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Hematological changes and structural morphological changes in the liver and spleen in mice against the background of acute toxic effects of cyclophosphan

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Abstract

Background. Disturbances in the body associated with bone marrow failure caused by the administration of lethal doses of cyclophosphan to animals make it possible to use this experimental model to solve important pathophysiological problems.

Aim. To reveal hematological changes and structural morphological changes in the liver and spleen in mice against the background of acute toxic effects of lethal doses of cyclophosphan.

Material and methods. The studies were performed on 200 outbred male mice weighing 27–29 g. Acute cytotoxic syndrome was modeled by a single intraperitoneal injection of a solution of cyclophosphan at doses of 500 and 750 mg/kg of body weight. Blood sampling for hematological studies was carried out on the 3rd, 6th, 10th and 15th days of intoxication, and the removal of the liver and spleen for morphological studies was performed on the 6th and 15th days. Statistical analysis was performed using Statistica 10 and MS Excel. Results were presented as mean (M) ± standard error of the mean (mx). To determine the significance of differences between groups with a significance level of $p < 0.05$, Fisher's test and Mann–Whitney U-test were used.

Results. Mortality against the background of acute cytotoxic action of cyclophosphan at doses of 500 and 750 mg/kg reached 83.3 and 100%. The initial content of leukocytes in the peripheral blood of mice was $(8.6 \pm 2.3) \times 10^9/l$, and on the 6th day of intoxication it decreased to $(1.0 \pm 0.3) \times 10^9/l$ and $(0.6 \pm 0.1) \times 10^9/l$ ($p = 0.01$). Blast cells of all hematopoietic lineages were present in blood smears. In the morphological picture of the spleen, the connective tissue stroma was sharply distinguished, and the white and red pulps were almost completely absent. Signs of acute toxic effects of cyclophosphan were found in the liver tissue.

Conclusion. The course of acute cytotoxic syndrome caused by cyclophosphan is accompanied by high mortality, the development of pancytopenia, pronounced destructive changes in the spleen tissue and toxic changes in the liver tissue.

Keywords: cytotoxicity, cyclophosphan, pancytopenia, hematological parameters, morphological changes, experimental modeling.

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Background

Pancytopenia is a significant issue in modern hematology because it is a sign of bone marrow failure [1, 2]. It can be caused by congenital and acquired disorders of hematopoiesis, exposure to ionizing radiation, contact with toxic substances, use of myelotoxic drugs, severe viral infections,

and myeloproliferative diseases [3–5]. The incidence of pancytopenia has not been accurately determined. It is a severe condition that is difficult to treat and affects all age groups [5–7].

Several experimental studies have demonstrated common associations of pathogenesis leading to interphase cell death and impaired mitotic

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activity, resulting in decreased formation of formal elements, despite the different mechanisms of the damaging effects of exogenous and endogenous factors that cause bone marrow failure [1, 7, 8].

Cytotoxic syndrome caused by cytostatics and acute radiation disease are the most successful experimental models for studying bone marrow failure [8, 9]. Pathological and anatomical changes resulting from toxic doses of cytostatics and ionizing radiation typically involve bone marrow damage and dystrophic changes in organs. Clinical changes may include hematological and hemorrhagic syndromes and syndromes of infectious complications and organic lesions of the central nervous system. These pathological processes are often associated with high mortality rates. Experimental models are highly sought after in military toxicology [5, 8, 9].

However, these models allow for the study of bone marrow failure, modifying effects of defense factors, and typical pathological processes under extreme environmental conditions. In our previous study on the medullary form of acute radiation disease model, we evaluated the modifying effect of low-intensity electromagnetic radiation on lesions with lethal doses of ionizing radiation [10]. During experimental modeling of myeloablative cytostatic chemotherapy with cyclophosphan in rats, researchers described chemical changes in the composition of blood and brain that could lead to impaired energy supply for neurological functions [11]. This study demonstrates the existence of common nonspecific reactions of hematopoiesis regulation in models of hypoplastic states and hematopoiesis-suppressing effects caused by cytostatics. This suggests the presence of common neuroendocrine mechanisms in the physiological and reparative regeneration of hematopoietic tissue [12].

The primary pharmacological effect of cyclophosphan is the alkylation of DNAs, which results in molecular destabilization and fragmentation, leading to the loss of its integrity. The nonselective action of cytostatics on body cells causes the development of a cytotoxic syndrome, which is accompanied by the suppression of hematopoiesis [13].

The cytotoxic effect of cyclophosphan corresponds to typical pathological processes that initiate endogenous programmed cell death. Based on this, the acute cytotoxic syndrome caused by cyclophosphan is of interest for experimental modeling to assess the modifying effect of defense factors and examine the restorative potential of internal organs in bone marrow aplasia. It can also help identify nonspecific compensatory and adaptive mechanisms that ensure the survival of a biobject

in the case of damaging effects of environmental factors [10].

