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PHARMACOLOGICAL EFFECTS OF THE SYNTHETIC ANALOGUE OF ANTIMICROBIAL PEPTIDE OF INDOLICIDIN 21 ON THE REGENERATION OF THERMAL WOUNDS IN THE EXPERIMENT

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Abstract

Introduction: Burn injuries in developed countries are among the most spread injuries of peacetime. The growth of resistance of microorganisms to existing antibiotics makes it necessary to develop some new generation antimicrobial drugs in the coming years. One possible way to solve the problem is to create drugs based on antibiotic peptides.

Methods: There were studied the effects of the use of the synthetic analogue of indolicidin 21 on the processes of regeneration of thermal wounds, the systemic inflammatory response, the functional activity of neutrophils and macrophages, lipid peroxidation processes for IIIA-B degree burns and II-III degree frostbites in rats.

Results: In the course of the study, we have established some stimulating effects of the synthetic analogue of the natural antimicrobial peptide indolicidin 21 on the processes of reparative skin regeneration after a local skin burn IIIA-B and a local frostbite of II-III degree, which are manifested in the acceleration of healing and prevention of purulent complications of thermal wounds. The use of the analogue of the indolicidin 21 has positive effects both on the functional activity of granulocytes of blood and macrophages, and on the systemic inflammatory reaction. The use of the synthetic analogue leads to an increase in the concentration of lipid peroxidation products in the blood plasma and skin supernatant throughout the experiment after a local burn, and up to 10 days after a local frostbite. The activity of antioxidant enzymes under the influence of the synthetic analogue of indolicidin was higher than the control values.

Conclusion: The study has revealed that the use of synthetic analogue of indolicidin 21 is an effective method of pharmacological correction of reparative processes in the skin with thermal damage. It was also found that the most effective use of the synthetic analogue of the peptide was with the dose of 500 μg/kg.

Keywords: burn; frostbite; antimicrobial peptide; indolicidin; lipid peroxidation; systemic inflammatory reaction.

Introduction

Burn injury is one of the most common types of peacetime injuries in developed countries. According to the data of the World Health Organization burns holds third place in the structure of injuries. Thermal damage accounts for 6% of all injuries, while the number of victims in industrialized countries is constantly increasing [1]. Annually in Russia recorded more than 800 000 cases of burns of varying severity, their frequency being 300-350 cases per 10 000 population [2]. The mortality for burns ranged from 1.5 to 5.9%, 85-90% of people with burns – people of working age and children [3].

Cold injury refers to the category of thermal damage and can manifest itself as a general cooling and frostbite of a limited area of the body, usually extremities. Among injuries, cold damage ranged from 1-2% in regions with a temperate climate, for the regions of Siberia and the Far North the frequency of frostbite in the structure of injury is 6-10% [4].

Treatment of patients with frostbites today is difficult, complex and multifaceted medical and social problem. The severity of complications and the high incidence of disabilities in thermal trauma give to the problem a social significance, which is especially important for residents of the Siberian, Far Eastern and Northern regions of Russia, where frostbites can be considered as endemic states [5, 6, 7].

The most formidable complication is the attachment of a microbial infection, the development

of sepsis and multiple organ failure on the background of thermal damage to the skin [8, 9]

Antibiotic therapy is one of the more important part in the treatment of thermal injuries of the skin [8].

Many of the traditional antibiotics lose their active qualities due to a constant increase in the number of antibiotic-resistant strains. According to the data of the WHO, the growth of resistance of microorganisms to existing antibiotics makes it necessary to development of antimicrobial drugs of new generation in the coming years [10].

One possible way to solve the problem is to create drugs based on antibiotic peptides [11]. Particular attention is paid to the family of effector and signal biologically active substances – antimicrobial peptides (AMP), which provides protective and adaptive reactions for infection, stress [12], parasitic invasion [13], tumor growth [14].

Antimicrobial peptides have unique properties: they have a selective effect on bacteria, since their cationic molecules have a high affinity for bacterial membranes enriched with negatively charged components – lipopolysaccharide, etc. The development of resistance to AMP in bacteria is difficult due to the features of the mechanism of their bactericidal action – rapid, within minutes, increase the permeability of membranes of microorganisms, loss of their barrier function, leading to osmotic destruction of cells. AMP is not delayed and does not accumulate in the body, they are bound and inactivated by plasma proteins, destroyed by proteases. Antimicrobial peptides do not inhibit the function of the immune system, but they have different immunomodulatory effects, for example, the ability to stimulate the activity of natural killer (NC) cells, which can be useful in the development of drugs [15]. These features favorably distinguish natural peptide compounds from classical antibiotics [16]. Identification of modifications of peptides with optimal antimicrobial and immunomodulating properties opens the possibility of creating antimicrobial, antiviral, fungicidal drugs based on them [10].

Representatives of the groups of AMP that deserves attention are widespread in nature peptides of catelicidin family, which have high antimicrobial activity. These include compounds characterized by a high content of tryptophan – indolicidin and its synthetic analogues with different charges of the molecule and the location of hydrophobic and hydrophilic regions. One of them is a synthetic analogue 21 of indolicidin (Ind 21), which exhibits higher antimicrobial, low cytotoxic and hemolytic activity in comparison with the natural molecule and other synthetic analogues.

The purpose of this report is to study the effect of synthetic analogue 21 of natural antimicrobial peptide indolicidin on repair processes in skin and subcutaneous tissues, development of a systemic inflammatory reaction and processes of lipid peroxidation in experimental animals with local burns IIIA-B degree and frostbites of II-III degree.

Methods

The study was conducted on 440 sexually mature male albino rats Wistar, weighting 180-220 g, obtained from Stolbovaya filial of National Center of Biomedical Technologies of Federal Medicobiological Agency (Moscow, Russia). The animals were housed in groups of five rats per cage. The rats were acclimatized for two weeks before using them. The rats were maintained under control conditions: temperature 23±2 ^{o}C, light regime: 12 hours light: 12 hours darkness. The animals were provided with standard diet containing pelleted food and water ad libitum. The experimental protocol was based on the principals of Convention for defense of vertebrates animals, used for experimental and other purposes (Strasbourg, France). The University Animal Ethic Committee approved this experimental protocol.

