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ABSTRACT

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CHANGES IN HEMATOLOGICAL PARAMETERS DURING THE TREATMENT OF EXPERIMENTAL PURULENT GENERALIZED PERITONITIS WITH AG/CU NANOCOMPOSITES AND LOW-FREQUENCY ULTRASOUND

Introduction. Generalized purulent peritonitis remains a serious challenge for modern emergency abdominal surgery. Previous studies have confirmed that the combined use of Ag/Cu nanocomposites (Ag/Cu NCs) with low-frequency ultrasound (US) improves the treatment outcomes of generalized purulent peritonitis; however, it is important to examine hematological indicators that can show the possible toxic effect of Ag/Cu NCs as well as their impact on the course of the general infectious-inflammatory process. Aim: to investigate the dynamics of the hematological parameters in rats during the treatment of experimental generalized purulent peritonitis with Ag/Cu NCs and low-frequency US.

Methods. The study was conducted using an experimental model of generalized purulent peritonitis in 60 laboratory Wistar rats. The rats were divided into four groups: in the 1st group planned sanitation of the abdominal cavity was carried out with Ag/Cu NCs, in the 2nd group – with low-frequency US, in the 3rd group – with Ag/Cu NCs and low-frequency US, and in the 4th group, as the control – with a 0.05% chlorhexidine solution. During the experiment, various hematological indicators in the rats were examined, including hemoglobin and hematocrit levels, erythrocyte sedimentation rate (ESR), erythrocyte, leukocyte, and platelet counts, as well as the percentage of different types of blood cells: band and segmented neutrophils, eosinophils, monocytes, basophils, and lymphocytes. Additionally, the leukocyte shift index (LSI), immunoreactivity index (IRI), and nuclear intoxication index (NII) were calculated.

Results and discussion. A detailed study of the general blood test reveals that the use of Ag/Cu NCs in combination with low-frequency

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US enables faster normalization of the main hematological indicators, indicating an accelerated recovery of experimental rats with a generalized purulent peritonitis. From the 3rd day, significant differences were noted in the percentage of monocytes and segmented neutrophils, as well as the indices IRI and LSI, in the “Ag/Cu NCs + US” group compared to the control group ($p < 0.05$). From the 7th day, a significant decrease in the number of leukocytes, ESR, percentage of monocytes, eosinophils, band and segmented neutrophils was noted in the “Ag/Cu NCs + US” group ($p < 0.05$), indicating a decrease in inflammatory phenomena. On the 7th day, a significant increase in IRI and a decrease in LSI were observed in the “US” group, as well as a first decrease in NII in the “Ag/Cu NCs + US” group ($p < 0.05$).

Conclusions. The combined use of Ag/Cu NCs and low-frequency US accelerates the normalization of key hematological markers associated with inflammation, enhances the body's immune response, and reduces the manifestations of systemic endotoxicosis in the treatment of experimental purulent generalized peritonitis. The application of Ag/Cu NCs does not result in significant undesirable changes in the hematological indicators of Wistar rats, indicating a lack of acute general toxicity.

Keywords: peritonitis, Ag/Cu nanocomposites, low-frequency ultrasound, general blood test.

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ДИНАМІКА ГЕМАТОЛОГІЧНИХ ПОКАЗНИКІВ ПІД ЧАС ЛІКУВАННЯ ЕКСПЕРИМЕНТАЛЬНОГО ПОШИРЕНОГО ГНІЙНОГО ПЕРИТОНІТУ НАНОКОМПОЗИТАМИ AG/CU ТА НИЗЬКОЧАСТОТНИМ УЛЬТРАЗВУКОМ

Поширений гнійний перитоніт залишається серйозною проблемою в сучасній невідкладній абдомінальній хірургії. Попередні дослідження підтвердили, що комбіноване застосування нанокомпозитів Ag/Cu (НК Ag/Cu) з низькочастотним ультразвуком (УЗ) покращує результати лікування поширеного гнійного перитоніту; проте важливо дослідити гематологічні показники, які можуть свідчити за можливу токсичну дію НК Ag/Cu, а також їх вплив на перебіг загального інфекційно-запального процесу. Мета: дослідити динаміку гематологічних показників у щурів під час лікування експериментального поширеного гнійного перитоніту НК Ag/Cu та низькочастотним УЗ.