Therefore, to address fundamental pathophysiological issues, the hematologic and morphologic changes associated with medullary failure induced by lethal doses of cyclophosphan can be effectively used in experimental models [14].

Aim

This study aimed to reveal hematologic changes and structural morphological rearrangements in the liver and spleen of mice exposed to acute toxic effects of lethal doses of cyclophosphan.

Materials and methods

Experimental studies were conducted at the central research laboratory of the Volga Region Research Medical University using 200 white mongrel male ICR (CD-1) mice weighing 27–29 g, obtained from the Andreevka nursery (Moscow region). The animals were kept in standard vivarium conditions with an air temperature of 18°C–24°C and a relative humidity of 40%–80%. The animals had unrestricted access to food and water (dietary regimen—*ad libitum*).

Before the experiment, the animals were quarantined for 14 days. Following this, the mice were randomly assigned to groups, with sick and weakened animals being excluded from the study. Experiments were conducted following bioethics principles and in compliance with regulatory and legal requirements for research involving animals. These requirements are outlined in the Guide for Care and Use of Laboratory Animals (ILAR Publication, 2017, National Academy Press) and the interstate standard GOST 33216-2014 Guidelines for the Maintenance and Care of Laboratory Animals. Rules of Keeping and Care of Laboratory Rodents and Rabbits, as well as in RD-APK 3.10.07.02-09 Methodological Recommendations for Keeping Laboratory Animals in Vivariums of Research Institutes and Educational Institutions. These rules and requirements are approved by the Ethical Committee of the Volga Region Research Medical University (Protocol No. 11 dated June 09, 2021).

Acute cytotoxic syndrome was induced in all animals in the experimental groups by a single intraperitoneal injection of cyclophosphan working solution. In group 1, the drug was administered at a dose of 500 mg/kg body weight ($n = 90$), whereas in group 2, it was administered at a dose of 750 mg/kg ($n = 90$). Working solutions of cyclophosphan at the required concentrations were prepared on the spot. A 5% solution of cyclophosphan was used to achieve a concentration of 50 mg/mL, whereas a 7.5% solution was used to achieve

a concentration of 75 mg/mL. The solutions were then injected intraperitoneally at a rate of 0.1 mL per 10 g of mouse body weight. The control group (n = 20) was administered an intraperitoneal injection of 0.9% sodium chloride solution at a rate of 0.1 mL per 10 g of mouse body weight.

The animals were observed for 15 days. During the study, the daily lethality and average life expectancy of deceased mice were evaluated. For hematological study and leukocyte counting, blood was collected in tubes with K3 EDTA anticoagulant after decapitation on days 3, 6, 10, and 15 of cyclophosphan intoxication. Blood smears (three for each individual) were fixed using May–Grunwald staining followed by Romanovsky–Giemsa staining (Arbis, Russia). Leukocytes were counted at a magnification of $\times 1000$ with oil immersion using a Leica CME light-optical microscope, with 100 cells observed.

After cyclophosphan administration, the liver and spleen were removed on days 6 and 15 for pathomorphological study. The material was fixed in 10% neutral formalin solution, dehydrated in alcohols of ascending concentration, and embedded in paraffin. Paraffin sections, 5–7 μm thick, were obtained using an RM 2245 microtome (Leica, Germany), stained with hematoxylin and eosin, and examined using a DM 1000 microscope (Leica, Germany). The images were captured using a DFC 290 video camera manufactured by Leica, Germany. Mass ratios (MR), which represent the relative mass of the organ per 1 kg of animal weight, were calculated using the following formula:

$$\text{MR} = (m/M) \times 1000$$

where m is the mass of the organ (g) and M is the mass of the animal (g).

Statistical processed data are presented as mean (M) $\pm$ standard error of the mean (mx). The frequency of signs (%) was calculated using the software packages Statistica 10 and MS Excel, and the error of the mean was determined with a confidence interval of 95%. The reliability of the differences between the experimental groups was determined using Student's criterion, Fisher's criterion, and nonparametric Mann–Whitney U-criterion. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

The study found that the use of cyclophosphan at doses of 500 and 750 mg/kg resulted in the development of acute cytotoxic syndrome and high mortality rates among experimental animals. The cytotoxic effect of cyclophosphan was accompanied by a significant decrease in body weight, with

