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ANALYSIS OF THE SELECTION OF THE COVERING MATERIAL FOR THE TREATMENT OF THERMAL BURN OF THE SKIN

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Abstract.

Background: Despite the vast experience of treating burns in surgery, a large number of victims become disabled; therefore they represent a serious medical and social problem.

Methods: The study included 120 white outbred male rats, which were simulated an experimental 3-degree topical burn and were divided into 4 groups according to the type of treatment substance. The burn was simulated by immersing the depilated skin area in boiling water. Studies of wound healing were carried out in three series, respectively, on the third, seventh and tenth days.

Results: We found that the local application of chitosan derivatives at an early stage leads to healing of the wound defect in rats in our experimental model. This was associated with a decrease in endogenous intoxication, lipoperoxidation, and internucleosomal degradation compared to the control group.

Conclusion: Thermal damage is accompanied by increased internucleosomal skin degradation. Compared with levomecol, chitosan derivatives more effectively reduce the degree of internucleosomal degradation, especially on the 10th day of the experiment.

Keywords: skin regeneration, chitosan, internucleosomal degradation, endogenous intoxication, lipoperoxidation, healing

Introduction.

All over the world, burns and burn diseases are the cause of a large number of all injuries and mortality remains high, especially in the stages of toxemia. The main causes of mortality in the stage of acute burn toxemia are purulent-septic complications combined with endogenous intoxication of metabolic and microbial origin. They firmly hold the first place among the

reasons. At the same time, the risk of developing early sepsis increases, especially with deep burns on the surface of the body. Despite the vast experience in treating burns in surgery, a large number of victims become disabled, so they are a serious medical and social problem [61].

On a global scale, surgery conducts a number of scientific studies on the creation and use of drugs that stimulate the processes of reparative regeneration in the epithelial cells of the damaged focus. In this regard, the development and introduction into clinical practice of biosynthetic and synthetic film coatings, which enhance epithelization [47]. Among them, chitosan isolated from silkworm cocoons has a high regenerative property, which makes it possible to use it as a local medicine [51]. In this regard, scientific research is being carried out to create wound-healing gel compositions with high therapeutic efficacy. The creation of films specially designed for one or another phase of the burn is an urgent task [7, 46].

The introduction into medical practice of the optimal version of the covering material can shorten the regeneration and hospitalization time of patients, which is both from a medical point of view and social. Because in addition to saving money for a prolonged stay in a medical institution, healing of skin without scars and scars has an important feature for the full life of a person. The use of biomaterials as scaffolds to facilitate skin wound healing is a new and interesting field of regenerative medicine and biomedical research. In many respects, the regenerative potential of biological material is associated with its ability to modulate and suggests an inflammatory response. At the same time, all foreign materials once implanted into living tissue, to one degree or another, cause an immune response. The modern approach to the development of bioengineering structures for use in regenerative medicine should be aimed at using the properties of the inflammatory response, which improve healing but do not lead to negative chronic manifestations [10].

The aim of the study was to study the molecular mechanisms of reparative regeneration of skin cells during thermal injury with correction with chitosan derivatives.

Research objectives: to select active chitosan obtained from local raw materials in vitro using bacteriophage λ DNA, intact and burnt skin to determine the degree of chitosan binding to specific DNA regions;

to evaluate the effectiveness of the use of chitosan derivatives in preventing internucleosomal degradation, enhancing the processes of replication and transcription in skin cells during thermal injury;

to evaluate the effect of chitosan derivatives on the degree of endogenous intoxication and lipid peroxidation in the dynamics of thermal injury.

Wound healing is the most important general biological, medical and social problem that remains relevant to this day. The modern direction in the development of dressings is the rejection of universal means used throughout the entire period of the wound process, and the transition to dressings specifically designed for use in one or another phase in accordance with a specific clinical situation [12, 59].

In the first phase of the course of the wound process, the most promising is sorption-application therapy, which is based on wound cleansing due to physical adsorption. In this case, the most effective is the combination of capillary drainage and selective sorption. In the treatment of wounds with a pronounced purulent-necrotic component, it is advisable to use dressings that provide a complex sorption and pharmacotherapeutic action [30, 31]. In the regeneration phase, it is most advisable to use coatings based on biodegradable polymers that

stimulate healing processes, as well as products based on synthetic polymers containing special preparations that stimulate regeneration [24, 38, 40].

In the last decade, in the problem of creating dressings, there has been a steady progress associated with the development and introduction into clinical practice of biosynthetic and synthetic film coatings. Currently, the main requirements for wound coverings have been formulated [22, 70]. Among them are sterility, non-allergenic, non-toxic, non-traumatic, ability to take the form of a wound defect, vapor and gas permeability, impermeability to bacteria, good fixation on the wound surface [35, 42, 43].

Chitosan and its derivatives are promising in this regard. Chitosan-2-amino-2-deoxy-B-glucans, depending on their molecular weight and degree of acetylation, are used in medicine and cosmeceptics [49, 52, 54]. Various options for wound healing agents based on chitosan have been proposed, which have a stimulating effect on a number of vital functions, and, on the other hand, contain natural antioxidants and help suppress the activity of free radical oxidation, and also provide effective biocorrection at seven levels of organization of living matter [56, 57, 63].

When using these drugs, the following occurs:

at the molecular level - activation of the synthesis of amino acids, proteins, enzymes, immunoglobulins, neurotransmitters, nucleic acids, hormones, ATP; trapping reactive oxygen species and aggressive free radicals; binding of excess cholesterol and chlorine ions in the blood;

at the subcellular level - stabilization of membrane structures and receptor apparatus; maintaining adequate operation of ion channels, their selective permeability for potassium, sodium, calcium, chlorine ions;

at the cellular level - restoration of the sensitivity of cells to insulin, absorption of glucose, its inclusion in carbohydrate and energy metabolism; intensification of cellular respiration; activation of growth and division of healthy cells; the formation of immunocompetent cells and blood cells [56, 57, 60, 71];

at the tissue level - providing with glucosamine (a structural component of connective tissue); restoration and strengthening of the cartilaginous tissue of the articular surfaces, intervertebral discs, ligaments; restoration of the strength and elasticity of blood vessels, heart valves; regeneration of the mucous membranes of the gastrointestinal tract, skin, wound healing; restoration of the normal structure of the liver parenchyma, removal of fat in case of fatty hepatosis; stimulation of hair and nail growth [2, 3, 73];

at the level of individual organs - improvement of liver function; restoration of normal intestinal microflora; cleansing of cholesterol plaques and restoring the integrity of the vascular endothelium, the release of nitric oxide (a powerful vasodilating factor); activation of brain activity; increased strength of bones, joints [16];

at the systemic level - increasing the efficiency of digestion, activating all types of metabolism; improvement of the cardiovascular system; stimulation of all links of the immune system: antitelogenesis, phagocytosis of pathogenic agents, synthesis of immunoglobulins, endogenous interferon, cytokines; strengthening of the musculoskeletal system; stimulation of hematopoiesis; elimination of under-oxidized decomposition products, slags and toxins [9, 19, 23];

at the level of the whole organism - a complex healing and restorative effect on the whole organism as a whole: increasing vitality and adaptive properties, resistance to stress, resistance to adverse environmental factors, including bacterial, fungal and viral infections, ionizing radiation, the effect of toxins and heavy metals [4, 11, 33, 34, 58].