There was used a synthetic analogue 21 of the natural antimicrobial peptide indolicidin having the formula H-Lys-Lys-Pro-Trp-Lys-Trp-Pro-Lys-Lys-Pro-Trp-Arg-Arg-NH2 (Verta, St. Petersburg) – different from the natural molecule and other synthetic analogs by the charge of the molecule and the location of hydrophobic and hydrophilic regions, high content of tryptophan. Ind 21 is characterized by a higher antimicrobial, low cytotoxic activity and a lack of hemolytic effect in comparison with the natural molecule and other synthetic analogues.

As the comparison drug used xymedon – substance that was synthesized in Institute of organic and physical chemistry named after A. E. Arbuzov, Kazan. Xymedon (Crystal, Dzerzhinsk, Nizhny Novgorod region) has low toxicity, LD50 for experimental animals – from 6,500 to 20,000 mg/kg depending on the method of administration [17].

A synthetic analogue of the natural antimicrobial peptide indolicidin was injected to animals of the experimental groups after the thermal injury intraperitoneally in dose of 100 or 500 μg per 1 kg of body weight in 0,2 ml of saline daily for 5 days after thermal injury [18]. As a comparison drug in the study used xymedon, which were injected to animals intraperitoneally in a dose of 100 mg/kg in 0,2 ml of saline daily for 5 days after modeling of thermal injury. For experimental animals, according to the literature the standard therapeutic dosage is 100 mg/kg [17].

Eight of the experimental animals were taken from experience overdose 1, 4, 7, 10 and day 14 of the experiment for collecting blood and portion heat wound, adjacent tissues. The remaining ten animals of each series continued to monitor the processes of repair of burn and cold wounds to assess the rate of

complete healing, the development of local infectious complications.

In the work was used a burn injury model developed by the team of authors (Monovtsov I.A. et al., Patent of the Russian Federation No. 2210118).

The burn is reproduced as follows. The experimental animal (rat) on the day of the burn is previously removed wool in the back area. Under inhalation ether anesthesia, the rat is fixed on the machine. The hot air flow is created by the Zubr ZTF-2000D hot air source with a temperature regulator and is fed through a window in an asbestos plate corresponding to the burn area. The distance from the surface of the skin to the nozzle of the hot air source is 1 cm. The air temperature is 500 C, the exposure is 4 seconds. The surface area of the animal's body was calculated by the formula Lee (1986), which is a modification of the Rubner formula.

The histological picture of the burns obtained by this method corresponds to IIIA degree burns with IIIB degree sites.

In the work was used the model of the frostbite by the method of Boyko V.V., 2010: using metal weights 3,5x3,0 cm in size, which had previously been cooled in liquid nitrogen (boiling point – 196° C) and then applied to the previously depilated skin of the rat back for 2 minutes. Morphologically the picture corresponds to contact frostbite of II-III degree.

Evaluation of inflammatory-reparative processes in thermal wounds. After modeling the experimental thermal trauma, during visual inspection of burn and cold wounds attention was paid to the timing of the formation of the scab, the elimination of the edema of surrounding tissues, the timing of complete cleansing, the onset of granulation, the beginning of marginal epithelization, and complete healing of the wound, and recorded the development of infectious complications of wounds.

Morphological study. Histological examination of the thermal wound and adjacent areas was performed on the 1st, 4th, 7th, 10th and 14th days after modeling the thermal trauma. The intensity of leukocyte infiltration, the appearance of granulation tissue, and the structure of the epithelium were evaluated. Morphometric examination determined the volume fractions of necrotic tissue, balloon dystrophy, new epithelium and connective tissue. Fibroblasts, granulocytes, lymphocytes and macrophages were counted up to 200 cells from the population at the selected site within the wound defect under the leukocyte-fibrinous scab [19].

To determine the activity of phagocytes perform the study the phagocytic activity of blood neutrophils and adhesive ability of macrophages by standard techniques [20].

The parameters of free radical oxidation of lipids in blood plasma and supernatant of skin were studied by standard methods: content of lipid peroxidation products (AGP, MDA) and the activity of antioxidant enzymes (SOD, catalase), total antioxidant activity. Blood sampling for biochemical research was carried out on the day the animals were taken from the experiment. Blood sampling for biochemical research was carried out on the day the animals were taken from the experiment [21].

To assess the systemic inflammatory response in blood plasma, the level of C-reactive protein (CRP), A1-antitrypsin (A1AT), ceruloplasmin and fibrinogen was determined by standard methods [22, 23].

Statistical processing of the obtained data was carried out using the statistical processor "Microsoft® Excel 2010". As the main technique for determining the level of statistical significance of the differences, the definition of the confidence interval (t) was used, with a probability of 95% ($p \leq 0,05$) for the in vivo experimental medical-biological studies.

Results

In groups of experimental animals injected with an analogue of indolicidin, no lethality was observed, the repair rate of experimental wounds was significantly higher compared to the control group. On the 2-3 day of the experiment, burn wounds were covered with a scab. In the experimental group receiving xymedon, the formation of the scab was on the 3-4th day of the experiment.

After the modeling of cold injury during the three days of the experiment marked the development of necrosis and an increase of wound area in the control group. In the experimental groups after frostbite, early formation of the scab was established – by the 4-5th day, in the control group the scab was formed and covered the wound only by the 7th day of the experiment. Under the influence of xymedon, the scab was formed on the 6th-7th day of the experiment.

In the control groups, thermal wounds were complicated by the development of suppuration. Early purulent complications of wounds (5-7 days) accompanied the process of repair of burn wounds, while infection and suppuration of cold wounds were noted only on the 7-10th day of the experiment. In the experimental groups receiving the peptide, isolated cases of suppuration of wounds, mainly burns, were noted. In animals receiving xymedon, by the 7th day of the experiment, suppuration of wounds was more frequent than in animals receiving the peptide.

The rejection of the scab of burn wounds in the experimental groups was noted on the 10-14th day of the experiment. In the control group by the 14th day of the experiment a large wound surface was covered with a scab (*Figure 1*), that was rejected on the 18-20th day, with the use of xymedon – on the 14-16th day.