Матеріали та методи досліджень. Дослідження проводилося з використанням експериментальної моделі поширеного гнійного перитоніту на 60 лабораторних щурах лінії Вістар. Щури були розподілені на чотири групи: у 1-й групі планову санацію черевної порожнини проводили за допомогою НК Ag/Cu, у 2-й групі – за допомогою низькочастотного УЗ, у 3-й групі – за допомогою НК Ag/Cu та низькочастотного УЗ, а в 4-ій, контрольній групі – за допомогою 0,05 % розчину хлоргексидину. Під час експерименту у щурів досліджували наступні гематологічні показники: гемоглобін, гематокрит, швидкість осідання еритроцитів (ШОЕ), кількість еритроцитів, лейкоцитів та тромбоцитів, а також відсотки різних типів клітин крові: паличкоядерних та сегментоядерних нейтрофілів,

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еозинофілів, моноцитів, базофілів та лімфоцитів. Додатково розраховували індекс зсуву лейкоцитів крові (ІЗЛК), індекс імунореактивності (ІІР) та ядерний індекс ступеню ендотоксикозу (ЯІСЕ).

Результати та обговорення. Детальний аналіз показників загального аналізу крові показав, що використання НК Ag/Cu у поєднанні з низькочастотним УЗ дозволяє швидше нормалізувати основні гематологічні показники, що свідчить про прискорене одужання піддослідних щурів із поширеним гнійним перитонітом. Починаючи з 3-ої доби відзначалися достовірні відмінності у відсотковому співвідношенні моноцитів та сегментоядерних нейтрофілів, а також в індексах ІІР та ІЗЛК у групі «НК Ag/Cu + УЗ» порівняно з контрольною групою ($p < 0.05$). Починаючи з 7-ої доби, у групі «НК Ag/Cu + УЗ» відзначалося достовірне зниження кількості лейкоцитів, ШОЕ, відсоткового співвідношення моноцитів, еозинофілів, паличкоядерних та сегментоядерних нейтрофілів ($p < 0.05$), що свідчить про зменшення запальних явищ. На 7-у добу спостерігалося достовірне збільшення ІІР та зниження ІЗЛК у групі «УЗ», а також було вперше відмічено зниження ЯІСЕ у групі «НК Ag/Cu + УЗ» ($p < 0.05$).

Висновки. Поєднане застосування НК Ag/Cu та низькочастотного УЗ прискорює нормалізацію основних гематологічних маркерів, пов'язаних із запаленням, посилює імунну відповідь організму та зменшує прояви системного ендотоксикозу при лікуванні експериментального поширеного гнійного перитоніту. Застосування НК Ag/Cu не призводить до суттєвих небажаних змін в гематологічних показниках щурів Вістар, що свідчить за відсутність гострої загальної токсичної дії.

Ключові слова: перитоніт, нанокомпозити Ag/Cu, низькочастотний ультразвук, загальний аналіз крові.

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INTRODUCTION

Generalized purulent peritonitis remains a serious challenge for modern emergency abdominal surgery. The course of acute surgical diseases is complicated by peritonitis in 15-25% [1]. Despite the progress of modern surgery, postoperative mortality in peritonitis continues to remain high, 24-35%, and with the development of multiple organ failure syndrome, it reaches 80-100% [2, 3]. Treatment of peritonitis is further complicated by the presence of antibiotic-resistant microorganisms [4].