The antioxidant activity of chitosan oligosaccharides allows to normalize the processes of free radical oxidation of organic molecules and the level of peroxide radicals accumulating in the cells of the body, which is important for the construction and renewal of structural lipids of cell membranes, as well as for the production of a number of enzymes and hormones [4]. As a result, destructive oxidation processes in cells are inhibited and potential opportunities are created to restore their activity. As a result, destructive oxidation processes in cells are inhibited and potential opportunities are created to restore their activity [56, 57, 60].

A very important property of chitosan is its wound healing effect. Analysis of the data obtained when studying the effect of several dosage forms of chitosan (solution, gel, film) on the healing process of skin wounds in rats and rabbits showed that the use of the gel provides a reliable acceleration of the skin repair [73]. The study of the mechanisms of the stimulating effect of chitosan showed that it is based on the activation of the biological wound cleansing phase. This is explained, first of all, by an increase in the functional activity of phagocytes: acceleration of the migration of phagocytes into the wound (inflammation focus), as well as an increase in the phagocytic activity of macrophages due to an increase in the positive charge of their surface membrane and activation of the mechanisms of oxygen-dependent bactericidal activity [18, 41, 62].

Chitosan, in addition to stimulating proliferation in the first stages, is very useful in the final phase of healing - scar restructuring (its presence in the wound helps to avoid the formation of rough scars). It conducts air well to the surface to be healed [68]. In the local treatment of skin lesions, it is necessary to constantly protect the wound from pathogenic microflora, and therefore antibacterial agents are introduced into the coating composition.

The use of chitosan leads to the formation of regularly organized skin tissue and reduces abnormal healing [52].

It was found that chitosan activates the healing of burn and wound surfaces without scarring, as it stimulates the growth of collagen fibers of the skin, providing elasticity of the skin [5, 27, 28, 62]. In addition, when applied to a wound, chitosan has a hemostatic and analgesic effect. It stimulates the processes of migration, proliferation and differentiation of hematopoietic stem cells, contributing to faster bone marrow recovery [26]. This takes into account the properties of the polymer to suppress fibrosis, to exhibit a hemostatic effect, to be cleaved by lysozyme. The ability of glycans or their polymer compositions to form films is used in the preparation of wound healing materials, provided that drugs, enzymes, metabolites are additionally impregnated into the polymer mass to accelerate the regeneration of the skin; appropriate liquid and ointment means for external of application [21]. Research has been carried out on the use of chitosan for the healing of various wound lesions of the skin and connective tissues [36, 39, 62, 50].

Combined sponges made of collagen containing aminoglucan and impregnated with antioxidants, antiseptics (chlorhexidine-bigluconate and superoxide dismutase, etc.) are proposed. The antibacterial efficacy of sponges and a decrease in skin and soft tissue infiltration in the area of inflammation have been established. Shown is the acceleration of epithelialization of wounds, processes of growth of granulations, intensification of cleaning of wound surfaces [6, 17, 22, 65, 66]. Nanoparticles containing low molecular weight chitosan exhibit pronounced transfection into epithelial cells and a high level of gene expression in cells. This study opens up great prospects for the implementation in practical healthcare of such systems in the form of

drops for the treatment of corneal dystrophies, dry eye syndrome, immune-mediated and other diseases of the human eye [43].

Combining chitosan with other substances can help increase tissue regeneration [20, 22].

Biologically active composition for medical and cosmetic purposes, in the treatment of burns, as a food additive contains chitosan-gel or chitosan-suspension with a nanogranule size of no more than 100 nm and noble metal ions in an amount of no more than 10% (chitosan-gel 98.5; Ag 1.5.) [14, 28, 44, 60].

The results of clinical trials are presented. The composition stimulates wound healing, reduces skin irritation [2, 48].

Chitosan, a containing agent for the treatment of infected wounds and burns, exhibiting antibacterial properties, transforming their clinical course, and accelerating various stages of healing, including the development of granulation tissue, fibrillogenesis and epithelialization [38, 59].

The combined use of mesenchymal stem cells of adipose tissue and allo- or xeno-skin (chitosan films) in conditions of a shortage of donor resources can increase the efficiency of plastic surgery [69].

Burns and burn disease remain one of the urgent problems of modern medicine due to the high frequency of occurrence, both in war and in peacetime. Depending on the degree of damage and the extent of skin damage, the severity of the course, the risk of complications and mortality increase dramatically. The mechanism of burn disease is multifunctional and proceeds against the background of severe endogenous intoxication and multiple organ failure. The intensity of reparative processes depends on many factors that determine the compensatory capabilities of both the macroorganism and the skin and its appendages. The sequential course of regeneration processes of damaged skin areas dictates the need to create dressings or coatings, taking into account the peculiarities of the molecular mechanisms underlying the processes of reparative regeneration. Despite the achieved success in the treatment of burns, the dressings and coverings available in the arsenal of combustiologists have certain disadvantages. This dictates the need to create gel coatings that meet the basic requirements of specialists and have biodegradability. In this regard, chitosan derivatives isolated from the pupae of the silkworm *Bombyx mori* are promising.

Material and methods

Samples of the preparation Chitosan (ChS) were kindly provided by the Institute of Chemistry and Physics of Polymers of the Academy of Sciences of the Republic of Uzbekistan. Chitosan was standardized for the amount of nitrogen content. In this work, we used gels based on Bombyx mori chitosan, crosslinked with glutaraldehyde (GA) and filled with biologically active elements (BAE). Furacilin (FC) was used as BAE, an aqueous solution of which for the experiments was prepared by dissolving FC powder (Petrovich Y.A., 2008; Rashidova S.S. *et al*., 2009). The gelation process is accompanied by the formation of a network supramolecular structure, which, depending on the conformational state and chain packing, is characterized by different porosity. The results of freeze drying of the swollen sample showed that crosslinked chitosan contains about 0.5% FC in its composition.

To solve the set tasks, the thermal burn model was reproduced on 120 white outbred male rats (*Rattus norvtgicus f.domesticus*) weighing 140-160 g. In accordance with the requirement of the Declaration of Helsinki on the humane treatment of animals, the burns were performed under

light ether anesthesia In accordance with the requirement of the Declaration of Helsinki on the humane treatment of animals, the burns were performed under light ether anesthesia (Shakirov B.M. *et al.*, 2009).. A burn wound was modeled by immersing a previously depilated skin area (depilation of rats was carried out 3 days before applying a burn; first, the hair was sheared off with scissors with rounded ends) skin area. In order not to injure the skin at the place where the hair was cut, it was stretched with fingers I and II of the left hand. A depilator is used to completely remove hair.

