INDICATORS OF ENDOGENOUS INTOXICATION IN THE MODEL OF BURN INJURY IN CORRECTION WITH CHITOSAN DERIVATIVES

Bayqulov Azim Kenjayevich
Associate Professor, Department of Biological Chemistry,
Samarkand State Medical University, Samarkand, Uzbekistan.
E-mail: azimbaykulov81@mail.ru

Raxmonov Farxod Kholbayevich
Doctoral student, Department of Animal Physiology, Biochemistry and Pathological Physiology, Samarkand State University of Veterinary Medicine, Animal Husbandry and Biotechnology, Samarkand, Uzbekistan.
E-mail: farxod1313jon@gmail.com

Egamberdiyev Kamol Egamberdiyevich
Student of Samarkand State University of Veterinary Medicine, Animal Husbandry and Biotechnology, Samarkand, Uzbekistan.
E-mail: egamberdiyevkamol1497@gmail.com

ABSTRACT

Burns and burn injury is one of the main problems of modern medicine due to high mortality and disability. Wound healing is the most important general biological, medical, and social problem, which remains relevant to this day. The aim of the work was to study the indicators of endogenous intoxication on the model of burn injury in the correction with chitosan derivatives. In this work, Bombyx mori chitosan-based gels were used; the thermal burn model was reproduced on 120 outbred male rats weighing 140–160 g. The preparations were applied at a dosage of 1 mg/kg of animal body weight to a skin area with a thermal burn. An analysis of the data obtained during the study of the effect of several dosage forms of chitosan (solution, gel, film) on the process of skin wound healing in rats and rabbits showed that

Keywords: chitosan, thermal burn, malondialdehyde, hexenal dream, thymol test.

INTRODUCTION

The main causes of mortality in the stage of acute burn toxemia are purulent-septic complications in combination with endogenous intoxication of metabolic and microbial origin [4].

The modern trend 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 of its phases in accordance with a specific clinical situation. Chitosan and its derivatives are promising in this respect. Chitosan - 2-amino-2-deoxy-B-glucans (ChS), depending on the molecular weight and degree of acetylation, are used in medicine and cosmeceuticals. Various versions of wound healing agents based on chitosan have been proposed. It has a stimulating effect on a number of vital functions, and on the other hand, it contains natural antioxidants and helps to suppress the activity of free radical oxidation. They provide effective biocorrection at seven levels of organization of living matter [1].

The aim of the work was to study the indicators of endogenous intoxication on the model of burn injury in the correction with chitosan derivatives.

Material and methods.

In this work, gels based on chitosan Bombyx mori crosslinked with glutaraldehyde (GA) and filled with biologically active elements (BAE) were used. Furacilin (FC) was used as BAE, an aqueous solution of which was prepared for experiments by dissolving the powder of FC. The gelation process was accompanied by the formation of a network supramolecular structure, which, depending on the conformational state and chain stacking, is characterized by different porosity. The results of freeze drying of the swollen sample showed that cross-linked chitosan contains about 0.5% PC in its composition. Standardization of chitosan was carried out for nitrogen, the content of which in the samples was 90%. The preparations were synthesized at the Research Institute of Chemistry and Pharmaceuticals (Dir. Academician S.Sh. Rashidova) and kindly presented by Senior Researcher, Ph.D. Milusheva R.Yu. [eleven].

The thermal burn model was reproduced on 120 outbred male rats weighing 140-160 g. In accordance with the Declaration of Helsinki on the humane treatment of animals, the application of burns was carried out under light ether anesthesia [13]. Modeling of a burn wound was performed by immersing a previously depilated skin area [2]. 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 was used to completely remove the hair. The depilated area of the lower surface of the back was immersed in boiling water. Exposure time - 10 sec. With this mode, damage was achieved to all layers of the skin in the burn zone, which corresponds to a third-degree burn [3]. The area of the burn wound is 18-20 cm2. The percentage of the burn surface was calculated as the ratio of the burn area to the total surface of the animal's body, which was 18–20%. The used model of thermal burn in rats was adequate, because caused skin burns. The skin of unbaked rats was used as a control. The lethality of animals was 13.6%.

Two hours after the reproduction of the burn, the rats were divided into 4 groups and local wound treatment was carried out:

group 1 (25 rats) – treatment with Chitosan (Bombix Mori) (CZ) in 2% acetic acid + glutaraldehyde (GA) + furatsilin (F) (C3 + GA + F);

group 2 (25 rats) treated with chS in 2% acetic acid + HA (ChS + HA);

group 3 (25 rats) were treated with levomikol (ointment) (comparison group).

group 4 (25 rats) were treated with saline (control group).

