-arginine in the dose equal to 10 mgr/kg of body weight into rats with cobalt intoxication daily during 4 weeks. As the experiment was over we determined nitrogen oxide concentration in blood and parameters of oxidation processes activation. The obtained results revealed higher NO concentration in blood after L-arginine introduction into rats with cobalt intoxication in comparison to the data obtained for cobalt intoxication only ($p<0.001$). L-arginine introduction into rats being under long-term exposure



1.control; 2. N+L-arginine;
3.Co; 4. Co+L-arginine

a)

1.control; 2. N+L-NAME;
3. Co; 4. Co+L-NAME

b)

Figure. 1. Changes in malonic dialdehyde concentration and total NO metabolites concentration against the background of NO-L-arginine donor (a) and eNOS – L-NAME inhibitor under cobalt intoxication (b).

to cobalt chloride led to lower manifestation degree for imbalance in "free radical oxidation - antioxidant protection" system and to simultaneously higher concentration of total NO metabolites in blood serum. Introduction of eNOS–L-NAME inhibitor into intact poisoned rats showed that lipid peroxidation metabolites content increased with simultaneous reduction in total NO metabolites concentration.

The detected free radical oxidation activation after introducing eNOS – L-NAME inhibitor into intact and especially poisoned rats can be caused by so called "disjunction" of eNOS reductase and oxi-

dase domains when an enzyme produces active oxygen forms instead of NO. Consequently, NO concentration in blood serum can be determined by concentration of L-arginine synthesis substrate, endothelium NO-synthase expression, level of endogenic eNOS expression inhibitor and disorder in NO biological availability.

Impact exerted by oxidized low-density lipoproteins and lysophosphatidylcholine (a product of reaction catalyzed by phospholipase and associated with low-density lipoproteins) on L-arginine transportation into endothelium cells is another significant process which to a certain extent determines NO formation efficiency (figures 2-4).

The data analysis showed statistically authentic increased concentration of total cholesterol (105.8% higher, $p<0.001$) in blood serum of rats with cobalt intoxication. XC (cholesterol) distribution analysis in lipoproteins with various density reveled its level reducing by 25.3% in high-density lipoproteins and increasing by 186.3% in low-density lipoproteins, i.e. in atherogenic lipoprotein complexes.

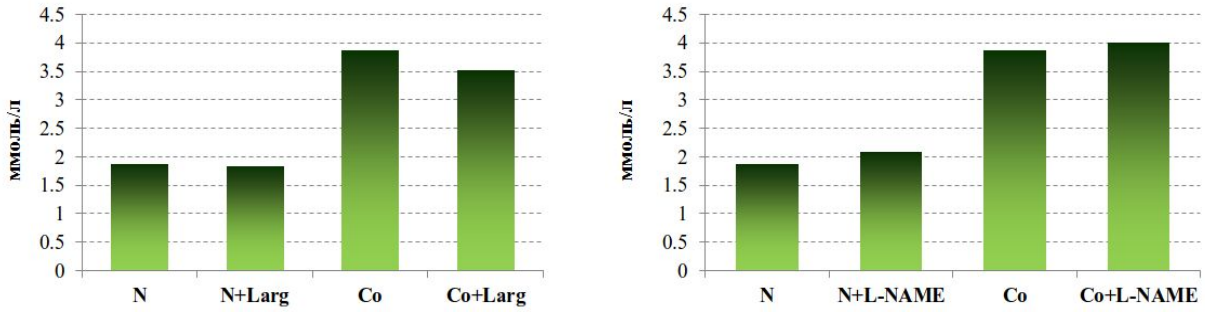


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Figure. 2. Changes in crude cholesterol concentration after introduction of NO-L-arginine donor (a) and eNOS – L-NAME inhibitor (b) under cobalt intoxication. (N-control)

