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Prevention of Toxic Coagulopathy to Ensure Life Safety in Industrialized Regions

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***Abstract* – Researchers of recent years have established a direct relationship between the increase in incidence and the level of anthropogenic pollution of the environment, while emphasizing the increase in the frequency of cardiovascular pathology and thrombohemorrhagic complications among the population living in areas ecologically unfavorable for lead. In this regard, it should be considered that the prevention of toxic coagulopathy is an important mechanism for ensuring life safety in industrialized regions. The purpose of this work was to study the preventive effect of melatonin on the hemostasis system indicators, the state of lipid peroxidation and antioxidant protection during chronic lead intoxication. Lead acetate solution was injected to Wistar rats at a dose of 40 mg/kg through a tube into the stomach, along with intragastric administration of a solution of melatonin at a dose of 5 mg/kg for two weeks, one and two months. Determined ADP-platelet aggregation, fibrinogen content, activated partial thromboplastin time, prothrombin time, the relative time of the polymerization of fibrin monomers, the activity of antithrombin III, while spontaneous euglobulin lysis, the concentration of soluble fibrin monomer complexes using standard techniques "technology standard", Russia. The level of D-dimers was determined by the reagents kit "INNOVANCE D-dimer", Siemens. To assess the intensity of lipid peroxidation processes, we determined the content of malondialdehyde in erythrocytes, hydroperoxides (diene conjugates and diene ketones) in blood plasma, superoxide dismutase activity, and catalase activity in erythrocytes (Solar-300, Belarus). Studies have shown that prophylactic administration of melatonin helps to reduce the severity of pathological changes in the parameters of vascular-platelet and coagulation hemostasis, anticoagulant and fibrinolytic systems, helps to reduce the level of thrombinemia. The revealed changes correlated with the restoration of the level of lipid peroxidation products and the activity of antioxidant blood enzymes. The results of the study allow us to recommend the use of melatonin for the optimization of therapeutic and preventive measures and the development of methods for the correction of hemostatic disorders when exposed to lead compounds on the body.**

Key words: melatonin, lead acetate, hemostasis, rats.

I. INTRODUCTION

Among the adverse environmental factors, a significant proportion of heavy metals. Researchers of recent years have established a direct relationship between the increase in incidence and the level of anthropogenic pollution of the environment [1], while emphasizing the increase in the frequency of cardiovascular disease and thrombohemorrhagic complications among the population living in areas ecologically unfavorable for lead [2, 3]. Against the background of external "well-being", activation of the processes of blood coagulation and hemolysis is found in workers in contact with lead. Laboratory manifestations of endothelial dysfunction in the serum of patients with microsaturnism are shown [4]. The literature data suggests that hemocoagulation changes when the lead is exposed to the body in the experiment and the clinic are the initial stages of DIC syndrome. Exposure to heavy metals is the most important factor determining the thrombogenic risk in people living in ecologically unfavorable regions [1, 5, 6]. In this regard, it should be considered that the prevention of toxic coagulopathy is an important mechanism for ensuring life safety in industrialized regions.

II. FORMULATION OF THE PROBLEM

As a means of preventing toxic coagulopathy in patients with chronic lead intoxication, we chose a synthetic analogue

of the hormone melatonin epiphysis Melaxen. Earlier in our laboratory, it was shown that the use of melatonin effectively prevents the pathological changes in the morphofunctional state of the kidneys, liver and heart during lead intoxication in rats in the experiment [2]. Data on the possibility of using melatonin to prevent disorders of the hemostatic system during lead intoxication, we have not found in the available literature.

III. PURPOSE OF THE STUDY

The study of the preventive effect of melatonin on the performance of the hemostasis system, the state of lipid peroxidation and antioxidant protection during prolonged lead acetate toxicity in rats in the experiment.

IV. MATERIALS AND METHODS

The work was performed on Wistar rats weighing 200–300 g. When conducting experiments on animals, they were guided by the "International Recommendations for Conducting Biomedical Research Using Animals" (1985) and the Rules of Laboratory Practice (Order of the Ministry of Health of the Russian Federation No. 267 of June 19, 2003).

The model of chronic lead intoxication was reproduced (Brin et al., 2017) by administering lead acetate to an animal in an effective dose of 40 mg/kg body weight through a probe into the stomach, every day for two weeks (20 individuals), one month (20 individuals) and two months (20 individuals). Half of the rats in each group were administered intragastrically melaxen at a dose of 5 mg/kg. Intact animals (10 individuals) and rats with isolated intragastric administration of the melaxen solution (10 individuals) served as controls.