The preparations were applied at a dosage of 1 mg/kg of animal body weight to a skin area with a thermal burn. Before applying the drug, the wound was treated with a 3% hydrogen peroxide solution. Levomikol ointment manufactured by Nizhpharm was used as a reference drug, which was applied in a thin layer to the burn site.in accordance with generally accepted methods [12].On days 3, 7 and 10 after treatment, 6 animals were decapitated from each group. Decapitation was performed under light ether anesthesia.

Before slaughter, a hexenal sleep (HS) test was performed to determine the detoxifying function of the liver. The content of malondialdehyde (MDA) and medium-weight molecules (MSM) was determined in the blood; relevant bioassays. Morphological studies were carried out in skin samples. The digital material was processed by the method of variation statistics.

Results and discussions.

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

In case of a burn injury, primary anatomical and functional changes, reactive-inflammatory phenomena and regenerative processes were observed in the affected area. In the control group, a burn bubble began to form in the area of thermal injury, redness was observed on the skin around the wound, which increased over time with signs of inflammation and necrosis. In animals of the 1st and 2nd groups, the expansion of the damaged surface over time was not observed. The formation of a wound scab was noted, and uniform healing was observed under its surface, signs of inflammation were not observed. In the 3rd group of animals on the first day after the experiment, there was a slight increase in the injured surface with signs of inflammation. An

analysis of the area of the wound in different groups showed a more pronounced decrease in the animals of the 1st group. The effect of drugs in the 2nd and 3rd groups coincided, while in the 4th group the regeneration lagged behind. As can be seen from the data presented, chitosan combined with GA and F had a more pronounced regenerating effect on damaged areas of the skin of experimental animals.

Earlier epithelialization of damaged areas of the skin of experimental animals with the use of CHS + HA + F (group 1) contributed to a decrease in the severity of EI, which was manifested by a decrease in the level of MSM in the blood serum of rats of the 1st group by 1.24; 1.52 and 1.45 times relative to the values of the control group of rats, respectively, after 3; 7 and 10 days from the start of the experiment. The same direction of changes was noted with the use of HZ + HA and levomikol: a decrease of 1.15 and 1.08 times after 3 days; 1.25 and 1.16 times - after 7 and 1.36 and 1.15 times after 10 days from the beginning of the experiment. At the same time, the high level of MDA in the 1st group decreased by 1.18; 1.31 and 1.53 times, in the 2nd - 1.08 times; 1.16 and 1.28 times, in the 3rd - 1.07 times; 1.10 and 1.19 times, respectively. Therefore, the use of chitosan gels containing furatsilin,

This may be due to the restoration of the detoxifying function of the liver of experimental animals, since this function of the liver in burned patients suffers significantly [5]. The studies carried out in this regard showed a significant decrease in the detoxifying function of the liver of the control group of animals, which was manifested by an increase in the duration of the HS to 121.3 + 6.8; 110.8+7.1 and 74.5+6.8 min, respectively, after 3, 7 and 10 days, with the value of this indicator in intact rats 27.4+3.1 min. In animals of the 1st group, the duration of HS was 73.4+6.5; 58.6+3.2 and 41.2+3.7 minutes, respectively. The same changes, but to a lesser extent, were noted in animals of the 2nd group, while in the 3rd group, the duration of the HS remained extended, amounting to 107.3+8.1; 81.2+8.1 and 59.6+4.1 minutes, respectively. Consequently,

It is known that with burns and burn disease, hepatorenal syndrome develops, causing the accumulation of toxins in the body [9]. This is confirmed by the lengthening of the duration of hexenal sleep observed by us in experimental animals. To clarify the syndromes of liver damage, we studied the dynamics of changes in the enzymes ALT and alkaline phosphatase, the content of bilirubin and the thymol test. As can be seen from the table, with a burn injury in animals of the control group, the development of a cytolytic syndrome (increased ALT and hyperbilirubinemia), cholestatic syndrome (increased activity of alkaline phosphatase), mesenchymal inflammation (increased thymol test) is noted. In the course of pharmacotherapy, the severity of the above syndromes is markedly reduced. This is more pronounced in animals of the 1st group, while in the 2nd and, especially in the 3rd groups, they

significantly exceeded the normative values. Apparently, the use of chitosan gels in combination with furacilin significantly reduced the involvement of the liver in the pathological process. Preservation of the integrity of hepatocytes led to the normal functioning of the detoxifying function of the liver and increased excretion of toxic metabolites, thereby contributing to a decrease in the severity of endotoxemia.

