

RELATIONSHIP BETWEEN HAEMOSTATIC AND MACRO-MICROHEMODYNAMIC PARAMETERS IN RATS UNDER EXPERIMENTAL EXPOSURE TO CADMIUM. MEANS OF PREVENTION

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ABSTRACT

In experiments on Wistar rats, it was shown that the prophylactic administration of melatonin at a dose of 5 mg/kg helps to reduce the manifestations of toxic coagulopathy, preventing the development of micro- and macrohemodynamic disorders in rats with chronic cadmium intoxication. Correlations between changes in indicators of the hemostasis system with the restoration of linear and volumetric blood flow rates and a decrease in the Pourcelot peripheral resistive index were revealed. These effects correlated with a decrease in the level of lipid peroxidation products and the activity of antioxidant enzymes in the blood under the influence of melatonin. The results of the experiments suggest further studies on using melatonin for the prevention and correction of the procoagulative and pathocirculatory effects of cadmium.

Keywords: cadmium, microcirculation, hemodynamics, melatonin, hemostasis

INTRODUCTION:

The available literature contains data on functional and pathomorphological changes in the cardiovascular system under the influence of the toxic effect of cadmium [1,2]. However, there is insufficient information on the mechanisms of angio- and cardiopathies development and the participation of hemocoagulation and lipid peroxidation processes in them under conditions of prolonged intoxication in experiment. It has been shown that oxidative damage is the main mechanism of the toxic effect of cadmium [3,4]. Literature data indicate that chronic intoxication can cause an increase in the procoagulant properties of biological membranes in blood cells and endothelium. Nowadays, there is no doubt about the relationship between the development of endothelial dysfunction, impaired microcirculation processes, and impaired blood clotting [7]. Hypercoagulability is not only a consequence of functional failure of the endothelium, but also an active participant in its development and progression. A necessary condition for the timely correction and prevention of pathological processes is a cumulative analysis of the indicators of the functional state of the micro-, macrohemodynamics and hemostasis systems.

The aim of the work was to experimentally study the prophylactic use of the hormone melatonin in the conditions of the formation of toxic coagulopathy, angio- and cardiopathy and the functional relationships between them under the influence of heavy metal compounds.

MATERIALS AND METHODS

The experiments were performed on 100 mature male Wistar inbred rats weighing 300-350 g, randomly

divided into ten groups: group 1 - control animals with daily administration of saline solution into the stomach through an atraumatic probe in the volume corresponding to the experimental series. 2nd, 3rd and 4th groups - rats with daily intragastric administration of cadmium sulfate in the form of an aqueous solution at a dose of 0.3 mg/kg (in terms of metal), for two weeks, one and two months. 5th, 6th and 7th groups - rats with daily intragastric administration of cadmium sulfate in the form of an aqueous solution at a dose of 0.3 mg / kg (in terms of metal), for two weeks, one and two months, which at the same time, the melatonin preparation melaxen (Unipharm, USA) at a dose of 5 mg/kg was administered intragastrically for prevention. In each group, the state of the system of macro- and microhemodynamics was studied by non-invasive methods, after which the activity of lipid peroxidation processes and the antioxidant defense system and the parameters of the hemostasis system were determined.

It is impossible to take blood from experimental animals when studying the state of hemodynamics in an acute experiment before the experiment, however, taking blood samples after manipulations is not recommended in coagulology [5,6], that is why three separate groups of experimental animals were distinguished for the study of systemic hemodynamics by invasive methods: 8th group - control with daily administration of saline solution into the stomach through an atraumatic probe in the volume corresponding to the experimental series, 9th group - rats with daily intragastric administration of cadmium sulfate at a dose of 0.3 mg / kg (in terms of metal), for two months; group 10 - rats with daily intragastric administration of cadmium for two months with prophylactic administration of melatonin at a dose of 5 mg/kg.