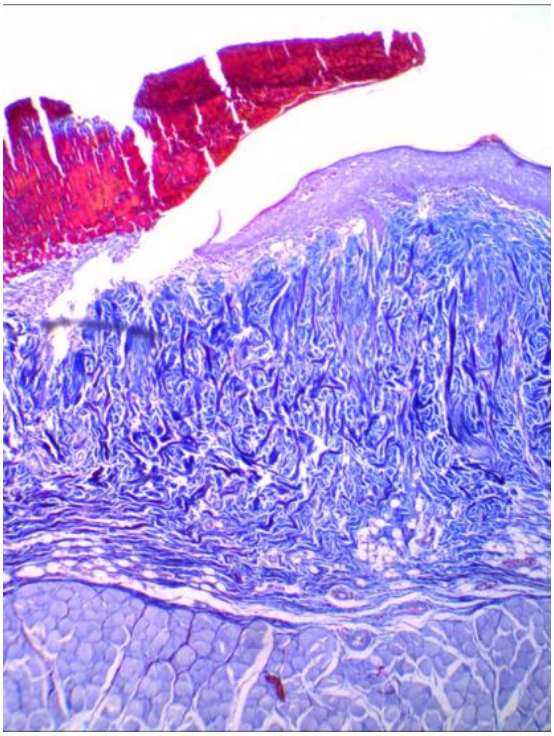

Fig. 1. The preparation of the burn wound site on the 14th day after the burn injury in the control group. Mallory, X 100. There is a "crawling" of the newly formed epithelium under the scab. The loose connective tissue is weakly expressed. The fibers of the mesh layer of the dermis and hypodermis are edematous

The complete epithelization of burn wounds in the experimental groups, receiving Ind 21, was terminated much earlier than in the control group. Complete epithelization with the formation of scars under the action of the peptide was completed by 14-18 days (*Figure 2*), whereas in the control group only by the 24-25th day of the experiment. In animals receiving xymedon, epithelization was completed by 18-20 days.

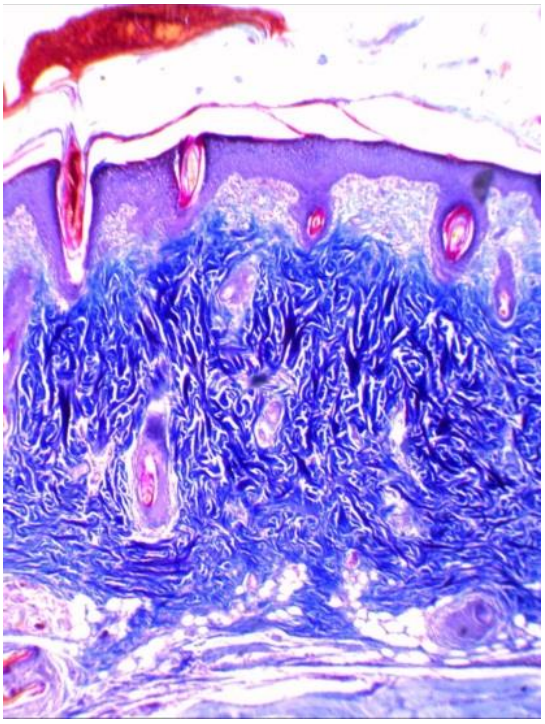

Fig. 2. The preparation of the burn wound site for the 14th day after the burn injury in the experimental group, which received a synthetic analogue of indolicidin at a dosage of 500 µg/kg. Mallory, X 100. There is no fibrous necrotic scab. The surface of the wound is covered with a pronounced layer of the newly formed epithelium. The underlying loose connective tissue is highly pronounced. The fibers of the mesh layer are structural

After contact local frostbite in the experimental groups receiving Ind 21, the process of epithelization with scar formation was completed by the 23-26th day of the experiment, in the control group – by the 34-35th day of the experiment, in animals, receiving xymedon – by 28-30 days.

Thus, the use of a synthetic analog of indolicidin has a stimulating effect on the processes of reparative regeneration, accelerates the epithelization and development of connective tissue in burns and frostbites, and prevents the development of purulent-septic complications.

Morphological investigation was carried out to confirm the positive influence of Ind 21 on the dynamics of wound repair.

The initial reaction of tissues to burn or cold trauma is edema, vasospasm in the wound area and adjacent tissues.

According to the literature, the first phase of the wound process is to remove necrotic tissue from the wound [24]. In the study groups receiving a synthetic analogue of indolicidin, a decrease in the proportion of necrotic tissues was observed from 4 days after the burn. At day 7, the volume of necrotic tissue in the wounds of the animals' experimental groups was significantly lower than in the group, receiving xymedon and in the control group. Such dynamics of wound cleansing in experimental animals is probably explained by a significant number of macrophages in the wound and their high functional activity. In the process of wound purification, the main function of macrophages is expressed in phagocytosis of necrotic tissues destroyed by leukocytes, decaying neutrophilic leukocytes, bacterial products, etc. [24].

The percentage of the neutrophils and lymphocytes in the cell infiltrate of the wounds of rats of test groups under the influence of Ind 21 on next day after the burn injury was significantly lower than in the control ones, and remained so for up to 7 days. Based on the main function of neutrophils – phagocytosis, we can assume, that under the influence of indolicidin bacterial contamination of the wound was less than in the control group.

After frostbite was noted progressing necrosis of the wound, spreading to all layers of the skin up to the hypoderm, marked dystrophic and necrotic changes of all layers of the epidermis and dermis. Vascular disturbances were noted: hyperemia, stasis and thrombosis of vessels at the site of exposure to the cold agent and in adjacent tissues.

Formation of the demarcation shaft, which delimited the focus of necrosis from adjacent tissues, in control animals and rats, receiving xymedon, were observed on the 7th day. At this same time, a scab formed. In the cell population, in the first week of the experiment, granulocytes prevailed. By the end of the experiment, granulocytes, macrophages, lymphocytes and fibroblasts were present in equal proportions, which also indicates delayed cold wound repair.

Under the influence of the peptide, the formation of the demarcation shaft and the scab after frostbite occurs at the end of the first week of the experiment and is accompanied by a change in the cell population of phagocyte cells by fibroblasts.

On the 4th day after the burn, a new epithelium and granulation tissue appeared, which indicates the onset of the second phase of the wound process – the repair phase. In the experimental groups, receiving Ind 21, the proportions of the new epithelium and granulation tissue were significantly higher in comparison with the control group, and from the 7th day of the experiment and in comparison with the group, receiving xymedon. In the experimental groups, the number of leukocytes and macrophages decreases, in the control group increases. In control animals, the number of fibroblasts was significantly lower in comparison with the animals of the experimental groups, in which the content of fibroblasts was significantly higher. An increase in the number of fibroblasts was noted in all groups in the experimental groups under the influence of the peptide.

