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Miramistin as an Antimicrobial Component in the Innovative Substance of Chitosan-Miramistin Complex (CMC) for the Treatment of Infected Wounds of Various Genesis

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Abstract:

One of modern approaches in the development of new-generation drugs is the design of original dosage forms based on substances of a known spectrum of action using modern innovative technologies that make it possible to obtain drugs with high therapeutic efficiency and with minimal side effects.

The invention relates to the pharmaceutical industry and is an innovative pharmaceutical substance, which can be used both independently and in combination with enzymes of the hydrolase class, that are capable of breaking down peptides and proteins, and anesthetic agents for the treatment of infected wounds of various genesis.

The chitosan-miramistin complex has multiresistance to antibiotics, enhances functional activity of immune cells by stimulating local nonspecific immunity, promotes antigen capturing by macrophages and its accumulation in the lysosomal fraction, and does not have a local irritant effect.

Keywords: chitosan-miramistin complex (CMC), chlorhexidine, lysozyme, miramistin, treatment of infected wounds of various genesis.

INTRODUCTION

In surgical practice, there is often a complicated course of the wound process, with the suppuration of the wound taking the first place among all possible complications. Suppuration occurs as a result of the development and progression of infection in damaged tissues – after a trauma, in postoperative wounds, which is associated with both external and internal causes, as well as in wounds formed after the drainage of various abscesses. Is by the interaction of macro- and microorganisms determines the development of purulent infection. The critical level of microorganisms that contribute to the development of infection is $10^5 – 10^6$ bacteria per 1 g of tissue. In wounds with damaged local tissue protection, the infectious process can develop at a lower level of microorganisms [1-3].

The formation of resistance of microorganisms to obsolete drugs widely used in clinics dictates the need to develop new drugs with wide range of activity against both aerobic and anaerobic components, and strictly corresponding to the phase of the wound process.

One of the modern approaches in the development of new generation drugs is the design of original dosage forms based on substances of known spectrum of action using modern innovative technologies that allow to obtain drugs with high therapeutic efficiency and, importantly, with minimal side effects. Such approach allows developing an innovative drug in a shorter time and with significantly lower economic costs [2, 4, 5].

There are many known technological concepts of a compound, active pharmaceutical substance or biologically active substance with matrix. But regardless of the type of interaction, active center of the substance should be free and the drug should be able to leave the carrier to affect the substrate, in our case – to enter the wound [3-6].

The use of drugs that have powerful antimicrobial action and at the same time are free from side effects, that are caused by antibiotics, plays an important role in solving the problem of wound healing. In addition to antibiotics antiseptics or bacteriolytic enzymes can be used, i.e. drugs that have bactericidal or bacteriostatic effects by lysing the cell wall of microorganisms [7, 8].

Miramistin and chlorhexidine are the most widely used antiseptics; lysozyme is a common representative of bacteriolytic enzymes. At the technology development stage for the innovative substance, it was decided to compare these drugs and choose the most suitable antimicrobial component for the new substance.

The choice of the antimicrobial component was made experimentally. For this purpose, polymer compositions based on acid-soluble chitosan were prepared: chitosan-miramistin; chitosan-lysozyme and chitosan-chlorhexidine. Antimicrobial preparations of lysozyme, chlorhexidine and miramistine in different concentrations were immobilized on the chitosan carrier.

Chlorhexidine bigluconate is used in the form of solutions of various concentrations and is a local antiseptic with predominantly bactericidal effect. Chlorhexidine bigluconate is close to proguanil by chemical structure and is a dichloride-containing biguanide derivative. The mechanism of action is based on its ability to change the properties of cell membrane of microorganism. After dissociation of chlorhexidine salts, the cations formed react with membranes of bacteria that have a negative charge. Lipophilic groups of the drug contribute to the disaggregation of lipoprotein membrane of bacteria, resulting in disturbance of osmotic equilibrium and loss of potassium and phosphorus from the bacterial cell. Under the influence of the drug, cytoplasmic membrane of the bacterium breaks down and its osmotic equilibrium is disturbed, resulting in the death of the bacterium.

The drug is effective against strains of the following microorganisms: Trichomonas vaginalis, Neisseria gonorrhoeae, Chlamidia spp., Bacteroides fragilis, Treponema pallidum, Gardnerella vaginalis. In addition, chlorhexidine bigluconate is active against Ureaplasma spp. and moderately active against some strains of Proteus spp. and Pseudomonas.spp. Viruses (except herpes virus) and spores of fungi are resistant to the action of the drug.