For non-invasive measurement of systolic and diastolic pressure, and heart rate after two weeks, one and two months, the state of hemodynamics was studied using the Systola system (Neurobotics, Russia) – tucked on top of the tail of rodents. To measure pressure, it is necessary to warm the animal to a temperature of 28–32 °C, previously for 10–15 minutes and throughout the entire procedure, for which we used an autonomous platform-thermostat for heating small animals "Flogiston" together with a retainer holding rodents container-limiter "Teremok". Heating the animal made it possible to ensure the circulation of blood in the tail in the required volume and stabilize the blood flow.

To study microcirculation parameters, doppler ultrasound (MM-D-K Minimax-Doppler-K, 20 MHz probe, St. Petersburg, Russia) was used. After adaptation of the animal at a temperature of 20°C (10 min), the indicators of the average linear velocity were determined according to the curve of the average weighted velocity (cm/s): V_{as} is the average velocity in systole, V_{am} is the average velocity over the vessel section, V_{akd} is the end-diastolic minimum velocity.

Volumetric velocity indicators: Q_{as} - systolic velocity according to the average velocity curve (ml / min); Q_{am} is the average velocity along the average velocity curve (ml/min); Indices: PI is the Gosling pulsation index, RI is the Purcelo peripheral resistance index indicative of the elasticity of vessels. Location - at the base of the tail.

After two months in an acute experiment, the state of systemic hemodynamics was assessed under conditions of zoletilol anaesthesia (5 mg per 100g weight). Measurement of the arterial pressure was made by a direct method. A plastic catheter 0.8 mm in diameter filled with 10% heparin solution was inserted into the femoral artery of a rat. The catheter was connected to a DDA electromanometer (Russia). Values of the heart rate and blood pressure were recorded on an MX-04 monitor (Russia). Mean arterial pressure (MAP) was calculated by calculation: $MAP = \text{diastolic pressure} + 1/3 \text{ pulse pressure}$. The minute volume of blood was determined by thermodilution, for which the thermistor MT-54M (Russia) was inserted through the left common carotid artery into the aortic arch. Through the right jugular vein, 0.2 ml of saline at a fixed temperature was injected into the right atrium using a catheter. Thermal dilution curves were recorded on an EPP-5 recorder (Russia). Cardiac index, stroke volume index and normalized peripheral vascular resistance were calculated using special formulas [11].

To study the hemostasis system, whole blood and blood plasma rich and poor in platelets were used. The number of platelets and their aggregation activity (ADP-induced, 10.0 µg/ml) were determined; fibrinogen content; activated partial thromboplastin time (APTT); prothrombin time (PT); fibrin monomer polymerization time to assess the state of the final stage of blood coagulation. The state of the anticoagulant system was assessed by the activity of AT(III) and protein C. The fibrinolytic link was assessed by the time of spontaneous euglobulin lysis time, the level of thrombinemia by the concentration of soluble complexes of fibrin monomers by a quantitative version of the phenanthroline test. Automatic coagulometer Helena - AC4, aggregometer Helena (Great Britain), diagnostic reagent kits "Helena" (UK), spectrophotometer "PV1251A", Solar, Belarus, diagnostic kits "Standard Technology", Barnaul were used.

To assess the intensity of lipid peroxidation processes, the content of malondialdehyde (MDA) in erythrocytes was studied by the method of V.B. GavriloVA, based on the interaction with thiobarbituric acid, hydroperoxides using diagnostic kits Agat-Med, Russia (PV1251A spectrophotometer, Solar, Belarus). The level of superoxide dismutase (SOD), which was determined by the method of adrenaline autoxidation, and the degree of catalase activity in the blood serum according to the method of E. Beutler, testified to the state of antioxidant protection.