It has been established, that epithelization of the wound after local frostbite starts from the 9 day of the experiment, under the influence of Ind 21 the epithelization of the wound begins at 7 days and proceeds more intensively. Already on the 14th day of the experiment in the animals of control group the wound was epithelialized just at some sites (*Figure 3*), in the animals of experimental groups the wound was epithelialized, with the exception of some small areas (*Figure 4*).

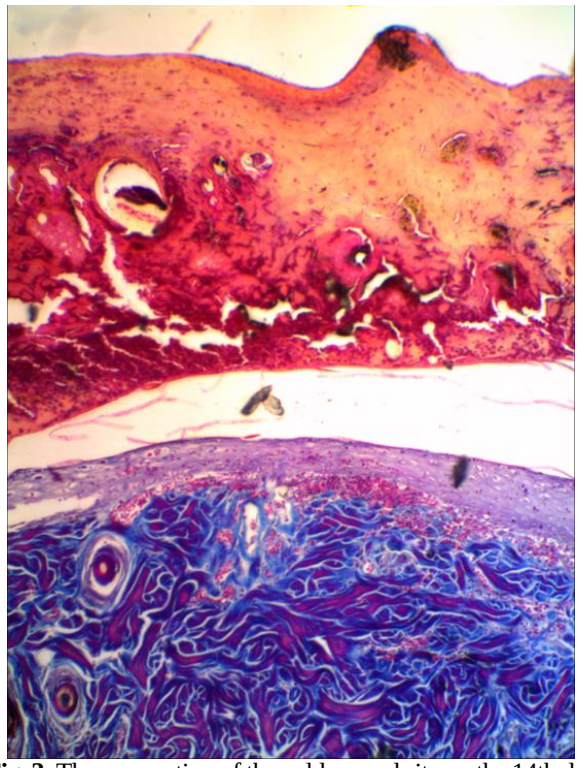

Fig. 3. The preparation of the cold wound site on the 14th day after the burn injury in the control group. Mallory, X 400. A fibrous necrotic scab formed. Epithelization is "crawling". The underlying loose connective tissue is slightly expressed. The fibers of the mesh layer of the dermis are edematous

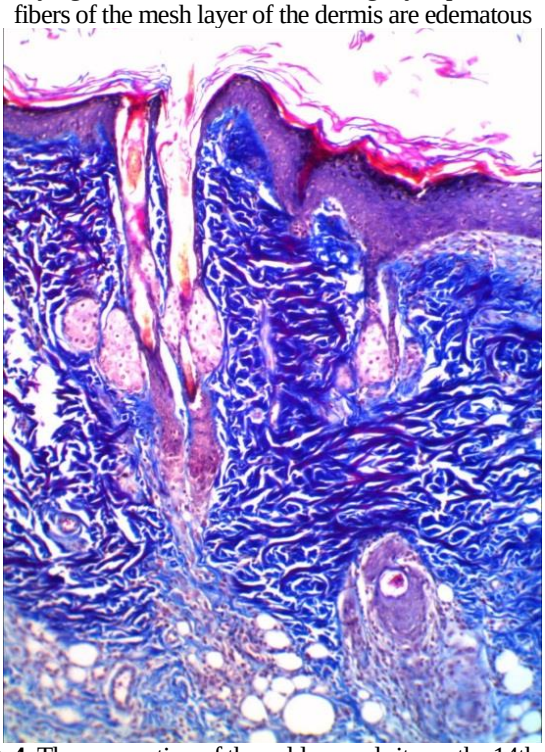

Fig. 4. The preparation of the cold wound site on the 14th day after the burn injury in the experimental group, which received a synthetic analogue of indolicidin at a dosage of 500 μg/kg. Mallory, X 400. There is no scab, the formed layer of the newly formed epithelium covers the fibrin films. The underlying loose connective tissue is highly pronounced. The fibers of the mesh layer are structural

The speed of epithelization is associated with granulation and is caused by the condition of the wound, metabolism, trophism, the degree and nature of the bacterial contamination. According to Garshin V.G., 1951, the most important condition for normal wound healing is the synchronization of the process of epithelization and maturation of granulation tissue.

The most intensive processes of epithelialization and formation of granulation tissue developed in animals after burn injury, from the tenth day of the experiment, which is confirmed by the data of clinical observation (reduction of wound area, appearance of scars). These changes correspond to the third phase of the wound process – the phase of scarring. In the control group, the wounds healed slowly, which is confirmed by histological examination, only in some cases was noted scar appearance on the 14th day of the experiment.

After frostbite and receiving indolicidin 21 at a dose of 500 μg/kg, the process of epithelization was more intense. On the 14th day single non-epithelialized sites were noted, the newly formed reticular layer of the dermis is represented by thin connective tissue fibers.

Under the influence of a synthetic analog of indolicidin in the cellular wound population, the number of macrophages and fibroblasts significantly increased from the first day after the thermal trauma, whereas the number of neutrophils and lymphocytes decreased. Edema and balloon dystrophy are less pronounced. Under the influence of the peptide, the epithelialization and formation of the granulation tissue were more intense and ended earlier than in the control animals and animals receiving xymedon.

It is established, that the use of Ind 21 with burn injury and local frostbite prevents lethality in the experimental groups.

Confirmed published data on the positive impact of the test peptide to the cells of granulocyte-macrophage immunity.

Thus, pharmacological correction with a synthetic analogue of indolicidin 21 is a promising direction in the treatment of burn and cold wounds. In our studies were established positive effects and mechanisms of stimulating influence Ind 21 on the regeneration of thermal wounds.

Revealed positive pharmacological effects of Ind 21 on the dynamics of acute-phase proteins, prooxidant-antioxidant balance, concentration of lipid peroxidation products, both at the system level in the blood plasma, and the local – in skin supernatant.

Were studied the pharmacological effects of indolicidin 21 on the intensity of the systemic

inflammatory reaction. The concentrations of C-reactive protein, ceruloplasmin, alpha-1-antitrypsin, and fibrinogen in the blood plasma of rats after thermal trauma were studied.

The injections of the indolicidin analogue at doses of 100 μg/kg and 500 μg/kg resulted in an increase in the concentrations of all the studied parameters of the acute phase proteins from the first to the fourth day of the experiment, whereas the use of xymedon at a dose of 100 mg/kg contributed to a decrease in the CRP concentration below the control values. It is known that neutrophils and macrophages stimulate the synthesis and secretion of hepatocytes of acute phase proteins. Probably, a significant increase in the concentration of acute phase proteins can be explained by stimulation of hepatocytes, mediated by an increase in activity of phagocytes under the influence of Ind 21.