At the end of the experimental period in animals, indicators of the functional state of the hemostasis system and the activity of lipid peroxidation were investigated. The material for the study was whole blood, as well as blood plasma, rich and poor in platelets. Stabilization and obtaining blood plasma samples was carried out considering international standards for clinical laboratory diagnostics for research in the field of hemostasis (Momot, 2006). The platelet aggregation activity (ADP inducer – 10.0 μ g/ml), the content of fibrinogen by the method of Clauss, activated partial thromboplastin time (APTT), prothrombin time (PT), relative polymerization time of fibrin monomer (VPM) (Momot, 2009), relative time of fibrin monomer polymerization (VPM) (Momot, 2009) were determined. antithrombin activity (ATIII), time of spontaneous euglobulin lysis, concentration of soluble fibrin – monomer complexes (FDM). The tests were performed on a Solar-300 spectrophotometer, an AP2110 aggregometer, a Solar CGL ‑ 2110 turbidimetric coagulometer (Belarus) using the Technology Standard diagnostic kits (Russia). The level of D-dimers was determined by the reagents kit "INNOVANCE D-dimer", Siemens. To assess the intensity of lipid peroxidation and antioxidant potential, the following were determined: the content of malonic dialdehyde (MDA) in erythrocytes, hydroperoxides (HP) (diene conjugates and diene ketones) in plasma (using isp. Potassium ferricyanide), superoxide dismutase activity (SOD), catalase activity in erythrocytes according to the method E. Beutler ("Solar-300", Belarus).

Statistical analysis was performed using Microsoft Excel and Statistica10.0 (StatSoft, Inc.). The data are presented as the median Me and (the 25th and 75th) percentile of the sample. The significance of differences was assessed using the non-parametric Mann-Whitney U-test. Differences were considered significant at a level of statistical significance of $p < 0.05$. Correlation analysis was performed using a nonparametric rank correlation coefficient (r_s) of Spearman.

V. THE DISCUSSION OF THE RESULTS

In recent years, researchers have increased interest in the hormone melatonin, which has a wide range of pharmacological effects – anti-stress, anti-toxic, desensitizing, anti-inflammatory, antioxidant, pronounced adaptogenic, etc. [10]. Unlike drugs used in hematology (antiplatelet agents, anticoagulants, etc.) that have a unidirectional effect, melatonin is a kind of modulator and exhibits a unique nonspecific ability to optimize any physiological processes in the body that can be enhanced by melatonin against the background of initially reduced indicators, but limits them growth in case of excessive activation. One of the most important properties of melatonin is its effect on the processes of thrombocytopoiesis, hemocoagulation and fibrinolysis. In vitro and in vivo experiments showed the effect of melatonin on the processes of spontaneous and induced (collagen, ADP) platelet aggregation mainly due to inhibition of thromboxane without changes in the activity of the prostacyclin system [10, 11]. The administration of melatonin prevented the development of hypercoagulation, prevented the development of DIC syndrome, reducing the level of thrombinemia [10, 13].

It was previously shown that the use of melatonin at a dose of 10 mg/kg effectively prevents morphofunctional changes in the internal organs in rats with the toxic effects of heavy metals [2]. At the same time, the introduction of melatonin in this dose influenced the vascular-platelet unit of the hemostasis system, suppressing the functional activity of platelets [13]. The correcting ability of melatonin at a dose of 1 mg/kg was shown against the background of hypoaggregation induced by the administration of acetylsalicylic acid and under conditions of hyperaggregation caused by the intraperitoneal administration of calcium chloride solution [13]. In this work, we used a dose of melatonin – 5 mg/kg to prevent chronic lead coagulopathy. The results of the study in the control group of rats treated for two months of the prepat Melaksen, indicate the absence of its effect on the blood coagulation system. Only a slight tendency to a decrease in the number of platelets was detected, the degree of ADP induced aggregation relative to the intact control did not change.