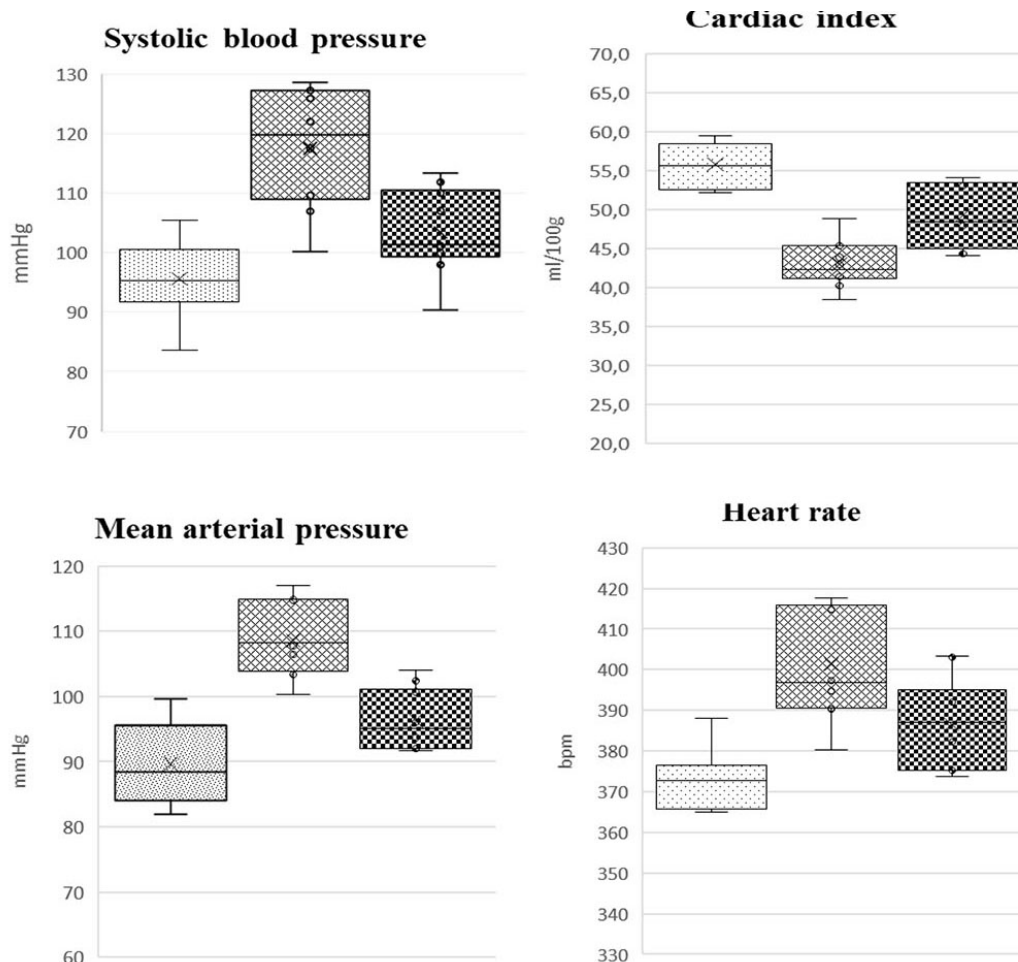
This study was carried out in accordance with the "Rules of Laboratory Practice in the Russian Federation" (Order of the Ministry of Health of the Russian Federation dated April 1, 2016 No. 199), "International Guidelines for Biomedical Research Using Animals" (1985) and Article 11 of the Helsinki Declaration of the World Medical Association (1964).

Statistical analysis was performed using MS Excel 2016 software and the Statistica 10.0 package (StatSoft, Inc.). Data are presented as median (Me) and interquartile range (25th and 75th percentiles) due to the small number of options in the sample. Comparison between groups was performed using the non-parametric Mann-Whitney test. Correlation analysis was performed using the rank correlation method according to the Spearman test. The significance level was $p < 0.05$.

RESULTS AND DISCUSSION

The study of the state of microcirculation showed that under the toxic effect of cadmium, by the end of the experiment, the indicators of maximum linear (V_{as} , $p \leq 0.05$) and volumetric systolic velocity (Q_{as} , $p \leq 0.01$) significantly decreased according to the average velocity curve. The mean velocity on the mean velocity curve V_{am} ($P \leq 0.05$) and end diastolic velocity on the mean velocity curve V_{Akd} ($P < 0.05$) also decreased. The Gosling index (PI), which reflects the elastic-elastic properties of the arteries, and the Pourcelot index (RI), which reflects the resistance to blood flow, were significantly higher than the control ($P < 0.05$ and $P < 0.001$).