It was established that after burn, starting from the 7th day of the experiment, the level of studied acute-phase proteins, with the exception of ceruloplasmin, did not differ in the experimental groups or was lower in comparison with the animals of the control group and the group, receiving xymedon. A different dynamics is shown for the experimental groups who underwent local cold trauma: the phase character of the change in the concentration of proteins of the acute phase was noted.

Differences in the dynamics of concentrations in the experimental groups in the modeling of burn and frostbite, apparently, can be explained by the different dynamics of wound repair. Thus, frostbite is characterized by a prolonged development of necrosis and a slowed down rate of tissue regeneration, which causes a prolonged inflammatory reaction at the site of the injury and, as a consequence, the production of acute phase proteins.

In our study has been shown that the use of the indolicidin analogue contributes to a significant increase in α-1-antitripsin concentration on the first day after the thermal burn, whereas for the initial stages of acute inflammation, a typical decrease in the levels of protease inhibitors, incl. A1AT [23]. This increase in A1AT level prevents secondary tissue damage at the burn wound site by elastase-like and chymotrypsin-like proteinases. Further significant decrease in A1AT in the experimental groups by the end of the second week of the experiment may be explained by a decrease in the intensity of the local inflammatory reaction, which is due to the action of the indolicidin analogue.

Ceruloplasmin is multiple oxidizer, inactivates superoxide anionic radicals [23]. We have shown, that by the 7th day of the experiment the concentration of ceruloplasmin was significantly lower in the experimental groups than in the control group, after which, starting from the 10th day, the content of this protein in the animals of the control and test groups did not differ. A significant increase in the level of fibrinogen during the seven days of the experiment was established in the groups receiving an analog of indolicidin. It can be assumed, that by increasing the concentration of fibrinogen, which is a source of fibrinopeptides with anti-inflammatory activity [23], indolicidin helps reduce the systemic inflammatory response of the body.

The immunomodulation properties of natural indolicidin and its synthetic analogue Ind 21 are described. It is of interest to study the effect of Ind 21 on the phagocytic-macrophagal link of immunity in burn and cold trauma. Neutrophilic granulocytes and macrophages directly affect the repair rate of the wound, providing elimination of the decay products of necrotic tissues and carrying out the first line of protection against infection, preventing, among other things, its generalization [25].

Information on changes in immunoreactivity in thermal injuries are contradictory. Most researchers note the activation of phagocytes, changes in complement activity, serum concentrations of immunoglobulins and opsonins, depression of the cell link of adaptive immunity, and information on the cytokine profile is ambiguous [26]. So far, no consensus has been reached on understanding the key mechanisms for changing the immune status for burns. It is believed that thermal trauma has specific patterns of changes in local and systemic immunity that determine post-burn changes and wound healing. Ambiguous information about the relationship of the immunoreactivity of the organism with the morphology of the lesion focus and the dynamics of wound healing in burns and frostbites [26].

In the work it was established, that after a thermal trauma the phagocytic activity of peripheral blood neutrophils was reduced throughout the entire period of the experiment, which was manifested by a decrease in all the studied parameters in the control groups: phagocytic index, phagocytic number and opsono-phagocytic index. However, after frostbite, the oppression of the neutrophilic link is more pronounced.

In the case of burn injury, the use of the indolicidin analogue led to an increase in the number of phagocytic neutrophils already on the 4th day after the burn, compared to the values of the control group and the group receiving xymedon. At the same time, an increase in the absorptive capacity of cells in this period is not established.

In the experimental groups exposed to frostbite, an increase in the number of phagocytic neutrophils was observed upon the influence of Ind 21 from the first day of the experiment, and an increase of their absorbent capacity. The number of phagocytizing neutrophils at all times of the experiment under the influence of the synthetic analog of indolicidin in the experimental groups was higher than in the control group and the group receiving xymedone at a dose of 100 mg/kg.

These changes are associated with an increase in the number of young neutrophils in the peripheral blood under the influence of indolicidin, which are functionally less valuable [27], which causes changes in the phagocytic index. On the other hand, it can be explained by the damaging effect of toxins, immunosuppressive factors and stress hormones on granulocytes and their precursors [28].

The stimulating effect of ind 21 on the phagocytic activity of neutrophils established in the study can be explained by the immunomodulating action of indolicidin 21 [15], as well as the bactericidal action of antimicrobial peptides.

Thus, it was established that the pharmacological effect of the peptide studied on the functional activity of phagocytes is more pronounced when it is applied at a dose of 500 μg/kg both on the model of burn and cold trauma. It is known that indolicidin has a low resistance to the action of peptidases [18]. The synthetic analogue used in the work has a longer half-life than the natural peptide, however, the administration of a larger dose leads to an increase in the duration of the drug, which probably causes its more pronounced effect.

In the experiments it was established that the modeling of local burn or frostbite leads to a decrease in the proportion of macrophages in the cellular population of peritoneal flushing and a decrease in the number of adhered macrophages (MF) in comparison with intact animals. In the group of animals after frostbite, the proportion of macrophages was significantly lower in comparison with intact animals and rats after the burn. Was detected, that using of Ind 21, a significant increase in the proportion of MF in the cellular population of peritoneal flushing from the first day of the experiment.

The relative amount of adhered macrophages in the experimental groups did not differ from the control values. Noted an increase in the proportion of adherent macrophages on the 4th day and a further decrease. In the experimental groups, the phase of the changes in the relative amount of adhered MF was established: an increase with a subsequent decrease and again an increase. Such dynamics was observed in the early stages after the modeling of the burn and at the late ones – with frostbite.

In the group of animals receiving xymedon at a dose of 100 mg/kg, an increase in the proportion of MF in the cellular population of peritoneal flushing was also noted. However, throughout the duration of the experiment, the relative amounts of MF and the adhered MF were lower than those in animals receiving Ind 21.

In this case, the most pronounced effect on the functional activity of MF was observed when using a synthetic analog of indolicidin at a dose of 500 μg/kg.

Thus, one of the pharmacological effects of the use of a synthetic analogue of indolicidin is to increase the proportion of macrophages in the population of cellular flushing, without significantly affecting their functional activity.