The conducted studies also testify to the effectiveness of the use of chitosan preparations in wound disease. An analysis of the data obtained from studying the effect of several dosage forms of chitosan (solution, gel, film) on the process of skin wound healing in rats and rabbits showed that the use of the gel provides a significant acceleration of the skin repair process [17].

Chitosan in the form of a sponge proved to be an effective stimulator of osteogenesis, which was shown when modeling a bone defect in the lower jaw in rabbits [18]. 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: an acceleration of the migration of phagocytes into a wound (a focus of inflammation), 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.

Of the currently existing wide range of polymer coatings for wounds and burns, absorbable coatings meet all medical and biological requirements to the greatest extent, and can be useful both in the early stages of wound and burn treatment, and at later stages. Therefore, the development of absorbable adhesive polymer coatings with different biodegradation periods is currently an important direction in the creation of effective applications for wounds and burns.

The natural polysaccharide chitosan has a wide spectrum of activity. Its derivatives regulate fibroblast proliferation and stimulate normal skin regeneration. The analgesic and antimicrobial action is due to the unique ability of chitosan to nonspecifically interact with pain receptors and the cell wall of microorganisms. One of the reasons for the effective effect of chitosan on wound healing is the stimulating effect on the immune system. it can be considered as an analogue of lipopolysaccharides of the cell walls of microorganisms that act as macrophage activators. A significant problem of post-burn areas are scars that form in places of skin regeneration. The use of chitin and its derivatives can significantly reduce the hypergrowth of granulation tissue. It is known that chitin derivatives have structural characteristics similar to skin glucosamines and can serve as a substrate for the growth of keratinocytes and fibroblasts [6]. The expansion of fundamental and applied research will certainly allow the use of chitosan-based polymers, which have

differentiated properties in terms of solubility, sorption, and bactericidal effect as a factor in increasing the biological value of drugs and their therapeutic effect.

A burn, even a local one, shifts the redox balance of the body in such a way that the content of hydroperoxides in the blood and liver increases and the processes of lipid peroxidation are intensified. Adequate effective therapy for burns, and in our case it is the use of chitosan-1, contributes to the normalization of these mechanisms and neutralizes metabolic disorders. Analyzing the listed methods of treatment of burn skin lesions, we have identified a special need for the implementation of the method we have developed for the treatment of burn skin lesions. The essence of the method of treatment lies in the fact that the application of a small amount of chitosan gel is applied to the dry burn surface. It is necessary to observe sterile conditions and the regimen of therapy. Comparative analysis of biochemical parameters of peripheral blood and liver in rats of the control and experimental groups showed that

In case of thermal injury, chitosan, in comparison with levomikol, has a more pronounced antitoxic effect, which is manifested by a decrease in the high level of MSM. The severity of hyperlipoperoxidation decreases to a greater extent with the use of chitosan derivatives.

REFERENCES

1. Alexandrova E.A., Kirilenko Yu.K., Sigiletov A.E., Rakhmanov R.S., Dushkova Z.G., Suvorov A.V., Cherkasova E.I. // Handbook of preparations Oligochit. Preparations of the oligochit(r) series reference manual. M.2005, p. 38.
2. Bogatova N.P. et al. The structure of endothelial cells of the lymphatic capillaries of the skin in the conditions of correction of the wound process in thermal burns. Bulletin of SO RAMS No. 1(115), 2005 s-37-42.
3. Buzlama A.V., Slivkin A.I. // Experimental preclinical study of the regenerative activity of the substance of plant polyphenols. Bulletin of VSU, series chemistry, biology, pharmacy, 2009. No. 1, pp. 101-107
4. Vazina I.R., Bugrov S.N., Sosin E.Yu. // Dynamics of mortality and causes of death over the last 40 years of the twentieth century. in the Russian Burn Center of the Ministry of Health of the Russian Federation: scientific publication. // Bulletin of Surgery.2004. Number 3. C-47-50.
5. Vildyaeva M.V. Rationale for the use of mexidant in the complex treatment of patients with thermal injury. Abstract of the dissertation for the degree of candidate of medical sciences. Saransk 2009.
6. Kryzhanovskaya E.V. // Biologically active substancesin veterinary medicine.Abstract. dissertations for a degreeDoctor of Biological Sciences. Shchelkovo 2008 S. 42.