The depilated area of the lower back surface (on the caudal dorsal, lumbar regions (reg.dorsi, reg.lumbaris, reg.glutea)) was immersed in boiling water. The exposure time is 10 seconds. With this mode, damage to all layers of the skin in the burn zone was achieved, which corresponds to a third degree burn (Axmatova N.K., 2008). The area of the burn wound is 12-16 cm^2. The percentage of the burn area was calculated as the ratio of the burn area to the total body surface of the animal, which was 18-20%. The used model of thermal burn in rats was adequate, because provided a skin burn. Skin of unburned rats was used as a control.

The studies were carried out in two series. In the first series, the restriction and the degree of binding of restrictive DNA's with new chitosan derivatives were studied in vitro. We used wild-type λ phage DNA and Hind III restriction endonuclease from "SibEnzyme"; 4 chitosan samples:

I preparation – (ChS I)
II preparation – (ChS II)
III preparation – (ChS III)
IV preparation – (ChS IV)

The most active chitosan sample was selected according to the degree of binding.

In the second series of experiments, we studied the effect of new chitosan derivatives obtained from Bombyx mori on the processes of skin regeneration during thermal injury in vivo.

The binding of the active sample of chitosan to DNA isolated from the damaged part of the skin during thermal burns was preliminarily checked. Burn models were reproduced according to the method described above in 100 rats, 20 rats constituted an intact group.

In an in vivo experiment, two hours after reproduction of the burn, the rats were divided into 4 groups, and local treatment was carried out:

1 - a group of 25 rats were treated with ChS 1

2 - a group of 25 rats were treated with ChS 2

3 - a group of 25 rats were treated with Levomekol (ointment);

4 - a group of 25 rats were treated with saline (0.9% sodium chloride solution);

5 - group of intact rats.

These preparations were applied at a dosage of 150 μg / cm2 of the skin area with thermal burn. Before applying the drug, the wound was treated with 3% hydrogen peroxide solution. Levomekol ointment produced by "Nizhpharm" was used as a comparison drug, which was applied in a thin layer to the burn site in accordance with generally accepted methods (Shakirov B.M. *et al.*, 2009).

The treatment was carried out once for the entire duration of the experiment. On the 3rd, 7th and 11th days after the treatment, 6 animals were decapitated from each group under light ether anesthesia, blood and skin of the animals were collected. In the blood, the content of malondialdehyde (MDA) and average weight molecules (MCM) was determined, and a thymol test was performed. Molecular biochemical analyzes were performed in skin samples.

Restriction of λ phage DNA was performed using the HindIII restriction enzyme (Shavandi A. *et al.*, 2017). The reaction mixture contained 0.2-1 μg of DNA in a volume of 20 μl. Water was added to the DNA solution to a volume of 18 μl and mixed. Then 2 μl of the corresponding 10-fold concentration buffer was added, mixed, 1 U of restriction enzyme was added, and mixed. The resulting mixture was incubated at 370C for 18 hours. The reaction was stopped by the addition of 0.5 M ethylenediamine tetraacetate (EDTA) (pH 7.6) to a final concentration of 10 mM. Electrophoresis of the products of DNA hydrolysis was carried out in 1.5% agarose gel. Electrophoresis was carried out at a voltage of 3 V / cm. Separation of DNA fragments was monitored under ultraviolet light (Eroshenko D.V. *et al.*, 2017; Lu S. *et al.*, 2008).

The binding of chitosan to restriction DNA fragments was carried out according to the Maniatis method as follows: 1 μg of λ phage DNA and 5 μl of chitosan I, II, III, and IV were added to the tubes, incubation for 15 minutes at 370C. After incubation, 15 μl of restriction buffer (50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 1 mM dithiothreitol), 0.5 μl HindIII were added to each tube. Incubation for 1 hour at 370C.

At the end of the reaction, each sample was centrifuged at 9000 rpm for 10 min. To precipitate the chitosan complex with bound DNA, the supernatant was taken and added 3 μl of the dye in the loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 40% sucrose in water). DNA electrophoresis was performed in 1.5% agarose gel (Lu S. *et al.*, 2008).

Electrophoresis was carried out in a horizontal gel using a variety of cuvettes in which the gel was placed on a separate glass plate. The plate was installed in such a way that the gel was under the very surface and the electrophoresis buffer. The resistance of the gel to the passing electric current differs little from the resistance of the buffer, therefore a significant fraction of the current passes through the gel (Lu S. *et al.*, 2008).

The buffer used for electrophoresis: - Tris - acetate; working solutions: 0.04 M tris - acetate (50X): 0.002 M EDTA; concentrated solutions (per 1 liter): 242 g of tris, 57.1 ml of glacial acetic acid, 100 ml of 0.5 EDTA, pH 8.0.

Fractionation of DNA preparations in agarose gel was carried out according to the Sharp method. The agarose gel was prepared as follows: the required amount of agarose was dissolved by heating in a water bath in a buffer containing 0.04M Tris-HC1 (pH 7.5), 0.005M sodium acetate, 0.001M EDTA. A buffer of the same composition was used as an electrode. The agarose solution was cooled to 500C, poured into an electrophoresis device (the thickness of the gel layer was 3 mm).

The samples after restriction were mixed with sucrose, EDTA, and bromine phenol blue dye (final concentrations were 5%, 0.01 M and 0.004%, respectively) and applied to the gel surface. Electrophoresis was performed overnight at room temperature at a voltage of 1 B / cm gel. The gel was stained in a solution of ethidium bromide (0.5 μg / ml) for 30-40 minutes and viewed under ultraviolet light. Bacteriophage DNA λ was used as a marker to determine the molecular weight of DNA fragments (Lu S. *et al.*, 2008).

High-molecular-weight DNA was isolated from the skin of normal rats and rats with grade III burns by a modified phenol extraction method [30; from. 532-541, 122; from. 479]. Leather samples were ground in a mortar to a fine powder in liquid nitrogen. The resulting powder of skin cells was homogenized in lysis buffer (20 mM Tris-HCl, pH 8.0; 10 mM EDTA, pH 8.0; 5% Triton X100), incubation in an ice bath for 30 min.

After incubation, the homogenate was centrifuged for 30 min. (13000g, 40C). After centrifugation, RNase (110 μg / ml) was added to the supernatant, incubated for 1 hour at 370 C.

Then, proteinase K (0.05 mg / ml) was added, incubation for 4 hours at 370. DNA was precipitated with 0.1 volume of 3M sodium acetate and 2 volumes of ethanol. DNA concentration was measured spectrophotometrically at 260 nm (Lu S. *et al.*, 2008).

To bind chitosan to DNA of skin cells, incubation mixtures were prepared containing 1, 3, and 5 μg of the analyzed DNA of normal skin cells and with a third degree burn. The control samples contained the corresponding DNA concentrations of normal skin cells with the addition of TE buffer pH 8.0 to a final volume of 15 μl.