The results of the experiments showed that long-term intake of cadmium into the body of animals led to pronounced disturbances in the pumping function of the heart, which was manifested by a decrease in both stroke ($p < 0,05$) and cardiac indices ($p < 0,05$) compared with the data in rats of the intact control group (figure 1). Two months later, a significant increase in mean arterial pressure was detected ($p < 0,001$). The main factor in the development of arterial hypertension was a significant increase in normalized peripheral vascular resistance ($p < 0,001$) compared to that in rats of the intact control group.



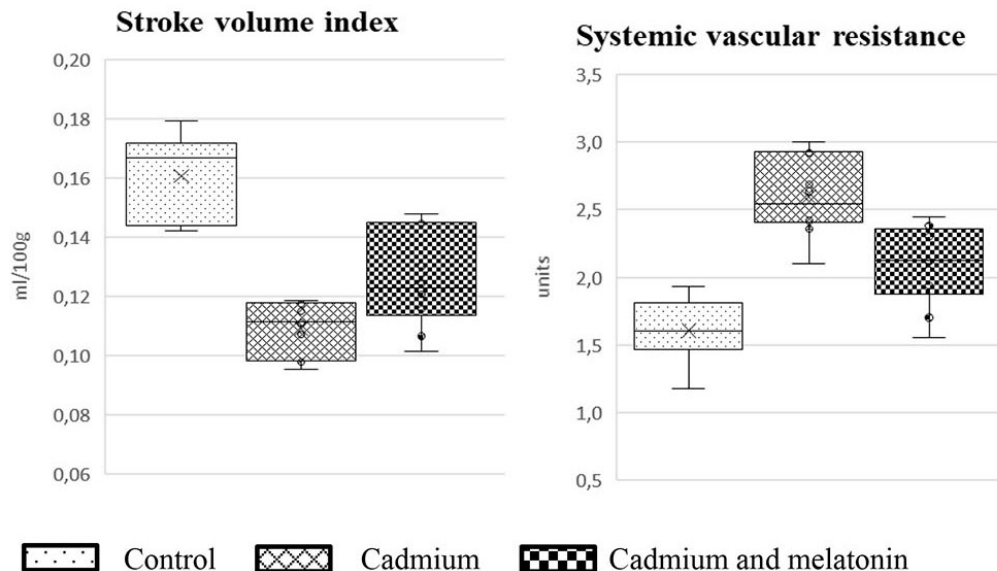


Figure 1 - Preventive effect of melatonin on changes in hemodynamic parameters in rats under the toxic effects of cadmium.

Literature data show that blood is a potential target for the toxic effects of heavy metals, as well as the main target organs - kidneys, liver, myocardium [9,12]. Entering the body, heavy metals bind to carrier proteins in the blood and are transported to the target organs. Changes in the structure and functioning of blood cells, erythrocytes, platelets, as well as changes in fibrin networks and endothelium under the influence of certain heavy metals are described [7,12], but a comprehensive study of all components of the hemostasis system and changes in the parameters of micro-macrohemodynamics caused by cadmium intoxication has not been carried out in dynamics.

The experiments have shown that after two weeks and one month of administration of cadmium sulfate, an increase in the concentration of fibrinogen, the number of platelets and their aggregation activity was detected (**Table 1**). At the same time, the activity of protein C and antithrombin III increased. Also, a shortening of the APTT was detected after one month ($p < 0.05$). The hypercoagulable orientation of the hemostasis system was compensated by the activation of the anticoagulant system and fibrinolysis processes. The time of spontaneous euglobulin lysis was shortened ($p < 0.05$). Two months later, the hypercoagulable direction of changes in the hemostasis system persisted, but depletion of the anticoagulant system and fibrinolysis was detected. The activity of protein C remained above the background, but a decrease in the activity of antithrombin III was detected ($p < 0.05$). The time of spontaneous euglobulin lysis was shortened ($p < 0.001$).