7. Kulmanova M.U., Sabirova R.A., Milusheva R.Yu. // Effect of chitosan on the protective barrier of the intestine in the development of chronic heliotrin hepatitis. // Postgraduate doctor. V .: Scientific book 2009 No. 6 (33) - p. 435-442.
8. Kulmanova M.U., Sabirova R.A., Milusheva R.Yu., Pulatova S.R., Rashidova S.Sh. // Changes in the composition of the mucous membrane of the gastrointestinal tract in chronic heliotrin hepatitis and its correction with chitosan. // Medical Journal of Uzbekistan.T. 2008 No. 5, p. 84-87.
9. Lavrov V.A. // Molecular mechanisms of inflammation in burn patients. RMAPO. - M. 2002. S-25.
10. Mubarakshina E.K., Amelina S.E., Yanin V.A., Novosolov V.I. // Influence of the contribution of key factors involved in the regeneration processes of the respiratory epithelium. Biology is a science of the XXI century. Collection of abstracts. Pushchino 2007. C-121.
11. Rashidova S.Sh., Milusheva R.Yu., // Chitin and chitosan Bombix mori: synthesis, properties and applications. - Tashkent: FAN, 2009. - 246 p.
12. Sanotsko IV // Methods for determining the toxicity and danger of chemicals / ed. I. V. Sanotsky. - M.: Medicine, 1970. - S. 108-114.
13. Stekolnikov L.I., Shakhtmeister I.Ya., Sevastyanov B.A., Shilov G.G., Soyukov E.A. Experimental study of the wound-healing effect of Ronidase. Collection of scientific papers Experimental - clinical aspects of reparative processes and their methods of stimulation. M. 1986. s-73-76
14. Baykulov A. Regeneration of experimental burn with the use of chitosan // IJDIIE Volume: 02 Issue: 01 | 2021. R 34-38.
15. Khabibullaev B.B. Correction of secondary immunodeficiencies with chitosan and its complexes in the experiment. Abstract of the dissertation of the candidate of medical sciences. T. 2005 25s.
16. Khadzhibaev A.M., Fayazov A.D., Shukurov S.I., Karabaev B.Kh., Ajiniyazov R.S., Ruzimurodov D.A. // Experience in organizing combustiological care in the system of emergency medical care of the Republic of Uzbekistan. Bulletin of emergency medicine. 2011. No. 3 P. 5-7.
17. Inoyatova F., Baykulov A.K. Preclinical study of drug forms based on chitosan // European science review. – Austria. - 2017. - No. 9-10.
Inoyatova F., A.K. Baykulov // Study of chitosan binding with specific DNA sites in the treatment of thermal burns. // Abstracts 9th international symposium on the chemistry of natural compounds. October 16-19, 2011 Urumqi Xinjiang China. P-72-73.

Table

Dynamics of changes in biochemical parameters of blood serum of experimental animals, M+m, n=6-7

groups	Deadlines (days)	MSM, c.u.	MDA, nmol/ml	ALT, µmol/l.h	AP mmol/tsp	Thymol test, units/ml	Bilirubin, µmol/l
Intact		0.211+0.018	0.273+0.014	1.20+0.12	0.82+0.06	2.18+0.11	3.1+0.33
1st	3	0.474+0.028	0.542+0.028	1.47+0.15	0.99+0.07	6.79+0.36	4.7+0.56
	7	0.354+0.027	0.436+0.040	1.34+0.13	0.95+0.08	5.12+0.22	3.2+0.53
	ten	0.287+0.018	0.329+0.021	1.31+0.14	0.93+0.05	3.78+0.21	5.3+0.54
2nd	3	0.512+0.046	0.589+0.043	1.51+0.14	1.03+0.05	7.95+0.56	8.1+0.89
	7	0.433+0.041	0.495+0.042	1.48+0.17	1.00+0.03	6.46+0.33	5.3+0.83
	ten	0.307+0.018	0.395+0.033	1.41+0.13	0.97+0.04	4.51+0.22	3.3+0.81
3rd	3	0.543+0.048	0.596+0.047	1.61+0.15	1.05+0.05	8.77+0.43	9.2+0.85
	7	0.466+0.039	0.518+0.036	1.66+0.16	1.03+0.06	7.91+0.66	8.7+0.95
	ten	0.363+0.024	0.423+0.032	1.51+0.17	0.98+0.04	5.08+0.29	7.0+0.93
4th	3	0.588+0.042	0.637+0.038	1.66+0.18	1.09+0.05	9.36+0.45	15.9+0.57
	7	0.541+0.034	0.572+0.033	1.85+0.17	1.14+0.03	10.97+0.52	12.8+0.66
	ten	0.416+0.027	0.504+0.041	1.77+0.18	1.11+0.04	5.33+0.28	13.5+0.68