STUDY OF ACUTE TOXICITY OF MONOCATIONIC CHLORIN e6 DERIVATIVE, A PERSPECTIVE PHOTOSENSITIZER FOR ANTIMICROBIAL AND ANTITUMOR PHOTODYNAMIC THERAPY

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Abstract

In this experimental work the acute toxicity of a chemically modified derivative of the natural pigment chlorophyll *a* called monocationic chlorin e6, which is a promising photosensitizer (PS) for antimicrobial and antitumor photodynamic therapy, was studied using white rats. The advantages of the PS under investigation are an intense absorption in the long-wavelength region of the visible spectrum, a sufficiently high quantum yield of singlet oxygen generation, pronounced amphiphilic properties along with an appropriate solubility in water, and a high level of photocytotoxicity in relation to both malignant *HeLa* cells and antibiotic-resistant hospital strains of *E. coli* bacteria., *P. aerugenosa* and others. It has been shown that the value of LD_{50} of the considered PS can be calculated as the value of 100 mg/kg. In the reproduced experimental model of acute toxicity, pathomorphological changes in the vital organs of laboratory animals indicate a pronounced vasopathic effect of the drug with the development of cerebral edema and respiratory distress syndrome, which have become the main signs of thanatogenesis.

Key words: antimicrobial photodynamic therapy, antitumor photodynamic therapy, photosensitizer, chlorins, acute toxicity.

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ИССЛЕДОВАНИЕ ОСТРОЙ ТОКСИЧНОСТИ МОНОКАТИОННОГО ПРОИЗВОДНОГО ХЛОРИНА e6 – ПЕРСПЕКТИВНОГО ФОТОСЕНСИБИЛИЗАТОРА ДЛЯ АНТИМИКРОБНОЙ И ПРОТИВООПУХОЛЕВОЙ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ

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Резюме

На белых крысах изучены особенности острой токсичности химически модифицированного производного природного пигмента хлорофилла *a* монокатионного хлорина е6 – перспективного фотосенсибилизатора (ФС) для антимикробной и противоопухолевой фотодинамической терапии. Преимуществами ФС являются интенсивное поглощение в длинноволновой области видимого спектра, достаточно высокий квантовый выход генерации синглетного кислорода, выраженные амфифильные свойства наряду с хорошей растворимостью в воде и высокий уровень фотоцитотоксичности в отношении как злокачественных клеток линии *HeLa*, так и антибиотикорезистентных госпитальных штаммов бактерий *E. coli*, *P. Aerugenosa* и других. Величина $ЛД_{50}$ для монокатионного хлорина е6 составляет 100 мг/кг массы тела. В воспроизведенной экспериментальной модели острой токсичности патоморфологические изменения жизненно важных органов лабораторных животных свидетельствуют о выраженном вазопатическом действии препарата с развитием отека головного мозга и респираторного дистресс-синдрома, ставшими основными звеньями танатогенеза.

Ключевые слова: антимикробная фотодинамическая терапия, противоопухолевая фотодинамическая терапия, фотосенсибилизатор, хлорины, острая токсичность.

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Introduction

Photodynamic therapy (PDT) is a sophisticated minimally invasive approach to the treatment of a wide range of oncological diseases and localized microbial infections, which can be used both independently and in combination with surgery, drug and radiation therapy [1–14]. Important advantages of PDT, in addition to its low invasiveness, are the lack of treatment resistance of tumor and microbial cells, as well as the ability of PDT to induce an immune response in the body [2, 4, 5, 9, 11].

The method is based on the selective accumulation in malignant and microbial cells of low-toxic pigments - photosensitizers (PS), capable of interacting with molecular oxygen $^3O_2$ under the action of visible light, triggering a cascade of photochemical reactions. This leads to efficient generation of reactive oxygen species (ROS) and destruction of pathogenic microorganisms or tumor cells [1–3, 10–15]. Depending on the nature of the PS, ROS generation can proceed according to one of two mechanisms: with the formation of singlet oxygen $^1O_2$ or oxygen-bearing radical species, in particular, OH and O_2 [1, 11, 15]. The luminescent red glow of malignant tissue under the action of visible radiation as a result of the selective accumulation of PS in it is used for fluorescence diagnostics (FD) of tumors [7, 12].