Under conditions of a significant increase in the level of soluble complexes of fibrin monomers, it is possible to ascertain the development of a state of thrombotic readiness. At the same time, the number of platelets and their aggregation activity remained elevated, the level of fibrinogen was significantly higher than the control.

The states of hypercoagulability and thrombinemia are clinically often accompanied by the expenditure of fibrinogen and thrombocytopenia, due to the «activation of blood coagulation processes» [5,6]. At the same time, it is known that long-term stimulation of the hemostasis system in chronic pathological processes and intoxication may be accompanied by the development of thrombinemia and a high level of acute-phase protein - fibrinogen, as well as an increase in the number of platelets [5,8], which was revealed in chronic intoxication in our studies.

Table 1 - Influence of melatonin on changes in the parameters of the hemostasis system in rats under the toxic effects of cadmium.

Parameter	Control	Cadmium	Cadmium and melatonin
		<i>1st line - after 2 weeks; 2nd - 1 month; 3rd - 2 months</i>	
Platelet count, 10 ⁹ /l	578 (514;595)	523(506;544) 653(605;677)** 642(605;681)**	525(506;559) 565(542;583) ΔΔ 610(532;636)
ADP-induced platelet aggregation, %	54,6 (50,3;61,8)	69(57;74)* 73(71;82)*** 75(70;81)***	59(51;65) 71(64;73)** 60(56;64) ΔΔ
Activated partial thromboplastin time, sec	27 (24;27)	27(24;30) 20(16;22)*** 31(30;32)***	29(26;30) 25(24;29) ΔΔ 30(28;31)*
Prothrombin time, sec	19 (16;22)	17(16;20) 16(13;22) 21(18;26)	18(14;21) 15(14;19) 23(20;24)
Protein C activity, %	110,5 (81,5;122,5)	160,3(145,4;163,0)*** 144,8(133,4;152,2)*** 136,2(124,0;150,6)**	127,4(122,1;137,3)*Δ 138,2(131,9;152,5)*** 141,1(121,7;155,8)**
Antithrombin III, %	107 (98;110)	120(113;127)** 103(99;108) 67(59;72)***	97(90;105) ΔΔΔ 110(109;114) Δ 90(88;92)**ΔΔΔ
Spontaneous euglobulin lysis time, min	468 (438;519)	484(454;514) 403(314;430)** 850(722;948)***	484(431;507) 422(354;521) 631(526;796)**Δ
Fibrinogen, g/l	2,17 (2,03;2,29)	4,14(3,63;4,74)*** 4,083(3,145;4,402)*** 2,665(2,524;4,699)**	3,42(2,45;4,63)** 2,78(2,17;3,43) 3,61(2,82;4,21)**
Fibrin monomer polymerization time, RU	1,60 (1,47;1,95)	1,81(1,40;1,94) 1,61(1,48;1,80) 1,33(1,11;1,59)*	1,55(1,28;1,67) 1,72(1,38;1,86) 1,64(1,47;2,04) Δ
Soluble complexes of fibrin monomers, mg/100ml	3,06 (2,99;3,55)	3,56(3,05;3,70) 3,53(3,40;3,61) 5,73(4,71;6,12)***	3,28(2,94;3,48) 3,30(3,00;3,77) 4,38(3,75;5,01)**Δ
Notes: p – level of statistical significance for the comparison of differences in parameters, */**/*** - p<0,05/0,01/0,001 – significance level for comparison of differences with the control group (intact), Δ/ΔΔ/ΔΔΔ - p<0,05/0,01/0,001 – significance level for comparison of differences with cadmium -only group. Data is presented as Me – median and (Q25; Q75) interquartile range.			

The relationship between lipid peroxidation processes and impaired blood coagulation is indicated by many researchers [13]. Fibrinogen is a highly vulnerable potential target for oxidizing agents. In vitro with induced oxidation, as well as in vivo with the development of oxidative stress, a violation of the spatial organization of fibrin and its viscoelastic and biochemical properties was shown. This can lead to the formation of abnormal fibrin with thin fibers and a finely porous structure, an increase in the resistance of the fibrin network to fibrinolysis [8]. These data support the concept of a relationship between oxidative stress, fibrin structure disorders, and the risk of cardiovascular disease [10].