Miramistin – benzyldimethyl-[3-myristoilamine)-propyl]ammonium chloride monohydrate has a pronounced bactericidal action against aerobic and anaerobic bacteria, Gram-positive bacteria (Staphylococcus, Streptococcus, Bacillus subtilis, Bacillus anthracoides) and Gram-negative organisms (Shigella, Pseudomonas aeruginosa, Escherichia coli, Salmonella), both in monocultures and in the form of associations – pseudomonas aeruginosa and staphylococcus aureus, escherichia and staphylococci, including hospital strains with multidrug resistance to antibiotics. Molecules of the drug affect the outer membrane of the microbial cell, which leads to its

destruction and death. Its biological effect is based on a direct effect on the cell membrane of microorganisms. Miramistin is able to inhibit the enzymatic activity of cells of pathogenic microorganisms, which is good for antibacterial activity, but it can significantly affect the enzymatic activity of drugs, since the chemical modification of the protein globule can affect the stability of enzymes. The results of experiments showed that miramistin does not lead to a drop in the enzymatic activity of the enzymes used, both native and immobilized in the chitosan gel.

Lysozyme is an enzyme of the hydrolase class, which functions as an antibacterial agent, catalyzing the hydrolysis of the polysaccharide in the cell walls of a number of bacteria. This polysaccharide is formed by alternating residues of N-acetylglucosamine and N-acetylmuramic acid, connected by a β-1, 4-glycosidic bond.

The bacteriolytic effect of lysozyme is well known and confirmed by long-term clinical application. Studies show that Gram-positive bacteria can be completely destroyed, while Gram-negative bacteria undergo bacteriolysis only when their cell wall is additionally destroyed.

Selective action of lysozyme on the cell walls of predominantly Gram-positive bacteria is associated with differences in the composition of the cell membranes of Gram-positive and Gram-negative microorganisms: Gram-positive microorganisms, unlike Gram-negative bacteria, do not contain aromatic amino acids - proline, histidine, arginine. Membrane of gram-negative bacteria contains more lipopolysaccharides. Biological role of lysozyme is not limited only to antibacterial action, it takes part in the protective, immune reactions of the body, in the processes of regeneration and healing of wounds.

In addition to the choice of antimicrobial component, as the main active substance in this composition, the choice of chitosan concentration was carried out. For this purpose, a series of solutions of chitosan with concentration of 0,5 to 1,5 wt% was prepared. Miramistine solutions, selected for the experiment, were also prepared at various concentrations from 0,01 to 0,05%. Reaction time and temperature were taken into account. The main goal of the experiment is to find the right ratio of carrier and active substance [9].

Thus, the task of preparing for development of the composition and formulation of a new pharmaceutical substance based on chitosan and miramistin, namely the substance with complex antimicrobial and regenerating activity for local therapy of infected wounds of various origins, was solved.

The object of this study is to develop a composition and formulation of a new pharmaceutical substance – chitosan-miramistin complex (CMC) to expand the assortment of drugs for treatment of infected wounds of different genesis.

RESULTS

To solve the problem we developed formulation of a pharmaceutical substance, which consists of active antimicrobial components and has a therapeutic effect on the wound.

The substance is an innovative complex of chitosan - miramistin (CMC). The complex is original in its composition and method of production. In available patent and literature sources no data was found on production of other similar compositions with myramistin based on glucosamine derivatives [10-13].

To improve stability and provide prolonged action of miramistin, it is expedient to immobilize it onto a carrier with formation of nonvalently bound complexes. Taking into account specific aspects of application, the following requirements are imposed on the proposed complex: biocompatibility with human tissues; absence of allergic reactions, pyrogenic and toxic effects on healthy tissues.

Chitosan was selected as an optimal carrier for the preparation of complexes with miramistin. Wound healing effect of chitosan can be explained by activation of the immune response through macrophage stimulation and the use of acetylglucosamine as a precursor of mucopolysaccharides, which directly participate in creation of biostructures, stimulate proliferation of fibroblasts and increase the release of mediators of immune response [13-17].

The resulting compositions in the form of a gel were subjected to freeze drying. After obtaining lyophilizate, antibacterial effect of the complexes was evaluated. The study of the antibacterial effect of chitosan - lysozyme, chitosan - chlorhexidine, chitosan - miramistin was carried out on strains of Esherichia coli and Staphylococcus aureus.

We used chitosan 1,0% solution. The solution was prepared by constant stirring at room temperature. After complete dissolution of chitosan, the solution was left for 24 hours at room temperature for further structuring.

The next stage was immobilization of antimicrobial preparations in different concentrations on chitosan carrier – lysozyme, chlorhexidine, miramistin.