Qualitative sanitation of the abdominal cavity plays a crucial role in the comprehensive treatment of purulent peritonitis. The use of sanitation programmed relaparotomy remains an effective method of treating intra-abdominal infections [5]. The quality of abdominal cavity sanitation has a significant impact on the progression of the disease and the patient's recovery. However, the sanitation of the abdominal cavity in cases of generalized peritonitis does not always allow for the complete elimination of the focus of intra-abdominal infection, especially when multiresistant microflora is added. Existing antiseptic solutions do not always produce the expected positive effect, which prompts us to seek new methods and treatment approaches that ensure adequate sanitation of the abdominal cavity and effectively impact its microflora. Nanostructured silver and copper exhibit a wide range of antibacterial properties, including those against multi-resistant microorganisms [6, 7]. Moreover, when used in combination, they display enhanced synergistic antibacterial action [8, 9]. Their high effectiveness in treating purulent surgical diseases has been repeatedly reported [10]. Low-frequency ultrasound (US) has undeniable advantages in the sanitation of purulent foci, as it helps cleanse purulent-necrotic tissues, exhibits anti-inflammatory properties, stimulates reparative processes, enhances the effect of antibacterial agents, and facilitates the direct delivery of active substances to the focus of inflammation [11–13].

Previous studies have confirmed that the combined use of Ag/Cu nanocomposites (Ag/Cu NCs) with low-frequency US improves the treatment outcomes of generalized purulent peritonitis [14]. But a mandatory condition for determining the pharmacological effectiveness of a drug is the study of its safety. Undesirable side effects of many antimicrobial agents include their toxic effects on the blood system, which manifest as the development of anemia, thrombocytopenia, leukopenia, agranulocytosis, and other conditions. In addition to studying the possible toxic effects, it is also advisable to examine the dynamics of hematological indicators, which reflect the stages of the general infectious-inflammatory process.

Objective: to investigate the dynamics of the hematological parameters in Wistar rats during the treatment of experimental generalized purulent peritonitis with Ag/Cu NCs and low-frequency US.

MATERIALS AND METHODS

All rat handling and experimental procedures fully adhere to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0. and European Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes. The experiment was conducted in accordance with the "General Ethical Principles of Animal Experiments" approved by the First National Congress on Bioethics (Kyiv, 2001) and the World Medical Association Declaration of Helsinki (2000). All procedures performed in the study were approved by the Commission on Bioethics in Experimental and Clinical Research of SumDU (protocol No. 1/02, 18 February 2025).

Modeling of experimental purulent generalized peritonitis was performed as described earlier [14]. All experimental animals were divided into 4 groups.

In the 1st experimental group (20 rats), planned sanitation of the abdominal cavity was performed using a 0.005% solution of Ag/Cu NCs through minilaparotomy access. Ag/Cu NCs (73.2±16.7 nm) were synthesized via the US-assisted polyol synthesis at the Department of Theoretical and Applied Chemistry of SSU.

In the 2nd experimental group (20 rats), planned sanitation of the abdominal cavity was performed through minilaparotomy access using low-frequency US (surgical device URSK 7N–22, frequency: 26.5 kHz, power: 0.2 W/cm², current strength: 10–12 mA) with saline solution.

In the 3rd experimental group (20 rats), planned sanitation of the abdominal cavity was carried out through minilaparotomy access using low-frequency US (surgical device URSK 7N–22) with 0.005% solution of Ag/Cu NCs.

In the 4th, control group (20 rats), routine sanitation of the abdominal cavity was performed using a 0.05% aqueous chlorhexidine solution through minilaparotomy access.

During the experiment, the following hematological parameters of rats were evaluated: hemoglobin, erythrocytes, leukocytes, erythrocyte sedimentation rate (ESR), platelets, hematocrit, percentages of band and segmented neutrophils, eosinophils, monocytes, basophils, and lymphocytes. Hemoglobin, hematocrit, erythrocytes, and leukocytes were determined on a hematological analyzer "ELite 3" (ERBA, Czech Republic). ESR was determined by the Westergren method, and platelets were counted by the Fonio method. The leukocyte formula was determined by the manual method with blood smear microscopy.

Leukocyte shift index (LSI), immunoreactivity index (IRI), and nuclear intoxication index (NII) were calculated using the formulas:

$LSI = (E + B + \Sigma N) / (M + L)$;

$IRI = (L + E) / M$;

$NII = (M + BNs) / SNs$;

where: E – eosinophils (%), B – basophils (%), N – neutrophils (%), M – monocytes (%), L – lymphocytes (%), BNs – band neutrophils (%), SNs – segmented neutrophils (%).