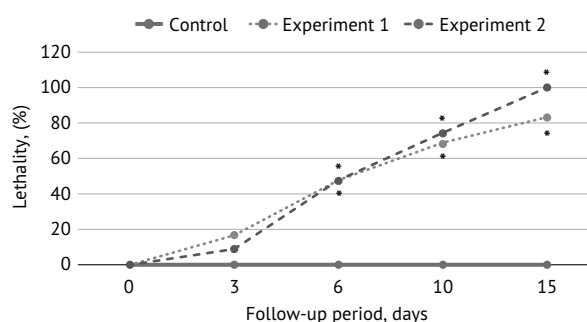


Fig. 1. Daily lethality of mice during acute toxic effect of cyclophosphan; experiment 1 involved a single intraperitoneal injection of cyclophosphan at a dose of 500 mg/kg; experiment 2 involved a single intraperitoneal injection of cyclophosphan at a dose of 750 mg/kg; *statistically significant differences compared with the control group, $p < 0.05$ (Fisher's criterion).

the experimental groups experiencing a reduction of >1.5 times compared with baseline and >2 times compared with the control group. The general functional state of mice was negatively affected by the acute toxic effect of cyclophosphan, as evidenced by dull and dirty wool color, liquid stool, alopecia, decreased and absent orientation-research reactions, reduced general motor activity, and loss of posture. Lethality rates were high in groups 1 and 2, with 47.8% dying within the first 6 days after receiving doses of 500 and 750 mg/kg of cyclophosphan. By day 15, lethality rates increased to 83.3% and 100%, respectively (Fig. 1).

During the follow-up period, no differences were found in the lethality and clinical course of acute intoxication with cyclophosphan in mice between groups 1 and 2. The average life expectancy in these groups were not statistically significantly different (7.2 ± 0.9 days; 6.8 ± 1.3 days; $p = 0.87$). However, from day 6 of observation, the lethality indices in the experimental groups showed statistically significant differences compared with that in the control group ($p = 0.01$) (Fig. 1).

After the administration of cyclophosphan at doses of 500 and 750 mg/kg, significant hematologic disorders and pronounced destructive morphological changes were observed in the spleen of mice. However, no significant changes in hemoglobin and erythrocyte indices in peripheral blood were found in the experimental groups when compared with baseline and control groups (Table 1). Erythrocytes have no proliferative activity and have a longer life expectancy than leukocytes, making them less susceptible to the cytotoxic effects of cyclophosphan than leukocytes.

Starting from day 3 of intoxication, blood smears of surviving mice showed the presence of basophilic, oxyphilic, and polychromatophilic erythroblasts, Jolly cells, anisocytosis, and

Table 1. Hematologic parameters in mice exposed to the acute cytotoxic action of cyclophosphan ($M \pm m$)

Day	Group	ER, $\times 10^{12}/L$	p	LC, $\times 10^9/L$	p	PLT, $\times 10^9/L$	p
0	Control	7,8 $\pm$ 0,2	—	8,6 $\pm$ 2,3	—	468,8 $\pm$ 51,1	—
	Experiment 1	8,3 $\pm$ 0,4	$p_1=0,88$	9,5 $\pm$ 2,1	$p_1=0,84$	472,5 $\pm$ 49,2	$p_1=0,84$
	Experiment 2	7,9 $\pm$ 0,1	$p_1=0,90$	8,7 $\pm$ 2,9	$p_1=0,86$	462,9 $\pm$ 47,1	$p_1=0,91$
3	Control	8,9 $\pm$ 0,1	$p_2=0,86$	9,6 $\pm$ 2,6	$p_2=0,87$	477,8 $\pm$ 52,6	$p_2=0,89$
	Experiment 1	9,1 $\pm$ 0,2	$p_{1,2}=0,89$	2,9 $\pm$ 0,7*#	$p_{1,2}=0,02$	457,8 $\pm$ 42,6	$p_{1,2}=0,87$
	Experiment 2	8,8 $\pm$ 0,5	$p_{1,2}=0,89$	3,6 $\pm$ 1,2*#	$p_{1,2}=0,03$	577,5 $\pm$ 93,6	$p_{1,2}=0,93$
6	Control	7,4 $\pm$ 0,8	$p_2=0,87$	7,7 $\pm$ 2,5	$p_2=0,71$	437 $\pm$ 96,4	$p_2=0,72$
	Experiment 1	9,7 $\pm$ 0,9	$p_{1,2}=0,67$	1,0 $\pm$ 0,3*#	$p_{1,2}=0,02$	229,6 $\pm$ 46,7*#	$p_{1,2}=0,03$
	Experiment 2	8,4 $\pm$ 0,4	$p_{1,2}=0,78$	0,6 $\pm$ 0,1*#	$p_{1,2}=0,02$	272,2 $\pm$ 44,4*#	$p_{1,2}=0,04$
10	Control	7,3 $\pm$ 0,7	$p_2=0,87$	9,1 $\pm$ 3,1	$p_2=0,87$	468,4 $\pm$ 47,2	$p_2=0,95$
	Experiment 1	8,9 $\pm$ 0,3	$p_{1,2}=0,85$	10,8 $\pm$ 3,0	$p_{1,2}=0,12$	692,5 $\pm$ 156,9	$p_{1,2}=0,34$
	Experiment 2	8,5 $\pm$ 0,5	$p_{1,2}=0,79$	5,2 $\pm$ 0,4	$p_{1,2}=0,09$	374,8 $\pm$ 105,3	$p_{1,2}=0,23$
15	Control	8,5 $\pm$ 0,4	$p_2=0,87$	8,6 $\pm$ 2,7	$p_2=0,92$	471,5 $\pm$ 58,2	$p_2=0,88$
	Experiment 1	8,2 $\pm$ 0,3	$p_{1,2}=0,81$	7,0 $\pm$ 1,7	$p_{1,2}=0,65$	757,8 $\pm$ 148,8	$p_{1,2}=0,09$
	Experiment 2	—	—	—	—	—	—