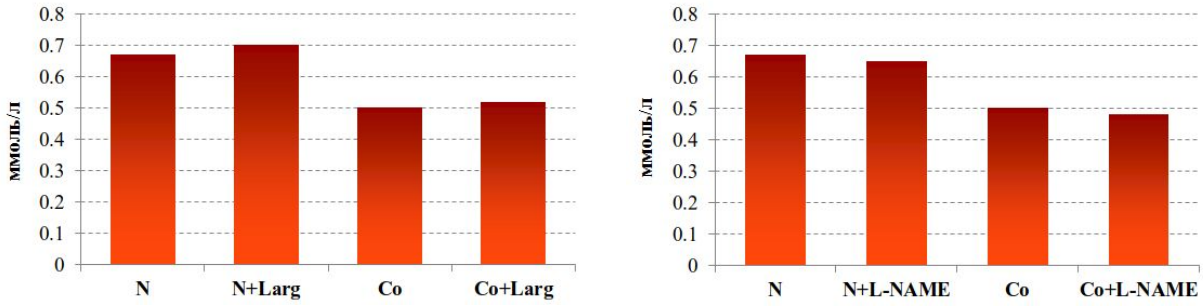

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Figure. 3. Changes in high density lipoproteins concentration against the background of NO-L-arginine donor (a) and eNOS – L-NAME inhibitor (b) under cobalt intoxication. (N-control)

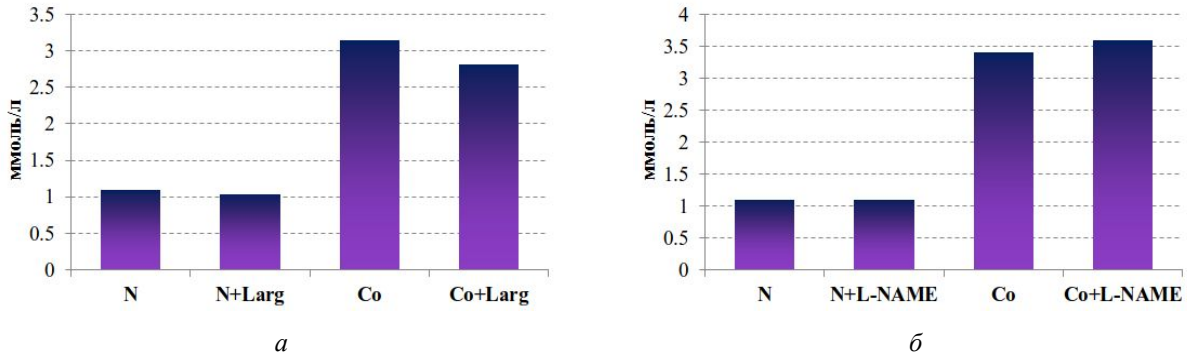

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Figure. 4. Changes in low0density lipoprotein concentrations against the background of NO-L-arginine donor (a) and eNOS – L-NAME inhibitor (b) under cobalt intoxication. (N-control)

When cobalt chloride impact is long-term, hypercholesterolemia and hyper-β-lipoproteinemia take place and it makes for vessels endothelium damage due to atherogenesis. We should presume that given the increased contents of oxygen reactive forms in blood, especially OH hydroxyl radical as the most capable to react, and malonic dialdehyde as a lipid peroxidation secondary product, such processes as oxidation modification of low-density lipoproteins (including lipid peroxidation and conjuncted dienes formation), β carbon modification, as well as enzymatic conversion of phospholipase phosphatidylcholine into lysophosphatidylcholine, take place. Conversed oxidized low-density lipoproteins (o.LDP) break L-arginine transportation from blood serum into endothelium cells; there appears also L-arginine and NO synthesis substrate deficiency: it results in lower NO formation and its biological availability.

We examined lipid peroxidation parameters, Na,K-ATP-ase membrane enzyme activity, as well as activity of such organic-specific enzymes as AlAT, AsAT, GGTP and excretory enzyme (alkaline phosphatase) in rats being long exposed to cobalt intoxication.