The development of an inflammatory reaction during thermal damage is accompanied by activation of lipid peroxidation processes, which is one of the leading mechanisms of secondary tissue alteration.

We have established that the modeling of local burn or frostbite causes accumulation of products of lipid peroxidation from the first day of the experiment, both in blood plasma and in the wound of control and experimental animals. In this case, after a burn injury, the concentrations of acylhydroperoxide (AGP) and malonic dialdehyde (MDA) in the experimental groups were higher than the control values and indices of the group receiving xymedon throughout the entire experiment (Table 1). A similar dynamics of accumulation of AGP and MDA was observed in the experimental groups, who underwent local cold trauma. Thus, the concentrations of lipid peroxidation products in plasma were significantly higher, than those of the control group on the first day of the experiment and differed insignificantly in dynamics. For the group of animals receiving xymedon, the concentrations of the products of lipid peroxidation both after burns and after cold trauma were lower or no different from those of the control group at all times of the experiment, significantly lower than those of the experimental groups used by Ind21 at the beginning of the experiment.

Table 1

The content of lipid peroxidation products in blood plasma of control and experimental groups after burn injury

Index group \ Day of the experiment		1st day	4th day	7th day	10th day	14th day
Content of AGP in plasma, s.u.	Control	0.2±0.04 t 0.08	0.3±0.04 t 0.08	0.3±0.02 t 0.04	0.1±0.01 t 0.02	0.1±0.01 t 0.03
	The group receiving xymedon at a dose of 100 mg/kg	0.2±0.02 t 0.04	0.2±0.01 t 0.02	0.2±0.01 t 0.02	0.1±0.01* t 0.01	0.1±0.01 t 0.03
	The group receiving an analogue of indolicidin at a dose of 100 μg/kg	0.4±0.1*^{x} t 0.1	0.3±0.03 t 0.1	0.5±0.04*^{x} t 0.1	0.3±0.04*^{x} t 0.1	0.3±0.03*^{x} t 0.1
	The group receiving an analogue of indolicidin at a dose of 500 μg/kg	0.6±0.1*^{x} t 0.1	0.4±0.02*^{x} t 0.1	0.3±0.03 t 0.1	0.2±0.02*^{x} t 0.04	0.2±0.02 t 0.1
Content of MDA in plasma, μmol/l	Control	1.5±0.1 t 0.3	1.7±0.2 t 0.4	1.8±0.2 t 0.5	1.8±0.1 t 0.2	1.7±0.2 t 0.3
	The group receiving xymedon at a dose of 100 mg/kg	1.3±0.1 t 0.2	1.6±0.1 t 0.2	1.6±0.1 t 0.3	1.5±0.2 t 0.3	1.4±0.1 t 0.2
	The group receiving an analogue of indolicidin at a dose of 100 μg/kg	2.8±0.2*^{x} t 0.4	2.8±0.2*^{x} t 0.4	3.3±0.1*^{x} t 0.3	1.9±0.2 t 0.5	2.0±0.2 ^{x} t 0.3
	The group receiving an analogue of indolicidin at a dose of 500 μg/kg	2.7±0.2* ^{x} t 0.4	3.4±0.2* ^{x} t 0.5	3.1±0.2* ^{x} t 0.4	2.3±0.1* ^{x} t 0.3	2.0±0.2 t 0.4

Note: * – differences in comparison with the control group are reliable;
x – differences in comparison with the group, receiving xymedon, are reliable.

In the supernatant of the injured area and adjacent intact skin of animals treated with Ind 21, the concentrations of AGP and MDA were significantly higher on the first day after local thermal trauma compared to the control values and indices of the group administered with xymedon. There was a significant decrease in the content of lipid peroxidation products studied in the wound for animals treated with Ind 21 at a dose of 500 μg/kg, compared with other experimental groups (Table 2).

Table 2

The effect of a synthetic analogue of indolicidin on the concentration of lipid peroxidation products in the skin /and adjacent tissues in control and experimental animals after local frostbite

Index group \ Day of the experiment		1st day	4th day	7th day	10th day	14th day
Content of AGP in the skin, s.u.	Control	1.3±0.1 t 0.2	1.6±0.1 t 0.1	1.0±0.1 t 0.1	2.6±0.1 t 0.2	1.0±0.1 t 0.2
	The group receiving xymedon at a dose of 100 mg/kg	1.2±0.1 t 0.1	1.5±0.1 t 0.1	1.0±0.1 t 0.2	2.0±0.1* t 0.1	1.0±0.1 t 0.2
	The group receiving an analogue of indolicidin at a dose of 100 μg/kg	1.9±0.1* t 0.1	1.6±0.1 t 0.2	1.8±0.1* t 0.2	1.9±0.1* t 0.1	1.2±0.1 ^{x} t 0.2
	The group receiving an analogue of indolicidin at a dose of 500 μg/kg	1.4±0.1 t 0.2	0.4±0.04*^{x} t 0.1	1.6±0.1* t 0.3	0.5±0.04*^{x} t 0.1	1.2±0.1 ^{x} t 0.1
Content of MDA in the skin, μmol/l	Control	4.1±0.2 t 0.3	6.0±0.3 t 0.7	5.2±0.2 t 0.4	7.5±0.3 t 0.5	4.4±0.2 t 0.4
	The group receiving xymedon at a dose of 100 mg/kg	4.1±0.1 t 0.3	5.7±0.2 t 0.3	4.8±0.2 t 0.5	5.9±0.3* t 0.5	4.2±0.2 t 0.3
	The group receiving an analogue of indolicidin at a dose of 100 μg/kg	5.3±0.2* ^{x} t 0.3	5.5±0.3 t 0.5	5.9±0.2 ^{x} t 0.5	6.5±0.2* t 0.4	4.0±0.2 t 0.5
	The group receiving an analogue of indolicidin at a dose of 500 μg/kg	5.4±0.2* ^{x} t 0.4	3.4±0.2* ^{x} t 0.4	3.3±0.2* ^{x} t 0.3	3.2±0.2* ^{x} t 0.4	3.9±0.1 t 0.3

Note: * – differences in comparison with the control group are reliable;
x – differences in comparison with the group, receiving xymedon, are reliable.

The activity of antioxidant enzymes of blood plasma after local burn and injection of Ind 21 was significantly higher in comparison with control animals from the first to the 7th day of the experiment. From the 7th day until the end of the experiment, catalase activity was lower, and the activity of superoxide dismutase (SOD) remained significantly higher than the control values.