Note: Experiment 1, single intraperitoneal injection of cyclophosphan at a dose of 500 mg/kg; experiment 2, single intraperitoneal injection of cyclophosphan at a dose of 750 mg/kg; ER, erythrocytes; LC, leukocytes; PLT, platelets; *statistically significant differences compared with the control group, $p_1 < 0.05$; #statistically significant differences compared with baseline, $p_2 < 0.05$ (Mann–Whitney test).

poikilocytosis. These findings suggest the release of morphologically immature erythrocytes with altered shapes and sizes into the peripheral bloodstream. These changes may result from both compensatory reactions of hematopoietic organs to the massive decay of blood cells under the influence of cyclophosphan and bone marrow damage. The acute toxic effect of cyclophosphan damages some histohematic barriers, including the hematocellular barrier of the red bone marrow, which is partly responsible for the release of immature forms.

In the experimental groups exposed to cyclophosphan on day 3 of intoxication, a significant decrease in leukocyte counts was observed, which was accompanied by thrombocytopenia on day 6 (Table 2). The initial leukocyte and thrombocyte levels in the peripheral blood of mice in the control group and groups 1 and 2 were within the physiological range and did not show any statistically significant differences ($p = 0.842$). During follow-up, the formal elements in the peripheral blood of the control group did not undergo significant changes. In addition, the analysis of the leukocyte formula did not reveal any deviations from the norm (Table 2).

After administering cyclophosphan at doses of 500 and 750 mg/kg on day 3 of intoxication, the total leukocyte count in the peripheral blood decreased by more than threefold in groups 1 and 2. On day 6, the leukocyte count in peripheral blood

was less than $1.0 \times 10^9/l$, which was statistically significantly different from the baseline and leukocyte counts in the control group ($p_1 = 0.015$; $p_2 = 0.023$; Table 2). However, on days 10 and 15, the surviving mice showed increased total leukocyte count in the peripheral blood, primarily because of an increase in young and morphologically immature leukocytes, as indicated by the leukocyte formula analysis (Table 2).

From day 3 of cyclophosphan intoxication and during observation, lymphocytopenia was observed in groups 1 and 2, and the lymphocyte count in the peripheral blood showed statistically significant differences when compared with baseline and control groups (Table 2). In addition, on days 3 and 10 of cyclophosphan intoxication in mice in groups 1 and 2, a significant and statistically significant increase in the content of segmented neutrophils was noted in the peripheral blood ($p_{1,2} = 0.017$). On day 15 of intoxication, similar changes were observed in mice in group 1. In group 2, a decrease in the proportion of rod-shaped neutrophils was recorded, and their content had statistically significant differences between the baseline and control groups (Table 2).

The ratio of neutrophils to lymphocytes in the peripheral blood can indicate an increase in neutrophils (cells of rapid immune response) and a decrease in lymphocytes (cells of more complex immune response). This may occur in response to

Table 2. Leukocytic formula parameters in mice exposed to the acute cytotoxic effect of cyclophosphan ($M \pm m$)