The experiments have found that daily administration of cadmium sulfate to rats for two months leads to the activation of lipid peroxidation processes, as evidenced by an increase in the concentration of hydroperoxides in erythrocyte membranes after 2 weeks, one and two months according to the study period (p<0.05; p< 0.001 and p<0.05) and an increase in malondialdehyde activity (p<0.01; p<0.01 and p<0.05). The suppression of the antioxidant defense system of cells was detected, as evidenced by a decrease in the activity of catalase (p<0.05; p<0.01) and superoxide dismutase (p<0.01; p<0.01) after one and two months.

The study of functional relationships between the processes of hemocoagulation with indicators of microcirculation, systemic hemodynamics and the state of lipid peroxidation processes and AOP enzymes revealed statistically significant relationships, which is shown in the correlation matrix (Table 2).

Table 2 - Spearman's rank correlation coefficients (r_s):

Parameter	Systolic blood pressure (mmHg)	Mean arterial pressure (mmHg)	Heart rate (bpm)	Cardiac index (ml/100g)	Stroke volume index (ml/100g)	Systemic vascular resistance (units)
Platelet count, 10 ⁹ /l	<u>0,46</u>	<u>0,58</u>	<u>-0,61</u>	<u>-0,67</u>	-0,34	<u>0,51</u>
ADP-induced platelet aggregation, %	0,37	<u>0,61</u>	<u>-0,62</u>	<u>-0,72</u>	0,32	<u>0,58</u>
Activated partial thromboplastin time, sec	<u>0,64</u>	<u>0,67</u>	<u>-0,64</u>	<u>-0,67</u>	<u>-0,67</u>	<u>0,51</u>
Prothrombin time, sec	0,34	0,17	-0,15	-0,21	<u>0,51</u>	0,08
Antithrombin III, %	<u>-0,61</u>	<u>-0,75</u>	<u>0,76</u>	<u>0,70</u>	-0,28	-0,24
Spontaneous euglobulin lysis time, min	<u>0,52</u>	<u>0,59</u>	<u>-0,80</u>	0,08	0,09	<u>0,72</u>
Fibrinogen, g/l	<u>0,58</u>	<u>0,69</u>	<u>-0,47</u>	<u>-0,66</u>	-0,38	-0,43
Fibrin monomer polymerization time, RU	-0,42	<u>-0,63</u>	0,32	0,36	-0,29	<u>-0,62</u>
Soluble complexes of fibrin monomers, mg/100ml	<u>0,61</u>	<u>0,69</u>	<u>-0,69</u>	<u>-0,83</u>	0,17	-0,37
Protein C activity, %	0,39	<u>0,58</u>	-0,47	<u>-0,70</u>	0,17	-0,37

a.) correlation coefficients of the hemostasis system with changes in hemodynamic parameters in rats with chronic cadmium intoxication for two months (significant correlations at $p < 0.05$);

Parameter	Systolic blood pressure (mmHg)	Mean arterial pressure (mmHg)	Heart rate (bpm)	Cardiac index (ml/100g)	Stroke volume index (ml/100g)	Systemic vascular resistance (units)
Platelet count, 10 ⁹ /l	0,08	0,31	-0,29	-0,40	<u>0,68</u>	-0,32
ADP-induced platelet aggregation, %	0,21	<u>0,45</u>	-0,41	<u>-0,57</u>	<u>0,64</u>	-0,44
Activated partial thromboplastin time, sec	0,05	0,34	-0,20	-0,21	-0,37	-0,16
Prothrombin time, sec	0,08	-0,04	0,08	0,19	0,06	<u>-0,58</u>
Antithrombin III, %	-0,34	<u>-0,55</u>	<u>0,76</u>	0,43	-0,08	-0,35
Spontaneous euglobulin lysis time, min	-0,11	0,22	-0,43	-0,18	-0,08	-0,35
Fibrinogen, g/l	0,09	-0,06	0,19	0,08	0,23	0,16
Fibrin monomer polymerization time, RU	-0,08	-0,36	<u>0,61</u>	0,22	-0,14	0,00
Soluble complexes of fibrin monomers, mg/100ml	0,27	0,27	<u>-0,54</u>	<u>-0,46</u>	-0,37	-0,16
Protein C activity, %	-0,32	-0,04	0,30	0,12	-0,41	<u>-0,62</u>

Note: significant correlations were identified at $p < 0.05$.

b.) correlation coefficients of the hemostasis system with changes in hemodynamic parameters under the influence of melatonin.