To date, a number of PS are used in clinical practice for the diagnosis and treatment of patients with oncological diseases and bacterial infections [1, 6, 8, 11, 16]. Most of the PSs are macroheterocyclic compounds of the class of porphyrins, phthalocyanines, chlorins, or bacteriochlorins [16–20], as well as a number of 5-aminolevulinic acid derivatives. It is important to note that almost all previously developed PDT preparations aimed at combating oncological diseases contain nonionic or anionic substituents in their molecules [11, 21].

Recent studies are aimed primarily at the development and testing of new, more effective third-generation PSs [12, 22]. In addition to intensive absorption of visible radiation in the area of the "therapeutic window" of biological tissues (600–850 nm) and efficient generation of ROS, the main modern requirements for PS are a good hydrophilic-lipophilic balance, which implies simultaneous water and fat solubility (amphiphilicity) of PS, low cost, stability of dosage forms during storage, and, most importantly, low dark and pronounced photocytotoxicity of drugs.

Antimicrobial PDT imposes a number of specific requirements on PS, the main of which is the presence of one or several cationic groups in the structure of the molecule, the positive charge of which significantly increases the affinity of drugs for the cell wall of microorganisms, primarily gram-negative pathogenic bacteria [9–12, 23], since the drugs used for antitumor PDT turned out to be ineffective in photoinactivation of gram-negative microorganisms [11].

Previously, we carried out a series of systematic multidisciplinary studies on the preparation and study of superficially active substances (SAS) soluble in water or aqueous solutions of PS of the porphyrin and chlorin series. The works included the synthesis of PS, evaluation of their generation of singlet oxygen [24–26], determination of solubility, hydrophilic–lipophilic balance, and study of the interaction of PS with potential carriers based on biocompatible polymers and micellar SAS [27–32]. The dark and photoinduced toxicity of drugs against tumor cells and conditionally pathogenic strains of microorganisms was also studied *in vitro* and *in vivo* [14, 25, 26, 33, 34]. Studies have shown that PS with cationic

groups have a pronounced photocytotoxicity against gram-negative bacterial microflora [27, 28, 33, 34] both *in vitro* and *in vivo*. It was found that monocationic derivatives of chlorin e6 can effectively inactivate tumor cell cultures *in vitro* [12, 35], while tricationic chlorins have a weak cytotoxic effect.

Several studied compounds, in particular, the monocationic derivative of chlorin e6 (compound I), have the best combination of characteristics in terms of the above requirements. This PS is fairly well soluble in bidistilled water (more than 1 mmol/kg at 25^{0}C), and in aqueous solutions of potential delivery vehicles - Tween 80 or polyvinylpyrrolidone (PVP), its solubility increases several times. It has intense absorption in the red region of the spectrum (660 nm) and in non-aqueous media effectively generates singlet oxygen with a quantum yield of ~0.6 [27], has a good hydrophilic-lipophilic balance with a distribution coefficient in the system "1-octanol/phosphate-buffer saline", equal to 8.6 ± 0.2 at a temperature of 298 K [27, 29], binds quite strongly to micelles of the nonionic SAS Tween 80 (K_b = 4.57 ± 0.22 in the PS concentration range ~0.01 mmol/kg), being localized mainly near the surface of micelles [30, 31], and also has a pronounced photoinduced antitumor effect (against *HeLa* cells *in vitro*) [35] and antimicrobial activity against both Gram-positive (*St. aureus*) and Gram-negative (*E. coli*) pathogenic flora *in vitro* [27, 33].

The survival index (%) of *HeLa* cancer cells *in vitro* at a PS content of 1 μmol/l after irradiation with red light (660 nm, dose 12 J/cm^2) was only 3.71±0.11 [33], which indicates a pronounced photoinduced antitumor activity of the drug. The authors showed the photocytotoxicity of compound I against archival strains of microorganisms [25, 31], and at a concentration of microbial cells of 10^3 CFU and irradiation with red light (660 nm, S_{PS} = 50 μmol/l) at a dose of 40 J/cm^2, complete inactivation of gram-positive microflora (*St. aureus*) was achieved, while the number of gram-negative bacteria (*E. coli*) did not decrease. The use of additives that contribute to the destabilization of the outer membrane of microorganisms (Tween 80, Trilon B) and/or an increase in PS concentration led to complete photoinactivation of microbes during the experiment [25].