Day	Group	BN, %	p	SN, %	p	LC, %	p
0	Control	5,3±1,1	—	22,7±1,6	—	66,4±2,5	—
	Experiment 1	6,1±0,9	$p_1=0,78$	26,4±1,3	$p_1=0,84$	62,1±3,2	$p_1=0,89$
	Experiment 2	5,4±1,8	$p_1=0,95$	21,4±1,5	$p_1=0,90$	61,4±4,3	$p_1=0,81$
3	Control	5,8±1,2	$p_2=0,89$	20,4±1,9	$p_2=0,86$	65,4±2,1	$p_2=0,93$
	Experiment 1	3,5±1,3	$p_{1,2}=0,12$	83,2±1,4*#	$p_{1,2}=0,02$	12,4±1,1*#	$p_{1,2}=0,02$
	Experiment 2	1,6±0,4*#	$p_{1,2}=0,01$	77,3±3,9*#	$p_{1,2}=0,02$	17,3±3,9*#	$p_{1,2}=0,02$
6	Control	4,3±0,3	$p_2=0,58$	34,3±7,0	$p_2=0,61$	61,3±7,4	$p_2=0,87$
	Experiment 1	3,6±0,7	$p_{1,2}=0,67$	38,3±9,7	$p_{1,2}=0,67$	49,7±7,5*#	$p_{1,2}=0,04$
	Experiment 2	0,9±0,2*#	$p_{1,2}=0,01$	28,5±9,5	$p_{1,2}=0,85$	64,2±9,4	$p_{1,2}=0,78$
10	Control	5,3±1,0	$p_2=0,98$	23,4±1,2	$p_2=0,87$	67,4±4,1	$p_2=0,95$
	Experiment 1	3,5±0,5	$p_{1,2}=0,19$	77,7±4,7*#	$p_{1,2}=0,02$	15,8±4,1*#	$p_{1,2}=0,01$
	Experiment 2	2,2±0,5*#	$p_{1,2}=0,03$	72,7±4,7*#	$p_{1,2}=0,02$	20,2±3,9*#	$p_{1,2}=0,02$
15	Control	5,9±1,7	$p_2=0,87$	24,4±2,0	$p_2=0,91$	64,8±2,2	$p_2=0,89$
	Experiment 1	5,2±1,4	$p_{1,2}=0,81$	69,4±2,7*#	$p_{1,2}=0,02$	19,1±1,7*#	$p_{1,2}=0,01$
	Experiment 2	—	—	—	—	—	—

Note: Experiment 1, single intraperitoneal injection of cyclophosphan at a dose of 500 mg/kg; experiment 2, single intraperitoneal injection of cyclophosphan at a dose of 750 mg/kg; BN, band neutrophils; LC, lymphocytes; SN, segmented neutrophils. *Statistically significant differences compared with the control group, $p_1 < 0.05$; #statistically significant differences compared with the initial value, $p_2 < 0.05$ (Mann–Whitney test).

increased levels of stress hormones and the cytotoxic effects of cyclophosphan, leading to the release of neutrophils into the bloodstream and depletion of lymphocytes with slow recovery. During the acute cytotoxic action of cyclophosphan, the neutrophil-to-lymphocyte ratio was high, with the former being 6–8 times higher than the latter (Table 2).

Immature hematopoietic sprouts of hematopoiesis were detected in peripheral blood smears, including erythrocytes with basophilic, oxyphilic, and polychromatophilic coloration, metamyelocytes, myelocytes, promyelocytes, blast cells, prolymphocytes, promegakaryocytes, and megakaryocytes. The cytotoxic effect of cyclophosphan on the peripheral blood form elements of the experimental animals was most pronounced from day 6 to day 10 of intoxication, which corresponded to the mass death of mice (Fig. 1).

However, successful compensatory and adaptive mechanisms were observed in surviving mice after cyclophosphan administration at a dose of 500 mg/kg, starting from day 15 of intoxication. Despite the presence of morphologically immature and altered blood cells in the peripheral bloodstream, the condition stabilized and ensured mouse survival.

Structural and morphological rearrangements were observed in the spleen and liver of mice exposed to the acute toxic effects of cyclophosphan, confirming its pronounced cytotoxic effect and

development of acute cytotoxic syndrome after a single intraperitoneal injection at doses of 500 and 750 mg/kg.

In intact mice, the spleen had a well-defined white pulp with large follicles, whereas the red pulp and venous sinuses contained moderate abundance of erythrocytes. The stroma was represented by sparse thin trabeculae running deep into the organ (Fig. 2a).

In response to the toxic effect of cyclophosphan at doses of 500 and 750 mg/kg, the same destructive morphological changes in the structure of the spleen were observed in mice in groups 1 and 2. On day 6 of intoxication, in the morphological structure of the spleen, the connective tissue stroma of the organ was sharply distinguished. Histological preparations revealed near-complete absence of red and white pulps, remnants of diffusely located lymphoid tissue, absence of erythrocytes in venous sinuses, and sparse structure of the spleen (Fig. 2b).

On day 15 of intoxication in the experimental groups, the spleen showed a significant increase in the connective tissue stroma and decreased red pulp content. However, the white pulp with large follicles and diffusely located lymphoid tissue was well defined. The structure of the spleen was less sparse than that on day 6 of intoxication. On day 15, structural changes in the spleen may indicate the activation of compensatory-adaptive mechanisms in surviving mice (Fig. 2c).