Currently, the role of melatonin in almost all vital processes of the body and the possibility of its use as a drug are being widely studied. It has been shown that the combination of traditional therapy regimens using melatonin is more effective in many pathological processes [14]. A pronounced adaptogenic, desensitizing, anti-inflammatory, hepatoprotective effect, anti-stress effects, etc. are described. The pronounced membrane-protective properties of melaxen are due to its significant antioxidant effect [14,15]. The use of melatonin at a dose of 5 mg/kg as a prophylactic agent was due to the fact that earlier experimental studies conducted in our laboratory showed the prophylactic efficacy of melatonin at this dose under long-term intoxication with heavy metals [1,4]. Given the diversity of the described properties of melatonin, we should expect high efficiency of melatonin in chronic intoxication, however, we did not find data on the possibility of its use in order to prevent disorders in the hemostasis system during prolonged exposure to cadmium on the body in the available literature.

The experiments have confirmed that prophylactic administration of the antioxidant melatonin to rats exposed to chronic cadmium intoxication prevented the development of disorders in the system of hemostasis and micro-macrohemodynamics during chronic intoxication (**Figure 1; Table 1**).

Melatonin reduces the severity of changes in vascular-platelet and coagulation hemostasis, restores the

activity of anticoagulant and fibrinolytic blood systems, reduces the degree of thrombinemia and oxidative stress. The administration of melatonin reduced the processes of peroxidation in cells, in comparison with the values in the group with the isolated introduction of a metal salt, and prevented a decrease in the activity of catalase ($p < 0.05$ after one month and $p < 0.05$ after two months) and SOD ($p < 0.01$ at one month and $p < 0.05$ at two months).

In rats with the prophylactic use of melatonin, a significant hypotensive effect was found, which was due to a decrease in vascular resistance compared with the data in rats that received only cadmium. The preventive effect of melatonin under conditions of cadmium poisoning was confirmed by the restoration of both stroke and cardiac indices relative to similar values in the group of animals that received only cadmium (Table 1). Previously, in our laboratory it was experimentally shown that melatonin prevents a decrease in α 1adrenoreactivity of the circulatory system and reactivity of the reninangiotensin system in rats under the toxic effect of cadmium, contributing to the restoration of the functional reserve of the myocardium [1,4].

The study of the state of microcirculation revealed that in animals treated with melatonin, less pronounced changes in the elastic-elastic properties of the arteries and vascular resistance were observed. Indicators of linear blood flow (Gosling index) and volumetric blood flow (Pourcelot index) significantly decreased in comparison with the group with isolated administration of cadmium ($P < 0.05$ and $P < 0.05$).

The results of statistical analysis revealed correlations between some parameters of hemodynamics and indicators of the hemostasis system (Table 2). Significant correlations were found between changes in parameters in the hemostasis system and restoration of linear and volumetric blood flow rates and a decrease in the Pourcelot peripheral resistance index. These effects correlated with a decrease in the level of lipid peroxidation products and the activity of antioxidant enzymes in the blood under the influence of melatonin.

CONCLUSION

Our findings demonstrate the relationship between the mechanisms of the pathocoagulative and pathocirculatory effects of cadmium during prolonged intoxication. For the first time, the relationship between the mechanisms of angio- and cardioprotective effects of melatonin and the mechanisms for preventing disorders of the state of cellular and plasma hemostasis, the anticoagulant system, fibrinolysis processes, and the level of thrombinemia under conditions of chronic exposure to cadmium has been established. It can be considered expedient to study the possibility of using melatonin in order to develop methods for the prevention and correction of the toxic effects of cadmium.

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