The study of the state of the hemostasis system in experimental animals already at the initial time of the experiments revealed the development of procoagulant tendencies with simultaneous activation of the anticoagulant and fibrinolytic units of the hemostasis system. From the data of table 1 it is seen that in rats after two weeks of intoxication, the degree of ADP-induced aggregation increased. A shortening of the activated partial thromboplastin time and prothrombin time, an increase in the fibrinogen concentration was detected. There was a statistically significant increase in the activity of AT (III) and a decrease in the time of

spontaneous euglobulin lysis. The results of the experiments confirm information about the presence of procoagulant activity in lead compounds [12]. The peculiarity of the metal is the cytotoxic effect, the destruction of red blood cells, an increase in the thromboplastic activity of the formed elements. It has been shown [14] that hemostasis disorders begin with a toxic effect on platelet membranes, which leads to changes in the adhesive and aggregation activity of platelets, the release of aggregation inducers and the secondary activation of coagulation hemostasis when exposed to lead. At the same time, the erythrocyte hemocoagulation factors play a significant role in the mechanisms of hemostatic potential increase [12].

TABLE I. THE EFFECT OF MELATONIN ON THE HEMOSTATIC SYSTEM IN RATS WITH CHRONIC LEAD ACETATE TOXICITY AT A DOSE OF 40 MG/KG BODY WEIGHT OF RATS

Options	Control	Lead Acetate	Lead Acetate and Melatonin
		1st line - in 2 weeks; 2nd - 1 month; 3rd - 2 months	
Platelet count, 10^9/л	566[510–573]	598[541–622] 416[385–459]*** 287[253–356]***	537[497–574]Δ 483[444–518]**/Δ 435[385–468]***/ΔΔΔ
ADP aggregation of platelets, %	59.9[54.5–63.1]	66.8[64.2–71.7]** 78.7[74.0–81.6]*** 73.9[68.0–78.6]***	62.5[54.8–64.4]ΔΔ 65.8[63.2–70.7]**/ΔΔΔ 65.6[61.4–69.5]*/Δ
APPT, sec	27.1[25.8–28.3]	22.4[21.8–23.7]*** 22.1[20.5–23.1]*** 34.4[32.6–35.3]***	25.9[24.6–26.9]ΔΔΔ 24.0[23.1–25.7]**/ΔΔ 30.4[28.6–31.1]**/ΔΔ
Prothrombin time, sec	18,6[16,9–19,3]	13,2[11.4–14.1]*** 20.1[19.5–22.2]** 24[23.0–25.4]***	15.1[13.5–17.0]**/Δ 15.9[15–17.5]*/ΔΔΔ 21[20.7–23.4]***/ΔΔ
Antithrombin (III) %	100.6[90.0–108.1]	125.8[116.0–134.2]*** 78.8[67.2–82.5]*** 65.6[59.4–80.0]***	113.8[99.4–123.4] 121.8[107.4–128.2]**/ΔΔΔ 87.3[77.0–93.3]**/ΔΔ
Spontaneous euglobulin lysis, min	507.9[467.2–556.5]	404.6[344.3–453.4]** 835.3[743.7–897.8]*** 857.3[834.0–991.5]***	465.4[397.7–501.3] 428.1[383.1–467.9]**/ΔΔΔ 706.5[658.7–771.5]***/ΔΔ
Fibrinogen, g\l	2.05[1.89–2.29]	3.29[3.15–3.66]*** 2.67[2.46–3.14]*** 1.49[1.26–1.66]***	2.77[2.44–3.10]***/ΔΔ 2.44 [2.15–2.61] 1.76[1.58–2.00]*/Δ
VPFM, relative units	1.02[0.92–1.09]	0.92[0.80–1.00] 0.70[0.65–0.81]*** 1.3[1.16–1.37]**	1.00[0.91–1.13] 0.91[0.81–1.05]ΔΔ 0.88[0.77–0.90]**/ΔΔ
PFCA, mg/100ml	3.22[2.90–3.68]	3.47[3.18–4.10] 4.66[4.18–5.46]*** 6.34[5.87–7.15]***	3.48[2.67–3.67] 3.86[3.02–4.7] 5.28[4.94–5.89]***ΔΔ
D-dimer, mg/l	1.34[0.63–2.25]	2.22[1.66–3.13] 2.49[1.73–3.39] 4.25[3.42–4.47]***	2.57[1.50–3.01] 1.80[1.10–2.73] 3.16[2.42–3.46]**/ΔΔ

[a.] Note: * – p ≤0.05 – the degree of reliability rel. intact control, Δ – p≤0.05 — with respect to the experience with lead at the appropriate time