Experimental samples contained the corresponding DNA concentrations of skin cells with a burn, adding 5 μl of chitosan-1 and TE buffer pH 8.0 to a final volume of 15 μl. Incubation for 1 hour at 37^0C. DNA electrophoresis was carried out in 1% agarose gel in tris-acetate buffer, pH 8.0 at a voltage of 60 V. The gel was photographed through the transmitted rays of a UF transilluminator.

Incorporation of ^{3}H-thymidine into DNA and ^{14}C-uridine into RNA. Tissue was selected from areas of normal skin and the edges of the inflammation focus of a burn injury in an equal volume, which was placed in an Eagle's medium and ground in a glass homogenizer. The cell suspension was filtered through a nylon filter, the number of cells in the Goryaev chamber was calculated, to 500 thousand.

To the cells was added 1 μCi of ^{3}H-thymidine, ^{14}C uridine was incubated for 2 hours in Eagle's medium in 3 ml with fetal calf serum 10% by volume, gentamicin 80 μg / ml. Then the cell suspension was centrifuged at 1500 turn/min for 5 min, 2.8 ml of the supernatant was removed, the remaining 200 μl of the precipitate was pepitated and applied to nitrocellulose filters 1.5x1.5 cm, the filters were dried and transferred into vials containing 3 ml of scintillation liquid.

The number of pulses per minute was measured on a Beckman counter. The results of the incorporation of isotopes into skin cells from the focus of inflammation of a burn injury were presented as a percentage of the incorporation of isotopes into cells of normal skin (Shakirov B.M. *et al.*, 2014).

The cell suspension was filtered through a nylon filter, washed and resuspended in Eagle's medium containing 1% fetal calf serum. If necessary, the cell suspension was stored at -20^0C. To study internucleosomal DNA degradation, cells (2x106) incubated with 100 nM olivomycin for 24-72 hours, precipitated at 1000 x g, the supernatant was centrifuged at 12000 x g for 15-20 minutes, the pellets were pooled and lysed in a buffer containing 20 mM Tris-HCl, pH 7.4, 0.35 M NaCl, 0.5% NP -40, 2 mM MgCl2, 1 mM dithiothreitol. DNA was extracted with a mixture of phenol and chloroform (1: 1) and precipitated with ethanol in the presence of 0.3 M sodium acetate at –20^0C. The precipitate was treated with RNAse A for 20 min. at 65^0C and analyzed by electrophoresis in 1.5% agarose gel.

The LPO intensity was determined by the content of malondialdehyde (MDA) (Andini I. *et al.*, 2017). The principle of the used determination method was that thiobarbituric acid (TBA) in an acidic medium interacts with low-molecular-weight dialdehydes (mainly malonic) to form a colored complex with a maximum light absorption at a wavelength of 535 nm. The samples obtained during the determination are photometric at wavelengths of 535 and 580 nm against n-butanol in a cuvette with an optical path length of 10 mm on an SF-46 spectrophotometer.

The extinction difference was multiplied by 53.2 (conversion factor). An increase in the level of TBA-active compounds in blood serum is a reflection of the activation of lipid

peroxidation processes and serves as a nonspecific marker of a wide range of pathological conditions, including infectious processes. The calculation of the content of products reacting with thiobarbituric acid was carried out taking into account the molar extinction of malonic dialdehyde, equal to 1.56 106 mol cm^{-1} in nmol MDA/g of protein.

Average mass molecules (AMM) were determined by the method of N.I. Gabrielian by the detection of the supernatant freed from coarse proteins, which was carried out after preliminary dilution, in which 4.5 ml of distilled water was added to 0.5 ml of the supernatant. The measurement was carried out on a spectrophotometer in UF light at a wavelength of 254 nm. The MMP level was expressed in units that are quantitatively equal to the extinction indices (Fedosov P.A. *et al.*, 2015).

Determination of the sorption sobility of erythrocytes (SSE). In medical practice, to assess endogenous intoxication, the method of A.Ya. Togaybaeva, based on the determination of the SSE of blood (Shakirov B.M., 2007). The method is based on the idea of an erythrocyte as a universal adsorbent. A.Ya. Togaybaeva is based on the ability of erythrocytes to absorb the methylene blue dye, depending on the functional state of their cell membrane, i.e. reflects the regenerative ability of erythrocytes. It is known that this ability changes as the permeability of the erythrocyte membrane changes: at the beginning of intoxication, it is reduced, and then, if its bilayer structure is damaged and the indicator interacts with intra-erythrocyte substances, the ESE increases above the norm, which indicates cellular disorganization. SSE was determined by the method of A.Ya. Togaybaeva et al. (Shakirov B.M., 2007). The technique is as follows: 1 ml of erythrocyte mass is placed in a centrifuge tube with 3 ml of methylene blue. Mix the contents of the test tube and leave the test tube for 10-12 minutes at room temperature. Then centrifuged for 10 minutes at 3000 rpm. The supernatant is transferred to a KFK-56 cuvette. Photometry with a red filter, wavelength 630 nm, against water. Photometry of the original dye solution is also performed.

The calculation is carried out according to the formula:

$$\mathbf{A\% = 100\% - (D_1 * 100\% / D_2)},$$

where: $\mathbf{D_1}$ - optical density index after incubation with erythrocytes, conv. units;

$\mathbf{D_2}$ - optical density of the original dye, conv. units;

A - sorption capacity of erythrocytes,%.

The data obtained in the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2003 software package, including the use of built-in statistical processing functions. Methods of variational parametric and nonparametric statistics were used with the calculation of the arithmetic mean of the studied indicator (M), standard deviation (σ), standard error of the mean (m), relative values (frequency,%), the statistical significance of the obtained measurements when comparing the mean values was determined by the criterion Student's (t), at a significance level of $P < 0.05$, the differences were taken as statistically significant.

Results and Discussion

Binding of chitosan to specific regions of DNA

A number of works show [12, 15, 32, 36] that chitosan as a polycation and its oligosaccharides are well suited for the role of carbohydrate vectors for DNA transfer, since chitosan is a natural polycationic polysaccharide that readily forms ionic complexes with DNA, with the degree of DNA binding occurring. However, the properties of chitosan in the compaction of DNA

fragments differing in molecular weight have not been fully studied. It was of interest to study the properties of chitosan by selective binding to specific regions of DNA or genes responsible for certain cell functions.

In this experiment, bacteriophage lambda DNA of wild type λ was used as a test DNA with a known physical and genetic map. Four types of chitosan were studied for binding to restriction DNA fragments - λ, cleaved by the Hind III enzyme (Fig. 1). The experimental results were analyzed by the polymorphism of restriction fragments, the absence and intensity of their luminescence in comparison with the marker fragmented DNA - λ / Hind III.

In the sample with chitosan-I, virtually complete elimination of DNA regions of different sizes from 23.1 to 2.0 kb is observed, which indicates the maximum binding of chitos-I DNA fragments (Fig. 3.1.1, lane 1). ChG-II is characterized by the binding of DNA fragments less than 9.4 kbp. (Fig. 3.1.1, lane 2), for X3-III and X3-IV - less than 6.6 kbp. (Figure 3.1.1, lanes 3,4). Analysis of the obtained restriction patterns allows us to conclude that the 4 types of chitosan differ markedly in binding to different DNA fragments, the highest binding was noted for chitosan I (Fig. 1).