One-way ANOVA with multiple comparisons was used to assess the difference between groups using GraphPad Prism 9.0 software, where a p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

No differences were observed in the hematological parameters of the experimental rats on the 1st day of the experiment. Leukocytosis with a percentage predominance of band neutrophils, segmented neutrophils, and monocytes was observed in all groups. (Table 1).

On the 3rd day of the experiment, a decrease in the level of leukocytes, platelets, ESR, monocytes, band and segmented neutrophils was noted in all groups, but without a statistically significant difference (Table 2, $p > 0.05$). The percentage of monocytes was significantly lower in the "Ag/Cu + US" group compared to the control group ($p < 0.05$). In the "Ag/Cu + US" group, an increase in IRI was noticeable on the 3rd day due to an increase in the percentage of lymphocytes (Table 2, $p < 0.05$), which indicates the activation of the immune response to inflammation (Fig. 1, $p < 0.05$). Additionally, a decrease in LSI was observed in the same group, indicating the onset of a decline in inflammatory process activity (Fig. 1, $p < 0.05$).

Table 1 – Hematological parameters in rats on the 1st day of the experiment

Indicators	Groups			
	Ag/Cu NCs	**US**	**Ag/Cu NCs + US**	**Chlorhexidine**
Hemoglobin (g/l)	103,2±6,4	105.3±7.5	106.6±7.7	104.4±10.1
Erythrocytes (10^{12}/l)	5.1±1.3	5.2±1.4	4.9±1.1	5.4±1.3
Leukocytes (10^9/l)	13.4±2.3	12.5±2.5	14.1±2.7	13.6±2.4
ESR (mm/h)	3.3±1.5	3.8±1.6	3.4±1.5	3.5±1.7
Platelets (10^9/l)	725.5±102.9	762.3±113.2	733.6±93.5	751.3±106.4
Hematocrit (%)	38.1±4.5	36.8±6.1	37.7±7.1	37.2±5.0
Band neutrophils (%)	11.6±2.7	11.7±3.3	10.8±3.4	12.1±4.1
Segmented neutrophils (%)	48.9±6.7	46.3±7.2	45.7±7.8	46.9±5.2
Eosinophils (%)	0.5±0.3	0.4±0.2	0.4±0.2	0.5±0.3
Monocytes (%)	15.2±3.5	16.5±3.2	15.8±2.6	16.0±2.4
Basophils (%)	0.7±0.3	0.8±0.4	0.9±0.4	0.6±0.2
Lymphocytes (%)	23.1±3.8	24.3±4.2	26.4±4.3	23.9±3.9

Table 2 – Hematological parameters in rats on the 3rd day of the experiment

Indicators	Groups			
	Ag/Cu NCs	**US**	**Ag/Cu NCs + US**	**Chlorhexidine**
Hemoglobin (g/l)	105.4±7.5	101.5±8.7	102.6±6.9	103.3±6.2
Erythrocytes (10^{12}/l)	4.9±1.2	5.0±1.4	5.1±1.2	5.2±1.3
Leukocytes (10^9/l)	12.7±2.4	12.0±2.3	11.4±2.6	12.9±2.8
ESR (mm/h)	3.2±1.1	3.5±1.6	3.0±1.5	3.2±1.2
Platelets (10^9/l)	658.6±98.6	675.4±101.7	632.9±95.0	644.3±112.6
Hematocrit (%)	36.4±5.3	34.2±5.1	32.1±4.0	35.6±4.6
Band neutrophils (%)	10.7±2.5	10.1±2.6	9.6±1.9	10.9±2.4
Segmented neutrophils (%)	45.6±5.6	40.7±6.1	38.5±5.7	44.7±4.3
Eosinophils (%)	0.8±0.4	0.9±0.3	1.2±0.5	0.7±0.4
Monocytes (%)	13.6±2.9	12.7±2.8	10.1±1.9 *	15.7±3.2
Basophils (%)	1.0±0.4	0.7±0.3	0.8±0.5	1.1±0.6
Lymphocytes (%)	23.3±4.1 #	34.9±5.0	39.8±5.2 *	26.9±3.3