The results of the conducted studies show that compound I has a good potential for use as a PS for PDT. This dictates the need to study the effects associated with the toxic effects of the drug on the living organism as a whole, its organs and systems. Previous studies indicate the extremely low toxicity of anionic PS for PDT based on chlorin e6 [8, 21, 22, 31]. In particular, one of the most commonly used PS of the chlorine series, photolon, has a lethal LD_{50} dose of about 180 mg/kg of body weight, which is 100 times higher than the commonly used doses during PDT [21]. However, the presence of a cationic group in compound I can significantly increase PS cytotoxicity. Thus, information about the features of its effects on the body is essential for further preclinical trials of the drug. The aim of this work is to study the acute toxicity of PS, estimate the LD_{50} value, and study the mechanisms of thanatogenesis of monocationic chlorin e6.

Materials and methods

Synthesis of chlorin (compound I) was carried out from methylpheophorbide *a* (compound II) according to the described two-stage procedure [25, 33, 34]. Methylpheophorbide *a* was obtained by demetallation and partial acid hydrolysis of chlorophyll *a* (compound III) extracted from the cyanobacterium *Spirulina Platensis* [35]. The purity of the obtained final product weighing more than 500 mg, spectrally identified by nuclear magnetic resonance (^{1}H NMR, Bruker 500 Avance III) and mass spectrometry (MALDI, MALDI-TOF Shimadzu Axima Confidence) was at least 95%.

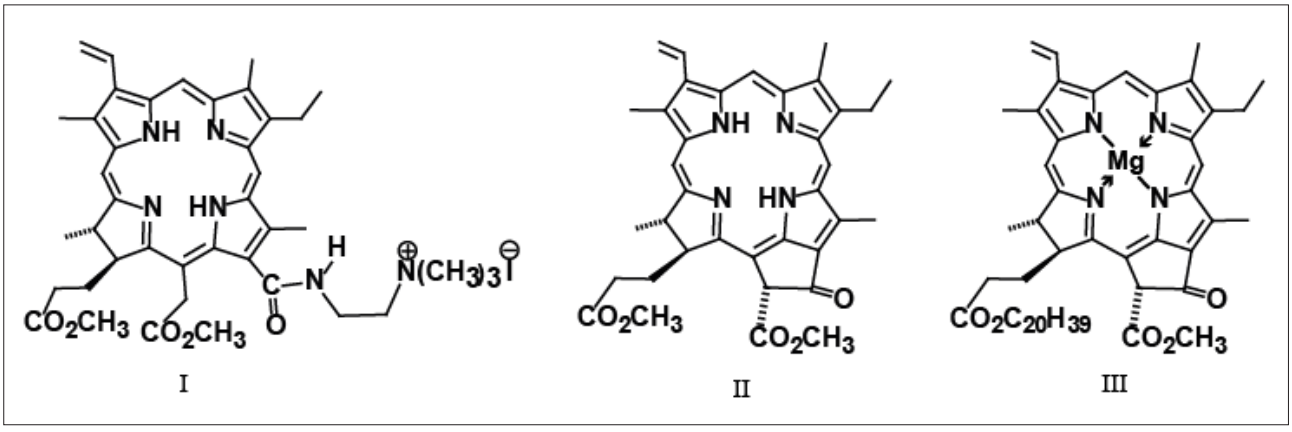

Рис. 1. Объект исследования и природные источники хлорина е6: I – хлорин е6 13(1)-N-(2-N'N'N'-триметиламмониоэтил иодид) амид 15(2), 17(3)-диметиловый эфир (соединение I); II – метилфеофорбид а; III – хлорофилл а.
Fig. 1. Objects of study and natural sources of chlorin e6: I – chlorin e6 13(1)-N-(2-N'N'N'-trimethylammonioethyl iodide) amide-15(2),17(3)-dimethyl ester (comp. I); II – methylpheophorbide a; III – chlorophyll a.

In a preliminary study of the drug in order to select doses for further determination of acute toxicity, 11 outbred female rats weighing from 200 to 230 g participated. The experiment was carried out in November - December.