Thus, in this experiment, the most pronounced DNA binding is observed in ChS-I. In this connection, in the future we investigated the binding of ChS-I with DNA isolated from skin cells with a third degree burn.

Figure 2 shows the results of electrophoretic mobility in an agarose gel of intact DNA and its combination with ChS-I (Figure 2, lane 1).

On the electrophoretogram (Fig. 2, lanes 2–4), there is a noticeable difference in the electrophoretic mobility of DNA from skin cells with grade III burns from intact DNA, depending on the increase in its concentration and fragmentation in the corresponding samples. In the same experiment, in parallel variants with the use of ChS-I in samples 5-7, on the electrophoretogram, a decrease in the concentration of fragmented DNA in these samples is observed (Fig. 3.1.2) The result of the combination of the use of DNA with ChS-I indicates that ChS-I binds finely cleaved DNA fragments

Internucleosomal DNA degradation analysis

In this experiment, the level of internucleosomal DNA degradation (IND-DNA) of skin cells with grade III burns treated with the studied drugs: ChS-1, ChS-2 and Levomikol was investigated. After treatment and appropriate decapitation of the animals, skin cells were isolated for IND-DNA analysis. Figure 3 shows the electrophoretic separation in a 2% agarose gel of nucleosomal DNA of the studied skin cells with burns after the application of this medicinal preparation.

On the third day after treatment with the studied drugs, the nucleosomal DNA is highly fragmented. Along the track (lanes 2-5), DNA splitting into small fragments is observed, with no difference between these samples.

On the 7th day (lanes 6-9), DNA fragmentation was reduced by 30-40% compared to DNA degradation on the third day, except for DNA samples of untreated skin cells (lane 9). In a comparative analysis of the degree of DNA fragmentation (lanes 6-8), when using ChZ-1, the level of fragmentation is reduced compared to the control group, the result of the variant of lane 6.

On the 11th day of the treated and untreated animals, a noticeable decrease in IND-DNA is observed in comparison with the results of the third day. With the use of ChS-1 and ChS-2, the

level of DNA fragmentation is significantly reduced. The level of IND-DNA, in the variant with levomikol, corresponds to the control variant (untreated).

The data obtained indicate that chitosan has a pronounced ability to inhibit IND-DNA and to absorb fragmented DNA molecules of skin cells. The ability of chitosan to bind to the DNA of cells leads to the activation of reparative processes in the nuclear apparatus of the cell, and to the regeneration of unfired cells.

With extensive and deep burns, deep shifts occur, both in the function and in the morphology of almost all major organs and systems, and the causal relationship of the violation is at the cellular and subcellular level, which greatly complicates the recognition of these subtle pathological processes. The pathogenesis of a burn is rather complex and not fully understood. With burns, significant biochemical, molecular genetics and humoral changes occur, leading to a violation of protein, carbohydrate, metabolism. Of great importance is the effect of high temperature on cell proteins, as a result of which proteins denature, protein synthesis is disrupted, and the processes of DNA replication and transcription are disrupted. In order to study the changes in the processes of DNA replication and transcription in burns under the influence of ChS-1, ChS-2 and levomikol, studies were carried out on the incorporation of ^{3}H-thymidine and ^{14}C-uridine (1).

As can be seen from the above data, in case of thermal injury in the early stages of the study, an increased inclusion of ^{3}H-thymidine and ^{14}C-uridine in the synthesis of DNA and RNA of skin cells is noted. Subsequently, these processes slow down, and by the 10th day of the experiment, the intensity of incorporation of ^{3}H-thymidine and ^{14}C-uridine into DNA and RNA is 25.5 ± 0.28 and 20.0 ± 0.35%.

Table 1 presents data on the effect of chitosan on the synthesis of DNA and RNA of skin cells with III degree burns. This figure shows that under the action of chitosan 1 on the third day the incorporation of ^{3}H-thymidine into DNA is 52%, on the 7th day - 72%, on the 11th day - 94%. The data obtained indicate the activation of DNA synthesis in intact skin cells. The incorporation of the labeled precursor into DNA without the use of ChS-1 (untreated) on the 3rd and 7th days is 48% and 27.8%, respectively. On the 11th day, the incorporation of ^{3}H-thymidine into DNA is 25.5%, i.e. there is a decrease in DNA synthesis by 74.5%.

The effect of ChS - 2 did not differ from the previous sample, although it was somewhat lower. On the third day, the incorporation of 3H-thymidine into DNA is 45.8%, on the 7th day - 60.4%, on the 11th day - 88.2%. The incorporation of the labeled precursor into DNA when using levomikol was 40.0; 58.8 and 75.5%, which is significantly lower than the values of the group of animals using chitosan.

Analysis of the results of the inclusion of ^{14}C-uridine in the synthesis of RNA showed that the intensity of its inclusion when using ChS-1 and ChS-2 up to 40.0 and 38.0% on the 3rd day, up to 67.2 and 57.4% - on 7th and up to 90 and 80% - on the 10th day of the experiment, respectively. When using levomikol these values were 32; 50 and 70%, which is significantly lower than the values of the group of rats treated with ChS. The incorporation of the labeled precursor into DNA in the control group on the 3rd and 7th days is 35% and 274.3%, respectively. On the 11th day, the incorporation of ^{14}C-uridine into RNA is 20%, i.e. there is a decrease in RNA synthesis in epithelial cells.

Consequently, in intact skin cells of rats with thermal injury, a slowdown in the processes of replication and transcription is noted. Levomikol only slightly increased the studied

indicators. We observed more pronounced intensification of DNA and RNA synthesis when using chitosan, especially chitosan-1.

Thus, the use of ChS-1 in the treatment of III degree burns leads to the restoration of DNA repair processes.

Effect of chitosan on the degree of endogenous intoxication

As can be seen from the results presented in Table 2, with 3-day treatment with ChS-1, SSE is 89%, which is 10.85% better than the performance of animals in the control group on the 3-day experiment. There is a clear tendency towards normalization of their level on the 7th and 10th day of treatment. The level of ESE in treated animals of the 1st group is only 22.14 and 11.4% higher than that in intact animals.

This effect differs in animals of the 2nd group, which were treated with the drug ChS-2, SSE with a slightly lower efficacy compared to the 1st group, but the result on the 3rd day exceeded the indicator by 8.17% compared to the control. In group 3, the optimization of indicators was low on the 3rd and 7th days, and on the 10th day it exceeded the indicators of the intact group by 31.09%, which was higher only in the control group.

Correction of experimental thermal trauma in the 1st group showed a decrease in SMP by 10.4; 41.3 and 57.4% on the 3rd, 7th and 10th day of correction. The content of SMP changed less significantly in the 2nd and 3rd study groups. Correction with drugs of the 3rd and 4th groups on the 3rd day of treatment was lower than the values of the control group by 1.09 and 1.05 times, on the 7th day at 1.85, 1.12, on the 10th day of correction at 13.0 and 5.7 times, respectively (Table 3).