With the increase in the time of toxic effects of lead, animals showed multidirectional changes in the parameters of the hemostasis system (Table 1). There was a decrease in the number of platelets and an increase in their aggregation function in a month. Fibrinogen concentration remained above control. In the plasma link of the hemostasis system, hypercoagulation along the internal pathway and hypocoagulation along the external pathway were recorded. Thrombin time did not differ from the control, however, a more sensitive marker of the state of the final hemocoagulation stage (HMFM) revealed a shortening of the polymerization time of fibrin monomers. A depletion of the reserve capacity of the anticoagulant and fibrinolytic systems of hemostasis was recorded, as evidenced by a decrease in the activity of AT (III) and a slowdown in the time of spontaneous euglobulin lysis. A significant increase in the concentration of RFMC was detected (Table 1). The data of the experiments clearly demonstrate the development of the state of thrombotic readiness, as determined by [8] as laboratory-detected hypercoagulation according to the so-called "global" coagulogram tests, an increase in the content of hemostasis activation markers in the blood, inhibition of anticoagulant and fibrinolytic activity. Researchers have previously found this at different doses and methods for introducing lead, using these and other indicators of the state of hemostasis [12]. In hypercoagulative syndrome, blood clots may not be observed in either the arterial or venous system or in the microcirculation system. However, this records the state of increased readiness of circulating blood to clot [8]. In such cases, there may be a few clinical signs of a prethrombotic state — an increase in blood viscosity, a slowing of the venous blood flow, and transient and initial signs of organ dysfunction. This is confirmed by the results of earlier experiments that demonstrate the development of dystrophic

and necrobiotic changes in the tissues of the liver, kidneys and heart under the toxic effects of lead at different times [2].

When lead intoxication in two months increased the degree of thrombocytopenia on the background of increasing platelet aggregation function. In these terms, the totality of several facts suggests the presence of signs of DIC syndrome. From the side of plasma hemostasis, according to chronometric tests, a shift of the hemostasiological profile of the blood towards hypocoagulation was registered, at the same time the anticoagulant and fibrinolytic mechanisms were depressed. At the final stage of coagulation, pronounced thrombinemia was recorded, which was confirmed by an increase in the concentration of D-dimers and FPC. In addition, a decrease in fibrinogen concentration was observed. Lengthening the time of polymerization of the fibrin-monomer complexes also confirms the fact of a decrease in fibrinogen in the blood plasma.

Experiments have shown that the prophylactic administration of melatonin against the background of chronic intoxication takes time and reduces the severity of pathological changes in the indices of cell and plasma hemostasis during all periods of experiments. The introduction of melatonin for two weeks contributed to the restoration of the functional activity of platelets. Hypercoagulation was maintained only along the external path of the coagulation system. The concentration of fibrinogen increased relative to the control but was significantly lower than in rats with isolated lead administration. The state of the anticoagulant and fibrinolytic systems did not differ from the level of intact control, but changes in comparison with the indices in lead rats were not detected at the appropriate time.

The prophylactic use of melatonin during the month was accompanied by the disappearance of most of the signs of thrombotic readiness — increased concentration of CPMC, inhibition of physiological anticoagulants, and fibrinolysis, recorded in rats with isolated lead administration. The concentration of fibrinogen was not significantly different from that of lead rats, however, the changes from the level of control were not significant. A shift in the hemostatic potential towards hypercoagulation according to the APTT and PV has been recorded, but it was compensated for by activation of the anticoagulant and fibrinolytic systems. The increase in the degree of ADP aggregation was maintained, the number of platelets decreased, but the changes were less pronounced.

The prophylactic administration of melatonin for two months revealed signs of stabilization of the hemostasiologic picture and a decrease in the likelihood of developing the state of thrombotic readiness described in rats with isolated lead administration. This was evidenced by a decrease in the severity of changes in RFMK and D-dimers, inhibition of physiological anticoagulants and fibrinolysis, recorded in lead rats (Table 1). Amid thrombocytopenia, platelet aggregation increased, but the changes were also less pronounced than in lead rats.

In the literature of recent years, information on the relationship between the activity of hemocoagulation systems and lipid peroxidation is widely presented. It has been shown that activation of blood coagulation processes, thrombinemia, is accompanied by an increase in the content of POL products in blood plasma, erythrocytes, leukocytes and platelets [12].