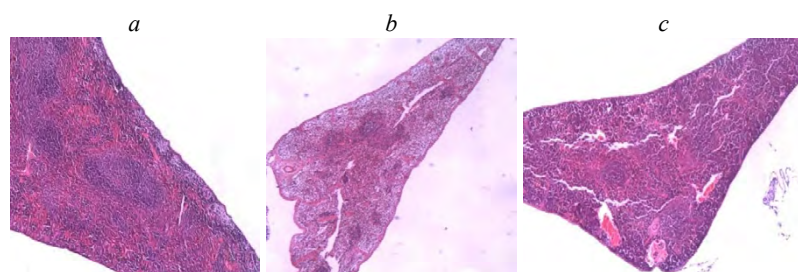


Fig. 2. Morphological picture of the spleen exposed to the cytotoxic action of cyclophosphan: *a*, the spleen in the control group; *b*, the spleen exposed to the toxic action of cyclophosphan at doses of 500 and 750 mg/kg on day 6 of intoxication; *c*, the spleen exposed to the toxic action of cyclophosphan at doses of 500 and 750 mg/kg on day 15 of intoxication. Hematoxylin and eosin staining of all preparations. Magnification $\times 4$.

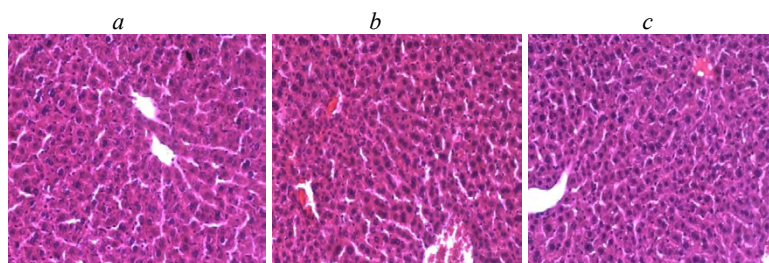


Fig. 3. Morphological picture of the liver exposed to the cytotoxic action of cyclophosphan: *a*, the liver in the control group; *b*, the liver exposed to the toxic action of cyclophosphan at doses of 500 and 750 mg/kg on day 6 of intoxication; *c*, the liver exposed to the toxic action of cyclophosphan at doses of 500 and 750 mg/kg on day 15 of intoxication. Hematoxylin and eosin staining was used for all the preparations. Magnification $\times 20$.

In the control group and intact animals, the liver morphology exhibited a lobular structure. The hepatocyte borders were well defined, and the cytoplasm was slightly oxyphilic and granular. No focal disorders of cytoplasmic tinctorial properties were observed. The nuclei were clear and contained a sufficient amount of chromatin, and the nuclear membrane was thin. The liver sinusoids and vessels of the portal tracts were moderately full of blood, and the bile duct lumen was free. The connective tissue of the stroma was poorly expressed (Fig. 3a).

In response to the toxic effects of cyclophosphan at doses of 500 and 750 mg/kg on day 6 of intoxication in groups 1 and 2, no significant pathomorphological rearrangements were observed in the liver structure. The liver structure exhibited a characteristic lobular structure with clear boundaries of hepatocytes and a granular, weakly oxyphilic cytoplasm. The nuclear membrane remained thin and the nuclei contained distinct nuclei; however, hyperchromatosis was present. The liver sinusoids and portal tract vessels were congested, and the lumen of the bile ducts was free. The stromal connective tissue was minimally expressed, and early morphological indications of fatty dystrophy were observed.

On day 6, morphological changes were observed in the liver tissue, indicating the acute toxic effect

of the drug on liver cells. However, these changes did not result in significant structural rearrangements of the tissues (Fig. 3b). In the experimental groups, Kupffer cell accumulation was observed in the liver preparations on day 15. This indicates the activation of innate immunity and proinflammatory reactions and active phagocytosis of toxins and endotoxins. The presence of multinucleated hepatocytes in the liver tissue can reflect the toxic effect of cyclophosphan and indicate the beginning of regeneration processes in surviving individuals (Fig. 3c).

During the acute cytotoxic effect of cyclophosphan, liver and spleen MR in mice were significantly decreased in the experimental groups compared with those in baseline and control groups (Table 3). On day 6, liver MR was more than 1.5 times lower in the experimental groups than in the baseline and control groups ($p_1 = 0.04$; $p_2 = 0.03$), and spleen MR was 5–6 times lower ($p_1 = 0.04$; $p_2 = 0.03$), which showed statistically significant differences.