Animals within 3 days led to an increase in the thymol test in blood serum by 55.7%, and under the influence of ChS-2 by 45.2% less than in the control group, respectively, and under the influence of levomikol, no significant changes in its content were revealed (table 4).

Positive changes in the thymol test index in blood serum were observed when correcting the experimental thermal burn in animals all groups on the 7th day of correction. ChS-1 leads to a 2.6-fold decrease in the thymol test, and in the study group using ChS-2, 1.9 times as compared with the control group.

With the correction of thermal injury in the studied animals of all groups within 10 days, there was a positive dynamics from the side of the studied indicator - thymol test. We have established a significant decrease in the thymol test index during correction with ChS-1 preparations; ChS-2 and levomikol ointment by 72.3; 73.6; and 17.1%, respectively, compared with the control group.

The results obtained showed that the correction of the experimental thermal burn in animals with the studied drugs for 3-, 7- and, especially, 10 days leads to a significant decrease in the SSE, MMP and thymol test, which indicates a more pronounced antitoxic effect of the studied drugs.

It should be noted that the correction of the studied pathology in the groups with the use of chitosan is the most pronounced decrease in the studied indicators compared with the levomikol group. From the above indicators it follows that the studied drugs most effectively affect the content of SSE, MMP and thymol test when corrected within 10 days. Chitosan is superior to levomikol in its action.

Thus, the treatment of experimental thermal injury with chitosan-1 for 10 days practically normalizes the indicators of average molecular peptides, the sorption capacity of erythrocytes and the thymol test, while under the action of levomikol only the indicators of average molecular

peptides. The effectiveness of the antitoxic effect of chitosan-1 is statistically significantly higher than that of levomikol ($p < 0.05$).

The effect of chitosan on the MDA content in blood serum during experimental thermal burn.

The assumption of a close relationship between the intensity of LPO processes and the level of certain lipids in the blood of patients with thermal burns can be confirmed or refuted by exposing these lipids to antitoxic drugs. In this connection, a single application of chitosan promoted a pronounced normalization of most indicators of endogenous intoxication; in this series of works, we investigated the effect of chitosan on the content of the final product POL – MDA in blood (tabl. 5).

As a result of the experiments, it was found that when correcting experimental thermal injury in animals, applying ChS-1 already after 3 days reduces the final LPO product in blood serum by 41.6%, and was 10.14 ± 1.61 nmol / ml, and after 7 and 10 days of treatment, its decrease was 60.2 and 64.4% compared with the control group. Under the influence of ChS-2, MDA in the blood serum of experimental animals decreases less significantly. Thus, the level of MDA on days 3, 7 and 10 of the study of the correction of this pathology decreased by 55.3; 58.4 and 59.4%, respectively.

Along with this, in rats with experimental thermal injury, ChS-2 does not cause pronounced stimulation of the content of lipid peroxidation products. On the 3rd day of its use, the MDA content in the blood serum decreases by 55.3%, on the 7th day by 38.6% compared with the group of experimental traumatic injury, through ChS-2 MDA compared with the control group is reduced by only 40.0%. With the correction with levomycol, there was a slight decrease in the MDA content on days 3 and 7 of the experiment in comparison with the control group, it reached 17.32 ± 1.74 and 13.24 ± 0.23 nmol / ml; 7.87 ± 0.17 nmol / ml on the 10th day of correction. This indicator in animals of the intact group was 5.85 ± 0.49 nmol / ml, experimental thermal burn - 17.85 ± 0.51 nmol / ml.

So, on the 10th day of the experiment of chitosan and levomikol, their difference in action is 2 times. The decrease in the MDA level under the influence of the studied drugs both on the 3rd, and 7th and 10th days of application was statistically significant in relation to the control group. However, the decrease in the MDA level under the action of chitosan was more significant than with levomycol, and this difference was also statistically significant.

The data obtained indicate that chitosan and levomikol act unidirectionally on the body's LPO, but chitosan, compared with levomikol, has a more pronounced antitoxic effect in thermal burns, especially when treated within 10 days of its administration, and it was expressed in a decrease in the MDA content

According to Azhikova A.K. (2010), the main condition for accelerating the rate of wound healing when using stem cells on a chitosan substrate was the weakening of the severity of the destructive-inflammatory phase of the wound process and the activation of the proliferative-reparative phase (Ajikova A.K., 2010). According to the author, the development of severe thermal injury is accompanied by the development of intoxication due to the entry into the blood of toxic substances formed in the lesion focus. Indeed, our studies have shown an increase in the content of MMP in the blood plasma of burnt animals and SSE. In our opinion, this is due to the absorption of cell degradation products from the affected focus and the attachment of microflora. The use of chitosan-1, due to the coating of the affected area with a protective layer,

prevents the formation of toxic compounds in the affected area, which leads to a decrease in the phenomena of endogenous intoxication.

Burn intoxication leads to the activation of free radical processes in the body of experimental animals. To assess this, we determined the level of MDA in blood plasma. Indeed, in our experiments, we observed an increase in LPO. Pharmacotherapy with chitosan-1 contributed to a decrease in the MDA level and restored the balance in the LPO-AOS system. The positive effect of chitosan in combination with furacilin revealed by us in the treatment of burn skin lesions dictates the need for their introduction into combustiology. This is carried out as follows: the application of a chitosan gel with furacilin is applied to a burn surface cleaned from necrotic masses under sterile conditions. It creates a layer impervious to bacteria and, therefore, accelerates the regenerative processes of the affected area. At the same time, not only the acceleration of the regeneration of the damaged focus, but also the biochemical parameters of blood plasma, is noted, which, in our opinion, is a good prognostic sign.

Thus, the studies carried out show that the studied drugs contribute to the normalization of disorders by the reproduced experimental thermal burn, the process of endogenous intoxication of the MDA content. Ten-day use of chitosan completely normalizes the content of the end product of lipid peroxidation - MDA in blood serum. The effectiveness of levomikol was less pronounced, and complete normalization of MDA was observed only on the 10th day of correction.

On the first day of reproduction of the experimental burn, the rats showed a state of acute burn disease: lethargy, weakness, shortness of breath, polydipsia, and polyuria. On the 3rd day, a burn scab was formed on the surface of the affected area, the condition of the animals began to improve. In rats of the 1st group in the course of treatment with ChS-1, the state gradually improved, they became more active with good appetite. The same direction of changes was noted in animals of the 2nd and 3rd groups, treated with chitosan-2 and levomycol, however, the phenomena of intoxication persisted. At the same time, in animals of the 4th group, the phenomena of intoxication persisted for a long time, a deterioration in the general condition was noted, due to the development of purulent-septic phenomena.