Antiseptics miramistin and chlorhexidine and bacteriolytic enzyme lysozyme in different quantities: 0,1 mg/ml, 0,5 mg/ml and 1,0 mg/ml were dissolved in 1,0% chitosan solution (pH 5,5), then stirred on magnetic stirrer at room temperature for two hours.

During the experiment freeze drying was chosen as an adequate drying method, as it is most gentle with respect to biologically active substances included in the composition.

After obtaining lyophilizate, antibacterial effect of the complexes was evaluated. The study of the antibacterial effect of chitosan - lysozyme, chitosan - chlorhexidine, chitosan - miramistin was carried out on strains of Esherichia coli and Staphylococcus aureus.

Antibacterial activity of the studied objects was investigated by the agar diffusion test. Bacteria were cultured on L-broth at 37 °C for 20 hours. Then, using the methods of quantitative estimation of the number of viable microorganisms (Koch's method), the number of cells in 1 ml of the initial suspension was determined. For further study dilutions were used, which gave a medium contamination on a petri dish 10^4 and 10^6 CFU/ml, which corresponds to contamination of a wound at initial and final stages of disease.

The obtained and thoroughly premixed dilutions were inoculated on the surface of agar plate in Petri dishes with a sterile pipette in amount of 0,1 ml. The volume of inoculated suspension was distributed over the surface of the medium with a sterile spatula.

In addition to surface growth on agar plate, we also used method of growth of microorganisms in agar layer. To implement this method we prepared flasks with molten L-agar with temperature not higher than 40 °C (pre-autoclaved), into which an inoculum of a given concentration was introduced. Then poured the contents of flasks into Petri dishes by 25-30 ml.

According to the results of experimental work miramistin is the optimal antimicrobial component of the complex. The choice of miramistin, which can be considered as a new generation antiseptic of wide spectrum of action, is due to the fact that its therapeutic effect will be more effective and complete in comparison with other drugs under study. In addition, preformulation studies show that miramistin and chemopsin in gel composition have an additive effect in the treatment of experimental purulent wounds.

CMC is a lyophilized mass in the form of lumps or plates of white or light yellow color, produced from acid-soluble chitosan.

The first stage of technological process is preparation of 1,0% solution of chitosan in 0,5% acetic acid. Process parameters: room temperature; concentration of acetic acid – 0,5 wt%; concentration of chitosan – 1,0 wt%.

At the second stage of technological process, the antimicrobial drug miramistin is immobilized onto high-molecular chitosan polysaccharide to form the chitosan-miramistin complex (CMC). Miramistin and chitosan molecules have functional groups, which form hydrogen bonds and hydrophobic contacts, resulting in the formation of polysaccharide conjugates of chitosan with miramistin. Process parameters: room temperature; immobilization time – 2 hours; miramistin concentration – 0,05 wt% (0,5 mg/ml); pH 4,5 – 5,5.

DISCUSSION

Thus, as a result of the experiments, the properties of the claimed pharmaceutical substance, its qualitative and quantitative composition were recorded and confirmed and technology of its production was established.

The optimal method of preparation of the composition with miramistin is the physical immobilization followed by freeze-drying. The optimal polymer for preparation of the composition is chitosan.

Resulting preferred composition of chitosan-miramistin complex (amount, in wt%): Miramistin - 0,05, Chitosan - 1,0, Acetic acid - 0,6, Purified water - up to 100,0.

CONCLUSION

The preferred composition and formulation of the developed innovative substance indicate that its production does not require high costs and is suitable for industrial manufacturing. This possibility determines the availability, prolonged action and application flexibility of the substance and conduces to the expansion of range of wound healing and antimicrobial agents. CMC has multiresistance to antibiotics, enhances functional activity of immune cells, stimulates local nonspecific immunity, promotes antigen capturing by macrophages and its accumulation in the lysosomal fraction, does not have local irritating effect and allergenic properties.