On day 15 of intoxication, liver MR in group 1 tended to increase, although it was statistically significantly lower than that in the baseline and control groups ($p_1 = 0.04$; $p_2 = 0.03$). In addition, spleen MR in group 1 on day 15 of intoxication was 1.5 times higher than the baseline values and MR in

Table 3. Mass ratios of liver and spleen in mice exposed to the acute cytotoxic effects of cyclophosphan ($M \pm m$)

Day	Group	Liver, $M \pm m$	p	Spleen, $M \pm m$	p
0	Control	70,29±2,67	—	6,45±0,66	—
	Experiment 1	71,23±3,01	$p_1=0,96$	6,58±0,49	$p_1=0,98$
	Experiment 2	69,87±2,95	$p_1=0,75$	6,03±0,38	$p_1=0,85$
6	Control	68,98±3,34	$p_2=0,96$	6,74±0,29	$p_2=0,93$
	Experiment 1	42,87±5,67*#	$p_1=0,04; p_2=0,03$	0,90±0,09*#	$p_1=0,04; p_2=0,03$
	Experiment 2	42,13±3,89*#	$p_1=0,04; p_2=0,03$	0,86±0,02*#	$p_1=0,04; p_2=0,03$
15	Control	71,28±2,42	$p_2=0,89$	6,98±0,15	$p_2=0,89$
	Experiment 1	53,71±3,20*#	$p_1=0,04; p_2=0,03$	10,82±1,89*##	$p_{1,2}=0,04; p_3=0,03$
	Experiment 2	—	—	—	—

Note: Experiment 1, single intraperitoneal injection of cyclophosphan at a dose of 500 mg/kg; experiment 2, single intraperitoneal injection of cyclophosphan at a dose of 750 mg/kg. *Statistically significant differences compared with the control group, $p_1 < 0.05$. #Statistically significant differences compared with baseline, $p_2 < 0.05$. ##Statistically significant differences compared with baseline, control group, and values of mass ratios on days 6 and 15, $p_3 < 0.05$; (Mann–Whitney test).

the control group, as well as 10 times higher than the MR in the experimental groups on day 6 of intoxication, which demonstrated statistically significant differences (Table 3).

Thus, cyclophosphan exhibits a cytotoxic effect on blood cells and hematopoietic organs, as evidenced by changes in the hematologic indices and structure and morphology of the spleen on days 6 and 15 of intoxication because the primary function of hematopoiesis is to produce a large number of cellular elements per unit time [1, 2, 5]. On day 6, spleen MR decreased because of the death of white and red pulp cells and decreased blood flow in the organ. On day 15, the spleen preparation clearly showed an increase in blood flow and appearance of white pulp, which was also reflected in the spleen MR. This increase in spleen MR may indicate the accumulation of decay products of form elements in the spleen. The appearance of white pulp and blood filling of the spleen, along with existing degenerative changes, may indicate the inclusion of compensatory and adaptive mechanisms.

Hematologic and morphologic changes in the liver and spleen may be attributed to the unique pharmacokinetics and pharmacodynamics of cyclophosphamide. Cyclophosphamide is inactive in vitro and requires the activation by liver microsomal enzymes to convert it into 4-hydroxycyclophosphamide. The cytotoxic effect of 4-hydroxycyclophosphamide is not specific to a particular phase of the cell cycle but acts throughout the cycle, leading to cell death [13].

Although cyclophosphan metabolites have pronounced damaging effects on body cells, particularly on rapidly dividing blood cells and spleen tissue, the cytotoxic effect affects liver cells to

a lesser extent on day 1 of intoxication. The lower proliferative activity of liver cells compared with that of hematopoietic organs may contribute to this phenomenon. In addition, drug metabolism occurs in the liver, which has a high regenerative capacity and quickly responds to toxic effects. This was supported by hyperchromatosis and multinucleated hepatocytes present in liver preparations on day 15.

The features revealed suggest that the liver tissue has a higher regenerative potential than other internal organs. This is crucial to maintain the viability of the organism when exposed to damaging factors. However, more pronounced morphological changes in the liver and the hepatotoxic effects of cyclophosphan may occur later [15].

Acute cytotoxic syndrome causes the death of leukocytes and other blood-forming elements, releasing biologically active substances with fibrinolytic and antifibrinolytic properties. This can result in the development of disseminated intravascular coagulation syndrome [1, 5, 9]. Different clinical manifestations appear in animals depending on the phase of intoxication. Hypercoagulability was observed in the early stages, whereas hypocoagulability was observed in the later stages.

These conditions contribute to circulatory disorders and tissue hypoxia, which leads to multiorgan failure and death of mice on day 1 of cyclophosphan intoxication, before the development of complications characteristic of acute cytotoxic syndrome. However, successful nonspecific compensatory and adaptive mechanisms in response to the toxic effects of cyclophosphan, in which the liver plays an important role, initiate the restoration of functions that regulate hematopoiesis in the spleen.