Two rats were injected with a potential carrier (Tween-80) in 1 ml of 1% and 3% aqueous solution containing 10 and 30 mg of the substance, respectively.

PS solutions were prepared by weight as follows: a weighed portion of solid PS was mixed with the calculated amount of Tween-80, then double-distilled water was slowly added to the resulting viscous mass, the solution was homogenized by ultrasound (Sonopulse ultrasonic homogenizer (Bandelin, Germany)), after which the resulting solutions were centrifuged (3000 rpm) to remove air bubbles.

The thus prepared aqueous solution of the study drug containing various doses of PS in accordance with the table, as well as 1% of the solubilizer Tween-80, was injected into 9 rats (Nos. 1 - 9). The injection volume in all cases was 1 ml. In rats No. 7 and No. 9, the drug was injected intraperitoneally, the rest - in the tail vein (Table).

Animals that received injections of Tween-80, including in amounts exceeding the working concentration of the solubilizer (1%), did not show any behavioral changes during the entire observation period, which indicated a low toxicity of this biocompatible SAS.

In rats that received a cationic chlorin preparation intravenously at a dose of more than 75 mg/kg of body weight, ear hyperemia developed the next day, and no other features associated with the route of administration were noted. One animal (No. 9, which received a dose of 150 mg/kg) died on the second day, an autopsy was performed on the day of death. Euthanasia of the rest of the animals was performed 2 weeks after PS injection by a sharp displacement of the cervical vertebrae, followed by autopsy and sampling of the brain, lungs, heart, liver, and kidneys for histological examination.

Таблица

Величина доз ФС, введенного крысам на первом этапе исследования

Table

The amount of PS doses administered in rats at the first stage of the study

№ животного Number of animal	Доза (мг/кг массы тела) Dose (mg/kg of body weight)
1	5
2	10
3	20
4	30
5	50
6	75
7	100
8	125
9	150

The main experiment, which was conducted in May-June 2021, involved 15 outbred female rats (nursery: Andreevka branch of the Federal State Budgetary Institution of Science "Center for Biomedical Technologies" of the Federal Medical and Biological Agency) weighing from 190 to 220 g.

The animals were divided into 3 groups of 5 rats each. A solution of monocationic chlorin e6 was administered once intraperitoneally in the morning at the following doses: group I - 100 mg/kg, group II - 125 mg/kg, group III - 150 mg/kg of body weight with subsequent observation.

In all dead animals on the day of death, in survivors - 14 days after the injection of monocationic chlorin e6, an autopsy and sampling for subsequent histological examination of the brain, lungs, heart, liver, kidneys and spleen was performed. The slaughter was performed by a sharp displacement of the cervical vertebrae.

After the removal of organs, a histological examination of the autopsy material was performed. In groups II and III, all animals died after 1.5–3 days, in group I, 2 rats died before the end of the experiment, on the 4th and 6th days, respectively. Autopsy of dead animals was carried out within the first day. The three rats that remained alive in group I on the 14th day were subjected to simultaneous decapitation followed by an immediate post-mortem examination. During autopsy, a craniotomy was performed, the entire brain was removed and fixed in 10% neutral formalin solution. One day later, using frontal incisions, the zone of the precentral gyrus of the forebrain, the cerebellum, and the brain stem were isolated. When opening the chest and abdominal cavities, the heart, liver, kidneys, adrenal glands, and spleen were also removed in their entirety and subjected to primary fixation. After secondary fixation and washing of the organ fragments, the material was dehydrated using 99% isopropyl alcohol. Pieces of organs were embedded in paraffin, and histological sections 5–6 μm thick, made on a Microm sledge microtome, were stained with hematoxylin and eosin.

Morphological analysis was carried out on a research microscope "Micros" MS-200, micrographs were obtained using a digital ocular camera DCM 900.

Results and discussion

When conducting a preliminary study on the second day after the injection, a lethal outcome was recorded only in a rat that received the drug at a dose of 150 mg/

kg of body weight, the rest of the animals survived. During the main experiment, almost immediately after the injection, some deterioration in the general condition of the animals was observed: lethargy, drowsiness, muscle weakness. The severity of the described symptoms correlated with the dose of the test compound received and increased with its increase.