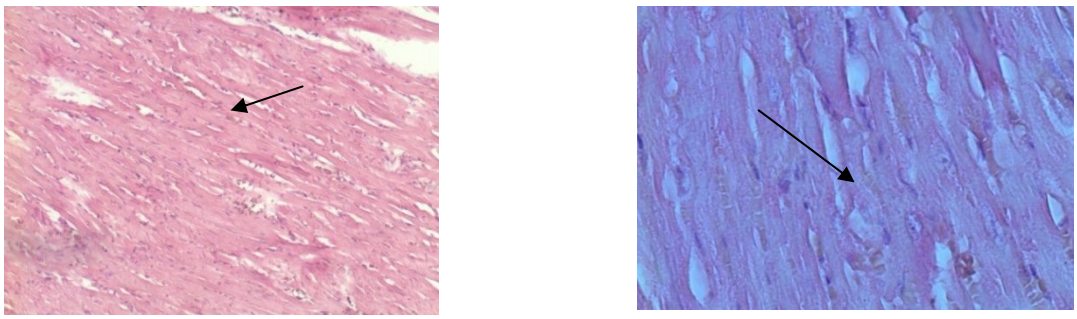
The obtained data showed lower Na,K-ATP-ase activity in both renal tissue layers ($p<0.001$), as well as in hepatic and myocardial tissue. We detected such evidence of cardiac hystiocytes damage as increased malonic dialdehyde concentration in them, lower Na,K-ATP-ase activity, as well as increased AsAT content in blood serum, this enzyme being the most organic-specific for cardiac muscle.

Toxic effects become apparent in endothelium dysfunction evolvement and visceral organs disorders (kidneys, liver and cardiac muscle).In addition to functional changes, cobalt intoxication causes changes in morphological structure of vessels endothelium and internal organs cells. The obtained results were confirmed by histological data received for renal, hepatic and myocardial tissue and endothelium of micro-circulatory channel vessels (Figure 5).

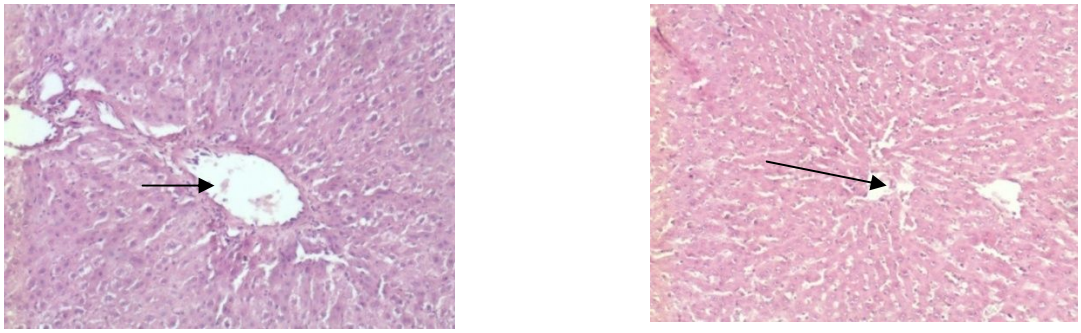
The sections of internal organs tissues taken from rats with cobalt intoxication after introduction of L-arginine bore the evidence of partial cardiac hystiocytes fragmentation; moderate parenchymatous-protein dystrophy and partial vanishing of glycogen in hepatocytes; moderate hyaline drop dystrophy of tubules and urinary areas dilation; moderate thickening of micro-circulatory channel vessels.

But as we took tissues from rats with cobalt intoxication after L-NAME introduction (figures to the right) we saw more apparent changes, such as fragmentation and disintegration of cardiac hystiocytes; cross striation disappearance in most cardiac hystiocytes; urinary area dilation; glomerule hyperemia; visible hyaline-drop dystrophy of tubules; visible parenchymatous-protein dystrophy of hepatic tissue, moderate fatty degeneration (drops in the form of transparent inclusions); moderate thickening of vascular walls endothelium and vascular wall roughness.