*Notes: * – statistical difference compared to the "Chlorhexidine" group, $p < 0.05$*

On the 7th day of the experiment, a significant decrease in the number of leukocytes, ESR, percentage of monocytes, band and segmented neutrophils was noted in the "Ag/Cu NCs + US" group, which indicates a decrease in inflammatory phenomena in the abdominal cavity (Table 3, $p < 0.05$). After 7 days from the start of treatment, an increase in circulating eosinophil count was first noted in "Ag/Cu NCs + US" and "US" groups (Table 3, $p < 0.05$). In the "Ag/Cu NCs" group, a decrease in segmented neutrophils was noted compared to the control group, as well as higher indicators of leukocytes, monocytes, and segmented neutrophils compared to the "Ag/Cu NCs + US" group (Table 3, $p < 0.05$), which indicates an active, ongoing inflammatory process. A smaller shift in the leukocyte formula (LSI) and an increase in immunoreactivity (IRI) were observed in groups "Ag/Cu NCs + US" and "US" in contrast to the control group; however, a decrease in systemic endotoxicosis (NII) was noted only in group "Ag/Cu NCs + US" (Fig. 1, 2, 3, $p < 0.05$).

On the 14th day of the experiment, normalization of hematological parameters continued in all studied groups, as indicated in Table 4. However, the most significant changes were observed in 2nd and 3rd groups, where low-frequency US was applied. It is noteworthy that in these same groups, a significant decrease in platelet levels was observed for the first time. The IRIs showed significantly higher values in all studied groups compared to the control, while a decrease in indices LSIs and NIIs was observed only for "Ag/Cu NCs + US" and "US" groups (Fig. 1, 2, 3, $p < 0.05$).

Table 3 – Hematological parameters in rats on the 7th day of the experiment

Indicators	Groups			
	Ag/Cu NCs	US	Ag/Cu NCs + US	Chlorhexidine
Hemoglobin (g/l)	104.5±6.4	101.6±7.6	109.9±8.1	105.4±7.7
Erythrocytes (10^{12}/l)	5.1±1.2	5.1±1.3	5.5±0.8	5.4±0.6
Leukocytes (10^{9}/l)	10.4±1.6 #	8.2±1.1	6.5±1.4 *	10.6±2.3
ESR (mm/h)	2.8±0.7	2.5±0.5	2.1±0.6 *	3.3±0.5
Platelets (10^{9}/l)	624.2±80.3	587.9±79.4	540.3±77.1	612.6±85.6
Hematocrit (%)	34.6±4.1	36.4±5.0	34.7±4.2	35.5±3.6
Band neutrophils (%)	6.3±1.2 #	4.8±0.8 *	3.1±0.6 *	8.7±1.5
Segmented neutrophils (%)	28.9±3.8 * #	24.6±3.4 * #	17.9±2.8 *	38.8±5.7
Eosinophils (%)	0.9±0.3 #	1.3±0.2 *	1.6±0.3 *	0.8±0.2
Monocytes (%)	10.3±2.2 #	7.6±1.0 *	5.4±1.6 *	12.7±1.8
Basophils (%)	1.0±0.2	0.9±0.2	1.3±0.3	1.2±0.2
Lymphocytes (%)	52.6±5.7 * #	60.8±4.6 * #	70.7±5.1 *	37.8±3.5

*Notes: * – statistical difference compared to the "Chlorhexidine" group, $p \leq 0.05$; # – statistical difference compared to the "Ag/Cu NCs + US" group, $p < 0.05$*

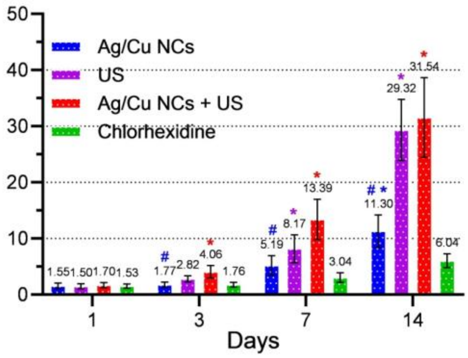

Fig. 1 – IRI indicators in the studied groups

** – statistical difference compared to the control group;*
 # – statistical difference compared to the "Ag/Cu NCs + US" group, $p < 0.05$