Over the next two days, all 10 animals of groups II and III died. In group I, after the administration of the drug, 2 rats died, the death was noted on the 3rd and 6th days. In another rat of group I, during the 5–6th day of the experiment, muscle weakness, shuddering, dishevelled and somewhat dulled hair were noted. In the rest of the animals of group I, no pronounced external changes were observed. Thus, in group I, 2 out of 5 rats died; the PS dose of 100 mg/kg of body weight can be considered close to LD_{50}. For more reliable conclusions, the number of observations should be increased.

During histological examination of the organs, the following data were obtained.

Group I (monocationic chlorin e6 at a dose of 100 mg/kg): in 3 rats that survived until the end of the experiment, moderately pronounced plethora of the postcapillary link of the microcirculatory bloodstream (MCB) was observed in the brain, accompanied by paretic expansion of venules, and perivascular edema of the nervous tissue of cerebral hemispheres. In the cortex of the precentral gyrus and in the cortex of the cerebellum, the stratification of layers is preserved, most of the pyramidal neurons and pear-shaped Purkinje cells with clear contours of the nuclei and cytoplasmic Nissl bodies with a uniform distribution of macroglial elements. The exception is brainstem neurons, some of which have the format of reversible ischemic damage in the form of a decrease in the volume of the cytoplasm, hyperchromia of nuclei, swelling of axons against the background of pericellular edema of the nervous tissue (Fig. 2).

At the macroscopic level, the cavity of the left ventricle of the heart is concentrically narrowed, the right ventricle is moderately dilated, contains liquid blood. Microscopic examination: the lumen of the MCB vessels are dilated, filled with erythrocytes, without signs of aggregation; contractile myocardial fibers of uniform color, with clear contours and cross striation.

Pasty lungs (doughy consistency), occupy 90% of the volume of the pleural cavities. In animals that died on the 3rd and 6th days from the beginning of the experiment, the microscopic picture is characterized by a moderately pronounced plethora of interalveolar septum, spasm of the bronchi of medium and small caliber, the lumens of which are partially or completely obstructed by mucus. In conditions of obstruction of the bronchial apparatus, the formation of foci of centric emphysema is observed (Fig. 3).

Moderately pronounced acute congestion is observed in the kidneys, the anses capillaires of the glomeruli contain erythrocytes, the lumen of the capsule is not expanded, the nephrocytes of the proximal and distal convoluted tubules are of the usual form with a uniform color of the cytoplasm, the lumen of the tubules is free.

Microscopic examination of the liver shows a moderately pronounced plethora of the central and portal veins, the histoarchitecture of the liver acini is preserved, the sinusoids are not dilated, with a free lumen, there are single histiocytes and lymphocytes in the stroma of the portal tracts. In the 2nd and 5th observations, periportal foci of fatty degeneration of hepatocytes with moderate lymphohistiocytic infiltration of the stroma are noted.

Group II (monocationic chlorin e6 at a dose of 125 mg/kg). In the study of the brain in all rats, signs of cir-

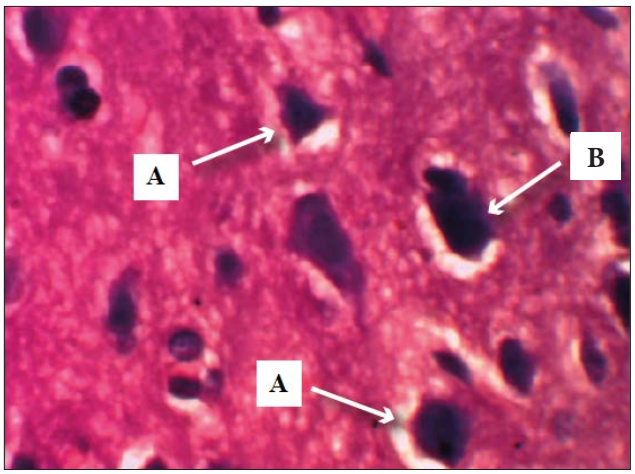

Рис. 2. Гистология. Плазмопикноз, гиперхромия ядер нейронов (А), набухание аксонального отростка нейрона (В). Окраска гематоксилином и эозином. Увеличение 1200.
Fig. 2. Histology. Plasmopyknosis, hyperchromia of neuron nuclei (A), swelling of the axonal process of a neuron (B). Stained with hematoxylin and eosin. Magnification 1200.