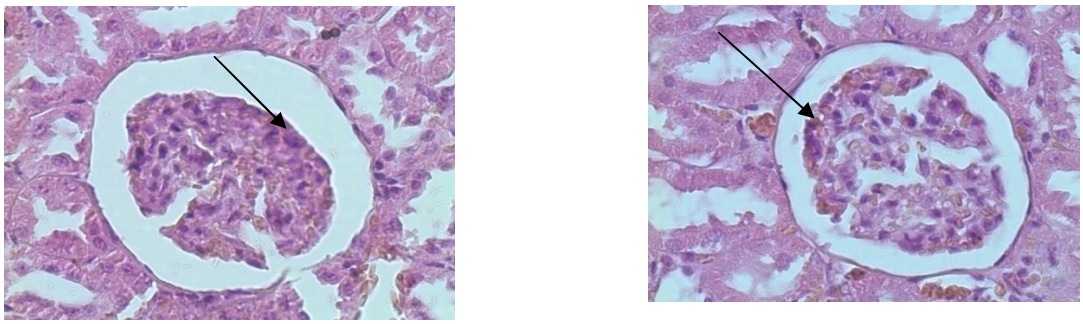
To correct the detected disorders we used coenzyme $Q_{10}$and its combination with L-arginine and L-NAME. Our research of impact exerted by $Q_{10}$and its combination with L-arginine on lipid peroxidation state revealed a significant lipid peroxidation depletion proved by statistically authentic decrease in malonic dialdehyde concentration ($p<0.001$) in blood after introduction of coenzyme Q_{10} and it combination with L-arginine.



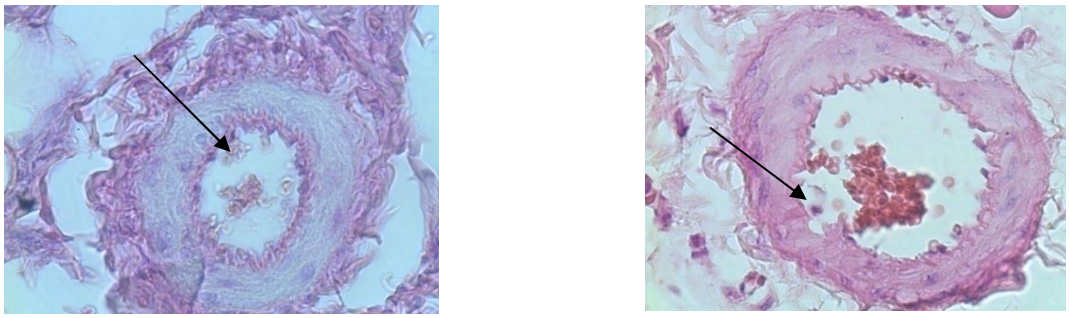
a)



b)



c)



d)

Figure 5. Changes in morphological structure of internal organs tissues and endothelium of micro-circulatory channel vessels in rats under cobalt intoxication after introduction of L-arginine (to the left) and

eNOS – L-NAME inhibitor (to the right). (painted with hematoxylin-eosin, magnification x400):
a) Myocardial tissue; b) Hepatic tissue; c) Renal tissue; d) Endothelium of micro-circulatory channel vessels

Q_{10} and its

In another research where L-NAME was used as eNOS enzyme inhibitor, endogenic antioxidant led to less significant lipid peroxidation intensity and smaller decrease in malonic dialdehyde concentration in erythrocytes. Our comparative analysis results of lipid peroxidation inhibition prove that Q_{10} and its combination with L-arginine are the most efficient.

Conclusion. Oxidation processes are activated under long-term induced cobalt intoxication. Malonic dialdehyde concentration increases in erythrocytes, in hepatic and myocardial tissues, in homogenates of cortical layer and medulla in kidneys. Imbalance occurs in anti-oxidation system and it is characterized with lower superoxide dismutase activity in erythrocytes and compensatory increase in catalase activity and ceruloplasmin concentration in blood serum. Oxidation processes activation is combined with lower concentration of total NO metabolites due to deficiency of L-arginine, a synthesis substrate, and higher concentration of expression inhibitor eNOS-ADMA detected by research under introduction of L-NAME endogenic inhibitor analogue.

We detected lower Na,K-ATP-ase activity in homogenates of renal, hepatic and myocardial tissues, as well as higher activity of some enzymes in blood serum, such as transminases (AlAT, AsAT), as well as GGTP and alkaline phosphatase, and it proves disorders in hydropathy of cells cytoplasmatic membranes and their increased penetrability.

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