Hematopoietic tissue can regenerate under the influence of nonspecific reactions of neuroendo-

crine regulation of hematopoiesis [12]. During acute toxicity of cyclophosphan, partial or complete recovery of hematopoietic functions reduces the high risk of complications such as infectious syndrome and organic lesions of the central nervous system [9–11].

Conclusions

The acute cytotoxic syndrome caused by cyclophosphan is associated with high mortality rates, development of pancytopenia, significant destructive changes in spleen tissue, and toxic changes in liver tissue.

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REFERENCES

1. Vladimirskaia EB. Normal hematopoiesis and its regulation. *Clinical oncohematology. Basic research and clinical practice*. 2015;8(2):109–119. (In Russ.) EDN: TYMYAP.
2. *Gematologiya*. Rukovodstvo dlya vrachei. (Hematology. A guideline for doctors.) NN Mamaeva, editor. 3d ed. St. Petersburg: SpeczLit; 2019. 639 p. (In Russ.)
3. Goldberg ED, Dygai AM, Zhdanov VV. The role of cytokines in dynamic theory of hemopoiesis. *Medical immunology*. 2001;3(4):487–497. (In Russ.) EDN: JUASED.
4. Goldberg ED, Dygai AM, Zhdanov VV. The mechanisms of blood system regulation under myelosuppressive influences. *Byulleten sibirskoy meditsiny*. 2002;1(2):7–16. (In Russ.) EDN: UPSQFF.
5. *Rukovodstvo po gematologii*. (A guideline of Hematology.) AI Vorob'ev, editor. 3d ed. M.: N'yudiamed; 2005. 450 p. (In Russ.)
6. *Transfuziologiya*. Natsional'noe rukovodstvo. (Transfusiology. National guidelines.) AA Ragimov, editor. Moscow: GEOTAR-Media; 2012. 1184 p. (In Russ.)
7. *Klinicheskaya onkogematologiya*. Rukovodstvo dlya vrachei. (Clinical Oncohematology. A guideline for doctors.) MA Volkova, editor. M.: Medicina; 2001. 576 p. (In Russ.)
8. Kutsenko SA, Butomo NV, Grebenyuk AN. *Voennaya toksikologiya, radiobiologiya i meditsinskaya zashhita*. (Military Toxicology, Radiobiology and Medical Defense.) St. Petersburg: Foliant; 2004. 528 p. (In Russ.)
9. Nikiforov AS, Ivanov IM, Sventickaya AM. Modeling of acute radiation bone marrow syndrome in the experiment on mice. *Medical-biological and social-psychological issues of safety in emergency situations*. 2017;4(6):66–73. (In Russ.) DOI: 10.25016/2541-7487-2017-0-4-66-73.
10. Kokaya GN, Kokaya AA, Kozyakov VP, Zavirsky AV, Zacepin VV, Basharin VA, Tsygan VN, Mavrenkov EM. Experimental evaluation of the radiomodifying efficiency of low-intensity electromagnetic radiation in acute X-ray irradiation of mice. *Bulletin of the Russian Military Medical Academy*. 2021;23(2):131–138 (In Russ.) DOI: 10.17816/brmma.65944.
11. Ivnitkiy YuYu, Shefer TV, Tyaptin AA, Rejnyuk VL. Changes in the chemical composition of blood and brain of rats under the conditions of modeling of the myeloablation regimen of cyclophosphamide administration. *Toxicological review*. 2019;156(3):14–18. (In Russ.) DOI: 10.36946/0869-7922-2019-3-13-18.
12. Dygai AM, Zhdanov VV. The theory of hemopoiesis regulation in health and disease. *Byulleten SO RAMN*. 2012;32(1):21–30. (In Russ.) EDN: OPUFHX.
13. Kukes VG, Sycheva DA. *Klinicheskaya farmakologiya*. Uchebnik. (Clinical Pharmacology. Textbook.) 6th ed. M.: GEOTAR-Media; 2021. 1024 p. (In Russ.)
14. Karkishchenko NN. *Osnovy' biomodelirovaniya*. (Fundamentals of Biomodeling.) M.: Mezhdakademicheskoe izdatel'stvo VPK; 2004. 608 p. (In Russ.)
15. Bhat N, Kalthur SG, Padmashali S, Monappa V. Toxic effects of different doses of Cyclophosphamide on liver and kidney tissue in swiss albino mice: A histopathological study. *Ethiop J Health Sci*. 2018;28(6):711–716. DOI: 10.4314/ejhs.v28i6.5.

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