In case of burn injury in the affected area, primary anatomical and functional changes, reactive-inflammatory phenomena and regenerative processes were observed. In the control group, in the area of thermal injury, the beginning of the formation of a burn bladder was noticeable, and redness was observed on the skin around the wound. Over time, there was an increase in the injured surface by 1.3-1.5 times with signs of inflammation and necrosis. In animals of the 1st and 2nd groups, the expansion of the damaged surface was not observed over time. The formation of a wound scab was noted, and uniform healing was observed under its surface, signs of inflammation were not observed. In the third group of animals on the first day after the experiment, there was a slight increase in the injured surface by 1.1 times with signs of inflammation.

In the experiment of chitosan binding to specific regions of DNA, the most pronounced DNA binding is observed chitosan-I. In this connection, in the future we investigated the binding of ChS-I with DNA isolated from skin cells with a third degree burn.

The data obtained from the analysis of internucleosomal DNA degradation indicate that chitosan has a pronounced ability to inhibit IND-DNA and to absorb fragmented DNA molecules of skin cells. The ability of chitosan to bind to the DNA of cells leads to the activation of reparative processes in the nuclear apparatus of the cell, and to the regeneration of unfired cells.

In intact skin cells of rats with thermal injury, a slowdown in the processes of replication and transcription is noted. Levomikol only slightly increased the studied indicators. We observed more pronounced intensification of DNA and RNA synthesis when using chitosan, especially chitosan-1.

Treatment of experimental thermal injury with chitosan-1 for 10 days practically normalizes the indicators of average molecular peptides, the sorption capacity of erythrocytes and the thymol test, while under the action of levomikol only the indicators of average molecular peptides. The effectiveness of the antitoxic effect of chitosan-1 is statistically significantly higher than that of levomikol ($p < 0.05$).

The studies carried out show that the studied drugs contribute to the normalization of disorders by the reproduced experimental thermal burn, the process of endogenous intoxication of the MDA content. Ten-day use of chitosan completely normalizes the content of the end product of lipid peroxidation - MDA in blood serum. The effectiveness of levomikol was less pronounced, and complete normalization of MDA was observed only on the 10th day of correction.

Conclusion

Derivatives of chitosan in in vitro studies are capable of binding to DNA molecules, which leads to the activation of DNA repair processes and regeneration of burnt tissue. The most effective drug is chitosan succinate.

1. Thermal injury is accompanied by an increase in internucleosomal skin degradation. Compared with levomecol, the ChS-1 and ChS-2 preparations more effectively reduce the degree of internucleosomal degradation, especially on the 10th day of the experiment.
2. The rate of incorporation of ^{3}H-thymidine in the treatment of ChS-1 is 52; 72 and 94%, respectively, for the periods of 3, 7 and 10 days, which is significantly higher than the values of the group of animals treated with levomecol and, especially without correction, on the 10th day of treatment. In this case, the rate of incorporation of ^{14}C-uridine increases by 1.25; 1.34 and 1.3 times compared with levomecol, which indicates gene expression.
3. In thermal injury, chitosan, in comparison with levomecol, has a more pronounced antitoxic effect, which is manifested by a decrease in the high level of MSM. The severity of hyperlipoperoxidation is reduced to a greater extent with the use of chitosan derivatives.

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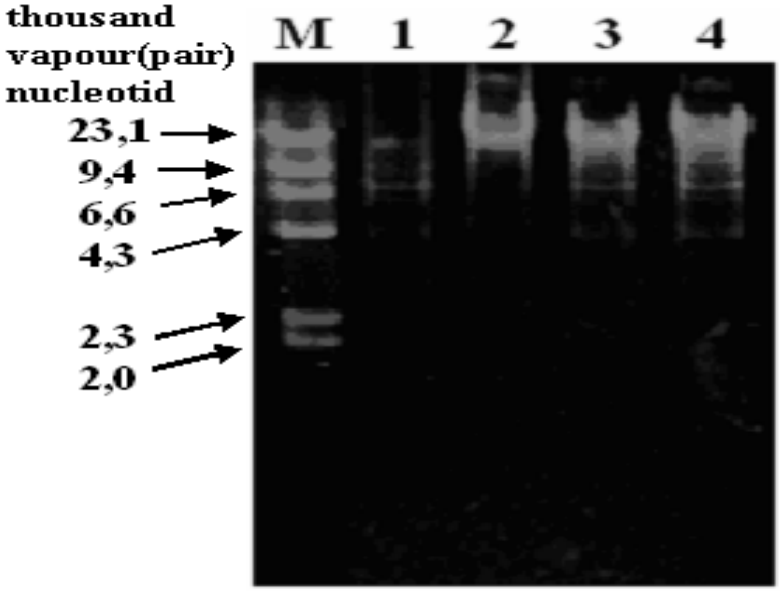
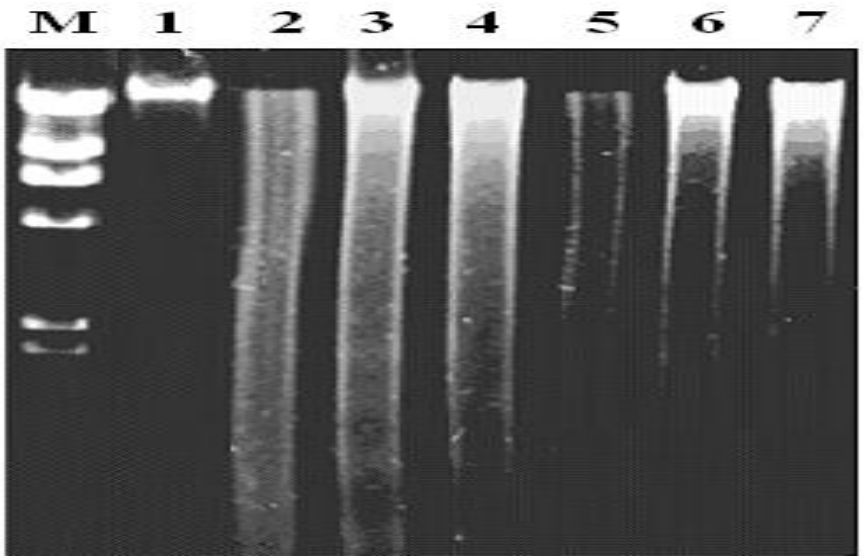
Appendix