Changes in the activity of antioxidant enzymes in the blood plasma of animals after frostbite and received Ind 21 were of a phase nature. At the beginning of the experiment, these indicators were below the control, then increased and, at the end of the experiment, again decreased and were significantly lower than the control.

We established that the activity of catalase and SOD were higher in the experimental groups subjected to thermal trauma and receiving the peptide, in the group administered peptide at a dose of 500 μg/kg.

A multidirectional change in the activity of antioxidant enzymes in the blood plasma in groups of animals after burn and cold trauma was determined. After modeling the burn in the groups using the peptide, catalase activity increased until the seventh day of the experiment and decreased to 14 days of the experiment. The activity of SOD was increased throughout the period. In animals treated with Ind 21 after local frostbite, the activity of catalase and SOD decreased, then increased, and on the 14th day again decreased significantly below the control values. Introduction Ind 21 after a local burn stimulated the production of free radicals by activated leukocytes, the accumulation of products of lipid peroxidation. After frostbite, this effect was noted only in late terms.

In the supernatant of the damaged area and borderline intact skin, catalase and SOD activity increased significantly from peptide 4 days after local burn and from 7 days after local frostbite. By the end of the second week of the experiment, the activity of antioxidant enzymes was significantly lower than the values of the control group.

With the introduction of Ind 21 at a dose of 500 μg/kg, data on a two-phase change in catalase activity in the wound were obtained: an increase on the fourth day of the experiment after a burn injury and a seventh after a cold injury followed by a decrease. It is known that a high content of hydrogen peroxide in the burn wound inhibits the development of regenerative processes, although a moderate increase in its concentration has a stimulating effect [29]. Given these data, it can be assumed that a high content of catalase in the wound in the first four days prevents an excessive increase in the concentration of hydrogen peroxide, and a decrease in the activity of the enzyme in the subsequent period, on the contrary, helps maintain a moderately high content of this substance, which can have a stimulating effect on the healing of burns wounds.

It is shown that the pharmacological effects of Ind 21 on the processes of lipid peroxidation, the activity of SOD and catalase are more pronounced when applied at a dose of 500 μg/kg.

Thus, we have established the pharmacological effects of the synthetic analogue of indolicidin, which are manifested by stimulation of repair of burn and cold wounds. This conclusion is confirmed by an earlier departure of the scab, the beginning of epithelialization of the damage zone, and the development of complete epithelization with the formation of young scars. The use of the studied peptide prevents the mortality of experimental animals, prevents the development of purulent-septic complications. The stimulating effect of the peptide is associated with activation of the functional activity and an increase in the number of neutrophils and macrophages, its effect on the prooxidant-antioxidant balance and the reaction of the acute phase of inflammation.

Conclusion

The study found that the use of synthetic analogue indolicidin 21 is an effective method of pharmacological correction of reparative processes in the skin with thermal damage. The stimulating effect of Ind 21 on the cells of the granulocyte-macrophage link was confirmed, the pharmacological effects of the studied peptide on the severity of the systemic inflammatory reaction, the processes of free radical oxidation, and the activity of antioxidant enzymes in the skin and blood plasma with local burns and frostbites were established.

The use of a synthetic analogue of indolicidin has shown its effectiveness in stimulating the processes of reparative regeneration in thermal wounds and in preventing purulent complications. Its use, especially at a dose of 500 mcg/kg, promoted the healing of thermal damage to the skin, both with local burns and with frostbite.

Pharmacological correction by using a synthetic analog of indolicidin has a stimulating effect on the processes of reparative regeneration, accelerates epithelization and the development of connective tissue in burns and frostbites. Under the influence of the peptide, complete epithelialization of burn wounds occurred at 8-10, and cold – for 9-11 days earlier than in control groups.

The use of a synthetic analogue of indolicidin prevents the development of purulent-septic

complications in thermal trauma. Thus, in the groups receiving the peptide studied, purulent complications of burn wounds were not observed, the incidence of infection of cold wounds was lower by 30%.

The pharmacological effect of a synthetic analog of indolicidin is manifested by a stimulating effect on the functional activity of neutrophils and the number of macrophages in burn and cold trauma.

The introduction of an analog of indolicidin at doses of 100 μg/kg and 500 μg/kg resulted in a marked increase in the concentrations of acute phase proteins (CRP 1.6-6.0 times, A1AT 1.3-2.2 times, ceruloplasmin 1.1-1.4 times, fibrinogen in 2.0-3.1 times) from the first to the fourth day of the experiment with a tendency to further decrease, which prevents secondary tissue damage at the site of the thermal wound by elastase-like and chymotrypsin-like proteinases.

The use of Ind 21 during thermal trauma leads to an increase in the concentration of lipid peroxidation products and supernatant of skin homogenate throughout the experiment after a local burn, up to 10 days after local frostbite, followed by a significant decrease in comparison with control animals. The activity of antioxidant enzymes under the influence of a synthetic analog of indolicidin was higher than the control values.

Conflicts of Interest: The authors have no conflict of interest to declare.

Reference

1. Sakharov S.P., Kozlov L.B. On background of ambustial trauma translocation of cultivated and uncultivated bacteria in an organism of rabbits of brand «Chinchilla». *Eastern Eur. Sci. J.* 2014. № 6: 56-60. [eLIBRARY] [Fulltext]

2. Ostrovsky N.V., Petrov V.V., Bystrova A.S., Musatskova M.V. Comparative estimation of medical preparation influence to termal burns of ii and iii degrees healing in experiment for local treatment of wounds. *Fundamental research.* 2014. № 6: 512-515. *(In Russian)* [eLIBRARY] [Fulltext]

3. Popov N.S., Demidova M.A., Shestakova V.G., Eliseeva T.I., Kazaishvili Yu.G. Morphological changes in the skin of rats with thermal burns in topical application of medical leeches extract. *Upper Volga Medical Journal.* 2014. Vol. 12, № 2: 45-50. *(In Russian)* [eLIBRARY] [Fulltext]

4. Sizonenko V.A. Cold trauma. *Bul. SB RAMS.* 2007. № 4: 56. *(In Russian)*

5. Eryukhin I.A., Gelfand B.R., Shlyapnikov S.A.. *Surgical infection: guidance.* (St. Petersburg: Peter, 2003), 864 p. *(In Russian)*