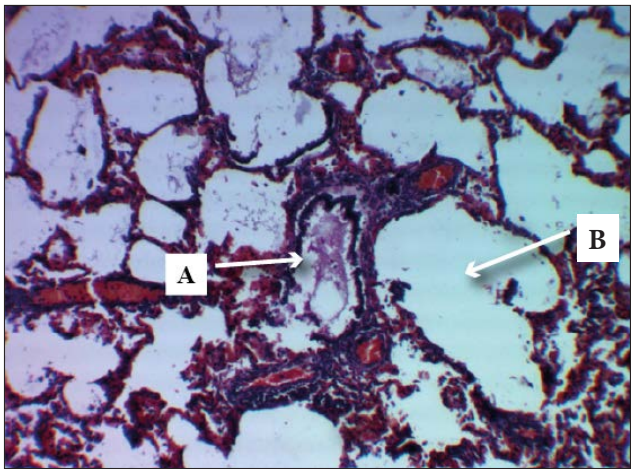

Рис. 3. Гистология. Просвет бронха обтурирован слизью (А), перибронхиальный очаг острой эмфиземы (В). Окраска гематоксилином и эозином. Увеличение 120.
Fig. 3. Histology. Bronchial lumen obturated with mucus (A), peribronchial focus of acute emphysema (B). Stained with hematoxylin and eosin. Magnification 120.

culatory disorders at the MCB level were revealed, which was expressed by aggregation of erythrocytes in the lumen of capillaries, moderately pronounced plethora and dilatation of venules, perivascular and pericellular edema of the nervous tissue (Fig. 4). Focal damage to pyramidal neurons of the cerebral cortex and Purkinje cells (pear-shaped neurons of the cerebellum) was observed, which was expressed by swelling of neurocytes, karyolysis, destruction of cytoplasmic organelles with a perifocal reaction of microglia. Brainstem neurons showed signs of ischemic changes in the form of nuclear hyperchromia with loss of nucleolar contours and a decrease in cell volume.

In the examination of the heart, there was an expansion of the cavities of the right and left ventricles, microscopically in all observations there was a violation of hemocirculation at the level of the microcirculatory bloodstream, which was characterized by aggregation of erythrocytes in the capillaries, plethora of venules, edema of the myocardial stroma. In the subendocardial parts of the myocardium of the left and right ventricles, foci of overcontraction (contractures) were found in the form of wavy contractile fibers with uneven coloring of the cytoplasm of cardiomyocytes.

Acute plethora, edema of the interalveolar septum with deformation and a decrease in the volume of the alveoli were observed in the lungs of experimental animals (Fig. 5).

Under conditions of significant extensive spasm of bronchial tubes and bronchioles, a violation of vascular permeability led to effusion and accumulation of fibrin on the bronchial mucosa in the form of "hyaline membranes".

In the study of the kidneys, moderate plethora of all sections is noted, in 4 observations the nephrocytes of the proximal convoluted tubules were in a state of hydropic (protein) degeneration, tubule lumen were narrowed.

In the liver, acute congestion was expressed by changes mainly in the centers of liver acini, where the central vein and sinusoids of the precentral zone were filled with blood and expanded. Hydropic degeneration of hepatocytes was focal in nature, histoarchitecture of the liver acini was preserved.

Changes in the spleen were characterized by a moderately pronounced plethora of red pulp.

Group III (monocationic chlorin e6 at a dose of 150 mg/kg). In the brain, against the background of hemostasis in the microcirculatory bloodstream vessels, pronounced perivascular and pericellular edema, neuronal damage was characterized by a decrease in cell volume, nuclear hyperchromia, and redistribution of Nissl bodies in the cytoplasm. In the brainstem, changes in single neurons were irreversible with signs of cell necrosis in the form of karyolysis, fragmentation of the cytoplasm, and perifocal reaction of microglia (Fig. 6).