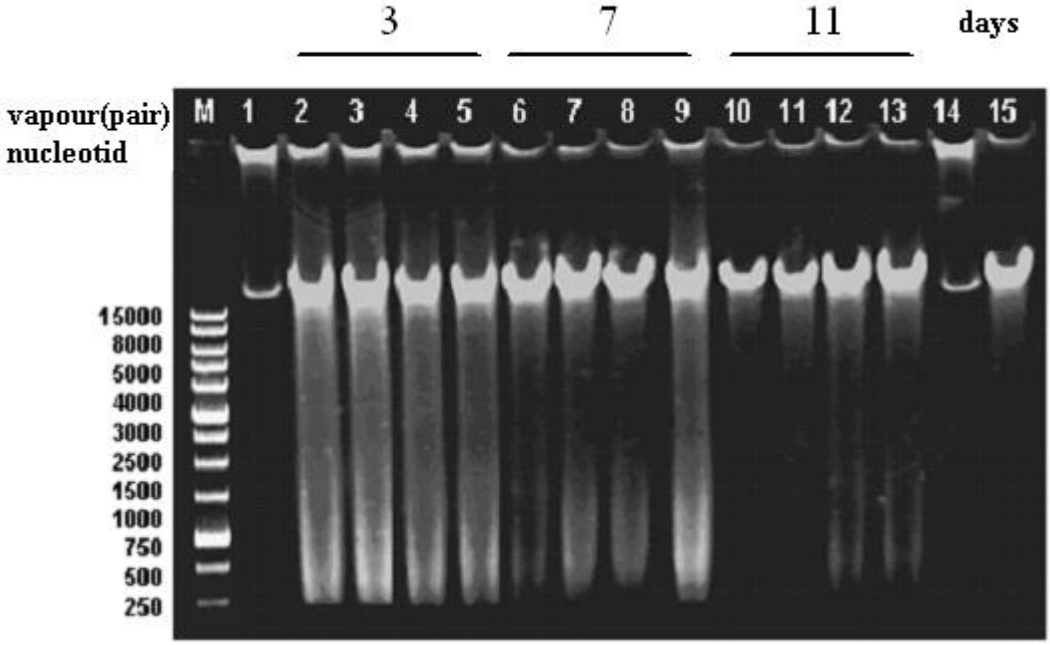
Track: M - marker λ / Hind III; 1 - chitosan I; 2 - chitosan II; 3 - chitosan III; 4 - chitosan IV.

Fig. 1. Electrophoretic separation of products of bacteriophage λ DNA hydrolysis by endonuclease Hind III in the presence of various types of chitosan.



Track: M - marker 1mkg λ / 20ED Hind III; 1 - 1 μg DNA from normal skin cells; 2,3,4 - 1, 3 and 5 μg, respectively, DNA of skin cells with a third degree burn; 5,6,7 - 1, 3 and 5 μg, respectively, DNA of skin cells with a burn of III degree using 5 μl of ChS-I.

Figure: 2. Electropherogram of chitosan-I binding with DNA of skin cells of white outbred rats (intact and on the 3rd day after burn).



Track: M - marker, 1, 14 - intact phage λ DNA; 2, 6, 10 –KhZ-1; 3, 7, 11 - KhZ-2; 4, 8, 12 - levomikol; 5, 9, 13 - untreated; 15 - intact DNA of skin cells, normal.

Figure: 3. Analysis of internucleosomal DNA degradation of skin cells with burns of III degree (intact and on the 3rd day after the burn).

Table 1

Influence of chitosan on the incorporation of ^{3}H-thymidine and ^{14}C-uridine into DNA and RNA of skin cells with grade III burns, M ± m, n = 10

Day	Inclusion of the labeled precursor, in% of the intact group							
	Chitosan – 1		Chitosan – 2		Levomikol		Control	
	^{3}H-thymidine	^{14}C-uridine	^{3}H-thymidine	^{14}C-uridine	^{3}H-thymidine	^{14}C-uridine	^{3}H-thymidine	^{14}C-uridine
3	52,0±0,39	40,0±0,34	45,8±0,18	38,0±0,28	40,0±0,33	32,0±0,21	48,0±0,35	35,0±0,37
7	72,0±0,59	67,2±0,26	60,4±0,45	57,4±0,25	58,8±0,16	50,0±0,10	27,8±0,33	23,4±0,18
10	94,0±0,32	90,0±0,37	88,2±0,47	80,0±0,32	75,5±0,21	70,0±0,24	25,5±0,28	20,0±0,35

* P <0.01 in relation to control

Table 3.4.1

Change in the sorption capacity of erythrocytes in the blood during the correction of experimental thermal injury, M ± m, n = 25

Groups		SSE, %		
		3-th day	7-th day	10-th day
1	ChS-1	89,12±0,02[a,c,d]	68,84±0,02 [a,c,d]	58,14±0,01 [a,c,d]
2	ChS-2	91,8±0,06 [a,b,d]	85,81±0,01 [a,b,d]	66,89±0,01 [a,b,d]
3	Levomikol	96,88±0,02[a,b,c]	90,87±0,02[a,b,c]	77,79±0,01 [a,b,c]
4	Control	99,97±0,005	90,84±0,12	87,78±0,007
5	Intact	46,7±0,02		

P <0.05: a - from the control group,

b, c, d - from indicators of 1-, 2- 3-groups, respectively.

Table 3.

Change in the content of average molecular peptides in blood serum upon correction of experimental thermal injury, M ± m, n = 25

Groups		MMP, conv.unit		
		3-th day	7-th day	10-th day
1	ChS-1	0,206±0,006[a,c,d]	0,152±0,005[a,c,d]	0,144±0,006[a,c,d]
2	ChS-2	0,225±0,004 [a,b,d]	0,202±0,005[a,b,d]	0,169±0,004[a,b,d]
3	Levomikol	0,215±0,006[a]	0,251±0,002[a]	0,138±0,003[a,b]
4	Control	0,230±0,006	0,259±0,002	0,338±0,003
5	Intact	0,142±0,001		

Note: P <0.05: a - from the control group, b, c, d, - from indicators of 1-, 2- 3-groups, respectively, significantly

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Table 4

Change in thymol test during correction of experimental thermal injury, M ± m, n = 25

Groups		Thymol test		
		3-th day	7-th day	10-th day
1	ChS-1	10,3±1,52[a,c,d]	4,1±0,23[a,c,d]	3,1±0,21[a,c,d]
2	ChS-2	17,1±0,9 [a,b,d]	10,7±0,89 [a,b,d]	3,4±0,31 [a,b,d]
3	Levomikol	18,3±1,74 [a,b,c]	11,2±0,23[a,b,c]	6,4±0,17[a,b,c]
4	Control	20,6±0,27	13,5±0,79	8,9±0,71
5	Intact	2,9±0,33		

Note: P <0.05: a - from the control group, b, c, d, - from the indices of groups 1-, 2- 3, respectively.

Table 5

Change in the content of malondialdehyde in blood serum during the correction of experimental thermal injury, M ± m, n = 25

Groups		MDA, nmol/ml		
		3-th day	7-th day	10-th day
1	ChS+GA+FC	10,14±1,61[a,c,d]	6,95±0,13[a,c,d]	6,35±0,16[a,c,d]
2	ChS+GA	7,75±0,5 [a,b,d]	7,26±0,69[a,b,d]	7,24±0,22[a,b,d]
3	Levomikol	17,32±1,74 [a,b,c]	13,24±0,23[a,b,c]	7,87±0,17[a,b,c]
4	Control	17,36±0,23	17,45±0,71	17,85±0,51
5	Intact	5,85±0,49		

Note: P <0.05: a - from the control group, b, c, d, - from indicators of 1-, 2- 3-groups, respectively, significantly