The results of the experiments showed that intragastric administration of lead acetate causes an increase in the activity of lipid peroxidation processes and inhibition of the antioxidant enzyme link with the development of oxidative stress (Table 2), confirming the previously obtained data (Bryn, 2017). An increase in the concentration of malonic dialdehyde over the time of the experiments, the depletion of the level of hydroperoxides in the blood plasma and a decrease in the activity of SOD and catalase (Table 2) were detected.

The prophylactic use of the natural antioxidant melaxen increased the level of catalase activity in erythrocytes while simultaneously reducing the concentration of malondialdehyde and restoring the level of hydroperoxides in the blood plasma, indicating a pronounced antioxidant effect of melaxen under conditions of chronic lead intoxication (Table 2).

TABLE II. THE EFFECT OF MELATONIN ON THE PERFORMANCE OF THE POL-AOD SYSTEM IN RATS WITH CHRONIC LEAD ACETATE TOXICITY AT A DOSE OF 40 MG/KG BODY WEIGHT OF RATS

Options	Intact control	Lead Acetate	Lead Acetate and melatonin
Malonic dialdehyde (μmol/l)	28.9[27.9–31.6]	32.6[30.7–34.8]** 35.3[33.8–37.7]*** 40.9[38.4–41.4]***	31.1[29.3–32.7] 33.6[31.8–35.0]**Δ 32.0[31.5–33.9]*ΔΔΔ
Hydroperoxide content (233nm)	0.0668 [0.0578–0.0775]	0.0808[0.0777–0.0934]** 0.0559[0.0535–0.0685] 0.0278[0.0175–0.0372]***	0.0744[0.0694–0.0867] 0.0758[0.0672–0.0837]ΔΔ 0.0429[0.0332–0.0494]***ΔΔ
Hydroperoxide content (278nm)	0.0432 [0.0365–0.0496]	0.0564[0.0503–0.0678]** 0.0250[0.0189–0.0344]** 0.0172[0.0122–0.0251]***	0.0466[0.0321–0.0492]ΔΔ 0.0349[0.0252–0.0406] 0.0262[0.0235–0.0355]**Δ
Superoxide dismutase activity (units of inhibitors, %)	69.36 [64.30–72.63]	78.77[75.86–86.65] ** 63.81[57.85–67.88] 55.89[49.50–61.15] **	76.22[69.35–80.50] 75.82[68.61–76.80]ΔΔ 63.90[56.17–67.57]*Δ
Catalase activity (* 10-4me/ghb)	7.43 [6.05–8.02]	8.71[7.19–9.06] 4.80[3.92–5.88] ** 4.33[3.54–5.18] ***	7.36[6.40–8.02] 9.21[7.34–9.48]*ΔΔΔ 5.90[4.41–6.87]*Δ

[b.] Note: see Table 1.

A correlation analysis of the results of the experiments revealed statistical correlations of the positive dynamics of hemostasiological parameters with the restoration of the activity of lipid peroxidation processes in rats against the background of prophylactic administration of melatonin.

A significant positive correlation was found between the recovery of catalase activity and the increased activity of antithrombin III (after 1 month $r_s = 0.64$; $P < 0.05$; and after two months $r_s = 0.58$; $P < 0.05$), as well as a high degree inverse correlation with the time of spontaneous euglobulin lysis (after 1 month $r_s = 0.61$; $P < 0.05$; and after two months $r_s = 0.57$; $P < 0.05$). Closely positively associated were changes in PPMC indices and MDA shifts ($r_s = 0.72$; $P < 0.05$) after one month, D-dimers and MDA shifts ($r_s = 0.55$; $P < 0.05$) two months later under influence of natural antioxidant melatonin.

Thus, the results of the study confirm the literature data on coagulopathy, activation of lipid peroxidation during lead intoxication and the pronounced antioxidant properties of melatonin, for the first time demonstrate the role of the hormone in the mechanisms for the prevention of toxic lead coagulopathy.

VI. Conclusion

- Prophylactic use of melatonin reduces the severity of changes in the parameters of vascular-platelet and coagulation hemostasis, restores the activity of the anticoagulant and fibrinolytic systems of the blood, helping to restore the hemostatic balance.
- Prophylactic administration of melatonin prevents the decrease in the activity of the antioxidant system, promotes the utilization of lipid peroxidation products and thereby eliminates the imbalance in the system of lipid peroxidation and antioxidant protection.
- The results of the study allow us to recommend the use of melatonin for the development of methods for correcting disorders of hemostasis when exposed to lead compounds on the body.

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