A macroscopic assessment of the heart revealed that the ventricular cavities were dilated, contained liquid blood, the myocardium had a flabby consistency, at the microscopic level, stasis of erythrocytes in capillaries, plethora of intramural veins, and myocardial stromal edema were observed. Focal contractile fibers had wavy changes, individual cardiomyocytes acquired a basophilic color.

The study of the lung tissue showed the presence of a pronounced plethora of the lungs stroma, against which the exudate of plasma proteins led to the formation of eosinophilic films (like hyaline membranes) on the inner surface of the alveoli and bronchial tubes. A pronounced spastic state

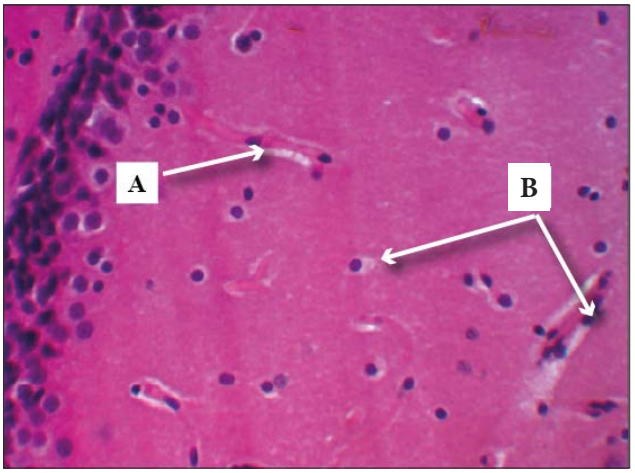

Рис. 4. Гистология. Гемостаз в капиллярах (А), периваскулярный и перицеллюлярный отек нервной ткани (В). Окраска гематоксилином и эозином. Увеличение 480.
Fig. 4. Histology. Hemostasis in the capillaries (A), perivascular and pericellular edema of the nervous tissue (B). Stained with hematoxylin and eosin. Magnification 480.

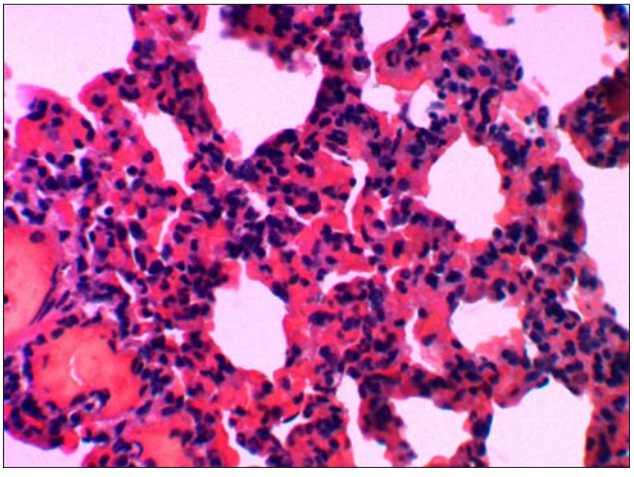

Рис. 5. Гистология. Острое полнокровие легкого, деформация альвеол. Окраска гематоксилином и эозином. Увеличение 480.
Fig. 5. Histology. Acute plethora of the lung, deformation of the alveoli. Stained with hematoxylin and eosin. Magnification 480.

of the bronchi is accompanied by peribronchial cuffing by lymphocytes with single eosinophils (Fig. 7).

In the kidneys against the background of acute venous plethora, hydropic degeneration of the proximal tubules epithelium has become widespread.

Morphological assessment of liver tissue in group III showed pronounced venous plethora of all parts of the liver acinus in the absence of significant damage from hepatocytes.

Changes in the spleen were comparable to the histological picture of plethora in groups I and II.