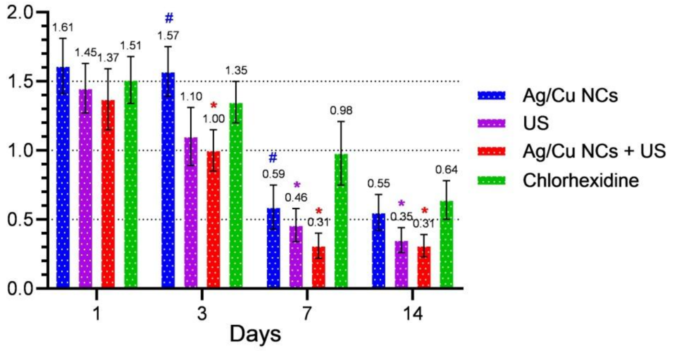

Fig. 2 – LSI indicators in the studied groups

** – statistical difference compared to the control group;*
 # – statistical difference compared to the "Ag/Cu NCs + US" group, $p < 0.05$

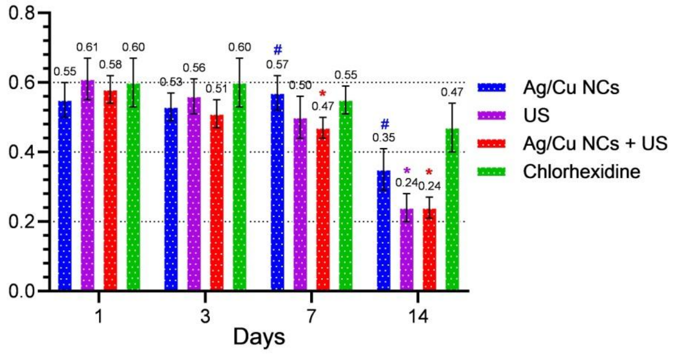

Fig. 3 – NII indicators in the studied groups

** – statistical difference compared to the control group;*
 # – statistical difference compared to the "Ag/Cu NCs + US" group, $p < 0.05$

Table 4 – Hematological parameters in rats on the 14th day of the experiment

Indicators	Groups			
	Ag/Cu NCs	**US**	**Ag/Cu NCs + US**	**Chlorhexidine**
Hemoglobin (g/l)	105.8±6.4	103.6±6.7	111.7±6.9	104.1±7.0
Erythrocytes (10^{12}/l)	5.8±0.8	6.1±1.1	5.7±0.8	5.9±0.7
Leukocytes (10^{9}/l)	7.6±1.2 #	4.6±0.7 * #	3.2±0.6 *	8.4±1.5
ESR (mm/h)	1.8±0.3 * #	1.3±0.2 *	1.1±0.2 *	2.6±0.4
Platelets (10^{9}/l)	447.3±73.7	383.6±64.6 *	372.2±41.8 *	521.2±64.7
Hematocrit (%)	37.5±4.2	39.2±6.3	41.0±5.6	38.8±6.5
Band neutrophils (%)	4.4±1.3 #	3.4±0.5 * #	2.1±0.4 *	5.9±1.1
Segmented neutrophils (%)	28.3±4.7 #	20.6±4.1 *	18.5±3.6 *	31.0±5.0
Eosinophils (%)	1.6±0.3	1.7±0.4	1.5±0.5	1.2±0.3
Monocytes (%)	5.4±1.1 * #	2.5±0.5 *	2.4±0.7 *	8.8±1.2
Basophils (%)	0.9±0.3	1.2±0.2	1.3±0.4	1.1±0.3
Lymphocytes (%)	59.4±4.9 #	71.6±6.2 *	74.2±5.8 *	52.0±5.3

*Notes: * – statistical difference compared to the "Chlorhexidine" group, $p \le 0.05$; # – statistical difference compared to the "Ag/Cu NCs + US" group, $p < 0.05$*

The dynamics of hematological parameters in rats correlate with the recovery dynamics of various treatment methods for generalized purulent peritonitis, as previously described [14]. The combined use of Ag/Cu NCs and low-frequency US resulted in faster recovery and improved key hematological markers associated with systemic bacterial inflammation, including monocytes and lymphocytes from day 3, and leukocytes, ESR, eosinophils, band and segmented neutrophils from day 7. Changes in the calculated indices indicate a significant increase in immune reactivity and the resolution of systemic endotoxicosis with this treatment method.