6. Ingram B.J., Raymond T.J. Recognition and treatment of freezing and nonfreezing cold injuries. *Curr. Sports. Med. Rep.* 2013. Vol. 12, № 2: 125-130, doi: 10.1249/JSR.0b013e3182877454. [PubMed] [Fulltext]

7. Bilgic S., Ozkan H., Ozenç S., Safaz I., Yildiz C. Treating frostbite. *Can Fam Physician*. 2008. №54(3): 361-3. [PubMed] [Fulltext]

8. Hassi J., Lehmuskallio E., Juhani J., Rytkönen M. Frostbite and other problems of skin expose to cold. *Duodecin.* 2005. Vol. 121, № 4: 454-461. [PubMed]

9. Jurkovich G.J. Environmental cold-induced injury. *Surg. Clin. North Am.* 2007. Vol. 87, № l: 247-267, doi: 10.1016/j.suc.2006.10.003. [PubMed] [Fulltext]

10. Chatterjee P., Fleck F. Political will mobilization to contain the development of antimicrobial resistance. *Bul. World Health Organization.* 2011. №89:168-169, doi:10.2471/BLT.11.030311. [Fulltext]

11. Guani-Guerra E., Santos-Mendoza T., Lugo-Reyes S.O., Terán L.M. Antimicrobial peptides: general overview and clinical implications in human health and disease. *Clin. Immunol.* 2010. Vol. 135, № 1: 1-11, doi: 10.1016/j.clim.2009.12.004. [PubMed] [Fulltext]

12. Aberg K.M., Radek K.A., Choi E.H., Kim D.K., Demerjian M., Hupe M., Kerbleski J., Gallo R.L., Ganz T., Mauro T., Feingold K.R., Elias P.M. Psychological stress downregulates epidermal antimicrobial peptide expression and increases severity of cutaneous infections in mice. *J Clin Invest.* 2007. №117(11): 3339-49, doi:10.1172/JCI31726. [PubMed] [Fulltext]

13. McGwire B.S., Kulkarni M.M. Interactions of antimicrobial peptides with Leishmania and trypanosomes and their functional role in host parasitism. *Exp Parasitol.* 2010. № 126(3): 397-405. doi: 10.1016/j.exppara.2010.02.006. [PubMed] [Fulltext]

14. Jin G. Kawsar H.I., Hirsch S.A., Zeng C., Jia X., Feng Z., Ghosh S.K., Zheng Q.Y., Zhou A., McIntyre T.M., Weinberg A. An Antimicrobial Peptide Regulates Tumor-Associated Macrophage Trafficking via the Chemokine Receptor CCR2, a Model for Tumorigenesis. *PLoS One.* 2010. Vol. 5, № 6: 10993, doi: 10.1371/journal.pone.0010993 [PubMed] [Fulltext]

15. Chernysh S., Irina K., Irina A. Anti-tumor activity of immunomodulatory peptide alloferon-1 in mouse tumor transplantation model. *Int. Immunopharmacol.* 2012. Vol. 12, № 1: 312-314, doi: 10.1016/j.intimp.2011.10.016 [PubMed] [Fulltext]

16. Gordon Y.J., Romanowski E.G., McDermott A.M. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr. Eye Res.* 2005. Vol. 30, № 7: 505-515, doi: 10.1080/02713680590968637. [PubMed] [Fulltext]

17. Izmailov S. G., Izmailov G. A., Averyanov M. Y., Reznik V. S. *Xymedon in clinical practice.* (N. Novgorod: publishing house of the NGMA, 2001), 185 p. *(In Russian)*

18. Smirnova M.P., Afonin V.G., Shpen' V.M., Tyagotin Y.V., Kolodkin N.I. Structure-function relationship between analogues of the antibacterial peptide indolicidin. I. Synthesis and biological activity of analogues with increased amphipathicity and elevated net positive charge of the molecule. *Russian Journal of*

Bioorganic Chemistry. 2004. Vol. 30. № 5: 409-416. *(In Russian)* [eLIBRARY]

19. Avtandilov G.G. *Fundamentals of quantitative pathological anatomy: Textbook.* (Moscow: Medicine, 2002), 238 p. *(In Russian)* [eLIBRARY]

20. Galankin V.N., Yunuskhodjaev E.Kh., Tokmakov A.M. A method for determining the phagocytic activity of polymorphonuclear neutrophils. *Arch. Pathologies.* 1987. Vol. 49, № 6: 77-79. *(In Russian)*

21. Korolyuk M.A., Ivanova L.I., Mayorova I.G., Tokarev V.E. Method for determination of catalase activity. *Clinical Lab. Diagnostics.* 1988. № 1: 16-19. *(In Russian)* [eLIBRARY]

22. Gusev E.Y., Yurchenko L.N., Chereshnev V.A., Zotova N.V. Methodology of research in systemic inflammation. *Cytokines and inflammation.* 2008. Vol. 7, № 1: 15-23. *(In Russian)* [eLIBRARY] [Fulltext]

23. Shevchenko O.P. Acute phase proteins of inflammation. *Laboratory.* 1996. № 1: 3-6. *(In Russian)* [Fulltext]

24. Kuzin M.I., Kostyuchenok B.M., *Wounds and wound infection: guide for doctors. Second edition, revised and enlarged.* (Moscow: Medicine, 1990), 592 p. *(In Russian)*

25. Kokryakov V.N., *Essays on innate immunity*. (St. Petersburg: Science, 2006), 261 p. *(In Russian)*

26. Osikov M.V., Dolgushin I.I., Telesheva L.F., Lihacheva A.G., *Pathogenetic justification of epidermal growth factor usage in thermal trauma.* (Moscow: Academy of Natural History, 2013), 152 p. *(In Russian)* [eLIBRARY] [Fulltext]

27. Pinegin B.V., Mayanskii A.N. Neutrophils: structure and functions. *Immunology.* 2007. № 6: 374-382. *(In Russian)* [eLIBRARY]

28. Mayansky A.N., Mayansky D.N., *Essays on the neutrophil and macrophage. Second edition, revised and enlarged*. (Novosibirsk : Science, 1989), 344 p. *(In Russian)*

29. Mikhal'chik E.V., Lipatova V.A., Ibragimova G.A., Korkina L.G., Piterskaya J.A., Pen'kov L.Yu. Activity of antioxidant enzymes in the wound in patients with deep burns. *Bulletin of Experimental Biology and Medicine.* 2009. Vol. 147. № 6: 753-756. *(In Russian)* [eLIBRARY]

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