Thus, changes in the brain, lungs and heart turned out to be morphologically significant. Apparently, the pronounced vasopathic effect of cationic chlorin was the main link in the pathogenesis, as a result of which edema and swelling of the brain progressed. The development of acute vascular encephalopathy was manifested by an increase in general neurological symptoms (at the stage of clinical observations) and correlated with the dose of the administered drug. Irreversible changes in the neurons of the brainstem revealed in rats of groups II and III indicate the development of a dislocation syndrome, which can be considered as the main cause of the death of experimental animals. Pathological evaluation of the lungs revealed widespread bronchospasm with mucus hypersecretion, the severity of which augmented with increasing dose of the drug. A picture of an acute allergic reaction is formed in combination with eosinophilic infiltration of the bronchial walls. The increased permeability of the vascular wall caused the formation of fibrin overlays on the inner surface of the alveoli and small bronchi (like hyaline membranes). Based on the totality of the described changes, it is possible to draw a conclusion about the development of acute respiratory distress syndrome, which has a certain significance in thanatogenesis. Reversible changes in the myocardium are most likely the result of exposure to an arrhythmogenic factor that is formed upon administration of the tested PS. The described morphological changes in the liver, kidneys and spleen in experimental animals are stereotyped in case of intoxication of various nature.

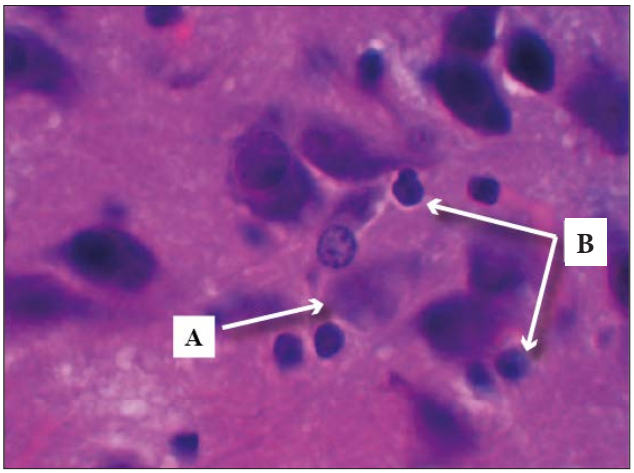

Рис. 6. Гистология. Погибший нейрон продолговатого мозга (A), перифокальная реакция микроглиальных элементов (B). Окраска гематоксилином и эозином. Увеличение 1200.
Fig. 6. Histology. Dead medulla neuron (A), perifocal reaction of microglial elements (B). Stained with hematoxylin and eosin. Magnification 1200.

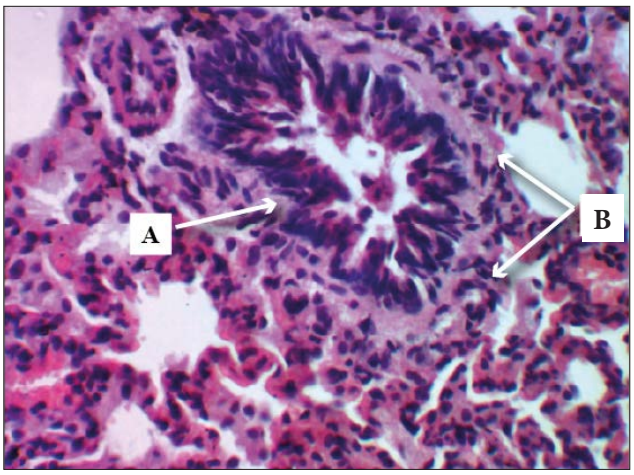

Рис. 7. Гистология. Выраженный бронхоспазм (A), эозинофилы в составе лейкоцитарного инфильтрата (B). Окраска гематоксилином и эозином. Увеличение 480.
Fig. 7. Histology. Severe bronchospasm (A), eosinophils in the leukocyte infiltrate (B). Stained with hematoxylin and eosin. Magnification 480.

Conclusion

1. The value of 100 mg/kg body weight can be considered as a preliminary value of LD_{50} for the studied monocationic chlorin (compound I). It is almost two times higher than that of anionic PS used in clinical practice, however, from a toxicological point of view, it is quite acceptable for continuing preclinical studies, since during PDT the dose of administered PS is usually in the range from 1 to 5 mg/kg. To obtain a more accurate LD_{50} value, the number of laboratory animals should be increased.

2. Intraperitoneal administration of the studied PS in toxic doses causes predominant damage to the brain, lungs, and myocardium.

3. Pathological changes in the vital organs of laboratory animals indicate a pronounced vasopathic effect of compound I with the development of cerebral edema and respiratory distress syndrome, which were the main links of thanatogenesis.

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