Reduced hemoglobin levels were observed throughout the treatment period as a consequence of the surgery. Over 14 days, no significant increase in hemoglobin levels was observed in any group, a consequence of reduced erythropoiesis against the background of the inflammatory process, which is a hallmark of anemia of inflammation. This phenomenon can be explained by the following mechanisms: cytokine-mediated suppression of erythroid progenitors (IL-6, TNF-α, IL-1β, and IFN-γ inhibit erythroid progenitor proliferation and induce apoptosis of erythroblasts), altered bone marrow microenvironment (the disruption of erythroblastic islands impairs erythroid maturation and survival), reduced responsiveness to erythropoietin (inflammation decreases erythropoietin synthesis in the kidneys and also reduces erythroid precursor sensitivity to erythropoietin), and shift toward granulopoiesis (hematopoietic progenitors are redirected from erythroid to myeloid lineages) [15–19]. Elevated platelet counts in all groups are a consequence of blood loss and non-specific inflammation following traumatic and infectious factors [20–21]. The percentage of hematocrit and basophils did not differ between sick and recovering animals, and it did not depend on the method of treatment.

Previously, we did not observe a pattern in eosinophil dynamics during the treatment of experimental purulent wounds [22], in contrast to the present study of generalized peritonitis, which reports a more pronounced systemic inflammatory response. Therefore, eosinopenia is a marker that reflects the systemic inflammatory burden, and the dynamics of eosinophils, along with other blood parameters, correlate (to a lesser extent) with the severity of generalized peritonitis and recovery during the treatment.

CONCLUSIONS

The combined use of Ag/Cu NCs and low-frequency US accelerates the normalization of key hematological markers associated with inflammation, enhances the body's immune response, and reduces the manifestations of systemic endotoxicosis in the treatment of experimental purulent generalized peritonitis. Additionally, the application of Ag/Cu NCs does not result in significant undesirable changes in the hematological indicators of Wistar rats, indicating a lack of acute general toxicity.

PROSPECTS FOR FUTURE RESEARCH

To comprehensively assess healing of the experimental generalized purulent peritonitis, a study of the biochemical and immunological blood parameters in laboratory rats is essential.

AUTHOR CONTRIBUTIONS

P.M. proposed the idea, formulated overarching research goals and aims, and conducted experiments on laboratory animals; conducted a statistical analysis of the studies, prepared and wrote the initial draft of the published work (including substantive translation); edited and approved the final version of the manuscript. I.D. was responsible for the management and coordination of the research activity planning and execution; developed the design and the main stages of the experiment; provided financial support for the project leading to this publication; and critically revised the article; edited and approved the final version of the manuscript. T.I. proposed a hypothesis regarding the effectiveness of Ag/Cu NCs against MDR bacteria in vivo; conducted experiments on laboratory animals; prepared bacterial cultures for the experiment; edited and approved the final manuscript version. R.P. synthesized Ag/Cu NCs and prepared them in the required concentrations; prepared tables 1, 2, and 3; wrote and edited sections Materials and Methods; edited and approved the final version of the manuscript. I.M. conducted research in a clinical diagnostic laboratory; wrote and edited the section Results, edited and approved the final version of the manuscript. V.H. conducted laboratory experiments; made a critical review, commentary, and revision of the manuscript; edited and expanded the literature review and discussion; prepared figures 1, 2, and 3; edited and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ARTIFICIAL INTELLIGENCE DISCLOSURE

No artificial intelligence technologies were used during manuscript writing or editing.

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