REFERENCES

1. Volodchenko, N.P., *Sovremennye aspekty lecheniia ran: Uchebnoe posobie* [Modern aspects of wound treatment: Textbook], Blagoveshchensk 2013.
2. Blatun, L.A., Mestnoe medikamentoznoe lechenie ran [Local medical treatment of wounds], *Surgery: Journal of N.I. Pirogov* 2011, *4*, 51-59.
3. Tolstykh, P.I., Derbenev, V.A., Filatov, V.N., Ryltcev, V.V., Medusheva, E.O., *Lechenie gnoinykh ran i iazv razlichnogo geneza s ispolzovaniem terapevticheskikh sistem immobilizovannogo tripsina (Posobie dlia vrachei MZ RF)* [Treatment of purulent wounds and ulcers of various genesis with the use of therapeutic systems of immobilized trypsin (The manual for doctors of Russian Federation Ministry of Health)], M. Dipak, Moscow 2013.
4. Izmailov, A.G., Dobrokvashin, S.V., Volkov, D.E., Pyrkov, V.A., Novye podhody v mestnom medikamentoznom lechenii ran miagkikh tkanei [New approaches in local medical treatment of soft tissue wounds], *Practical Medicine: Electronic Scientific Journal* 2015, *6*.
5. Leepatov, K.V., Etiopatogeneticheskie osobennosti hirurgicheskoi infektcii miagkikh tkanei [Etiopathogenetic features of surgical infection of soft tissues]. *Surgery* 2013, *5*, 48-54.
6. Lutcevich, O.E., Tamrazova, O.B., Shikunova, A.Iu., Sovremennyi vzgliad na patofiziologiiu i lechenie gnoinykh ran [Modern view on pathophysiology and treatment of purulent wounds], *Surgery* 2011, *5*, 72-77.
7. Mihalskii, V.V., Goriunov, S.Iu., Bogdanov, A.E., Zhilina S.V., Prividentcev, A.I., Anikin, A.I., Ulianina, A.A., Primenenie preparata Betadin v lechenii infitcirovannykh ran [The use of Betadine in treatment of infected wounds], *Russian Medical Journal* 2010, *18(28)*, 35-38.
8. Brkich, L.L., Pyatigorskaya, N.V., Development of a scientific methodological approach to expansion of product range for treatment of infected wounds, *Asian Journal of Pharmaceutics* 2017, *11(4)*, 731-736.
9. Dosadina, E.E., Brkich, L.L., Piatigorskai,a N.V., Ispol`zovanie hitozana v kachestve nositelia proteinaz i miramistina dlia polucheniia fermentsoderzhashchego gelia [The use of chitosan as a carrier of proteinases and miramistine for the preparation of an enzyme-containing gel], *Butlerov Communications* 2016, *48(10)*, 49-59.
10. Dosadina, E. E., Kulmet`eva, M.A., Dubovikova, O. E., Sintez i issledovanie svoi`stv kompleksa proteinaz, immobilizovannykh na polisaharidnykh nositeliakh dlia meditcinskogo ispolzovaniia [Synthesis and study of properties of proteinases complex, immobilized on polysaccharide carriers for medical use], *Butlerov Communications* 2016, *38(6)*, 1-10.
11. Kulmet`eva, M.A., Dosadina, E.E., Danilova, T.A., Vzaimodeistvie belkov (fermentov) proteoliticheskogo kompleksa iz gepatopankreasa kraba s rastvorom hitozana [Interaction of proteins (enzymes) of the proteolytic complex from crab hepatopancreas with chitosan solution], *Advances in Chemistry and Chemical Technology* 2016, *30(9)*, 13-15.
12. Shipovskaia, A.B., Zudina, I.V., Fomina. V.I., Novye antimikrobnye preparaty na osnove kompleksnoi soli hitozana s hiral`nym organicheskim ligandom [New antimicrobials based on the complex salt of chitosan with chiral organic ligand], *Butlerov Communications* 2015, *41(3)*, 82-94.
13. Dosadina, E.E., Kulmetyeva, M.A., Belov, A.A., The changing of enzymatic activity of hydrolases immobilized on natural polysaccharide matrix for purulent and burn wounds treatment during storing and exploitation, *Biointerface Research in Applied Chemistry* 2016, *6(3)*, 1291-1298.
14. Valueva, M.I., Khlystova, T.S., Gusev, I.V., Oltarzhevskaia, N.D., Lechebnye gidrogelevye materialy razlichnoi stepeni strukturirovaniia na osnove prirodnykh polimerov [Therapeutic hydrogel materials of different degree of structuring on the basis of natural polymers], *The News of Higher Educational Institutions: Technology of Light Industry* 2012, *3(17)*, 59-61.
15. Tang, Z.X., Qian, J.Q., Shi, L.E., Preparation of Chitosan Nanoparticles as Carrier for Immobilized Enzyme, *Applied Biochemistry and Biotechnology* 2007, *136*, 77-96.
16. Biró, E., Németh, A. S., Sisak, C., Preparation of chitosan particles suitable for enzyme immobilization, *J. Biochem. Biophys. Methods* 2008, *70*, 1240-1246.
17. Bhushan, B., *Encyclopedia of Nanotechnology*, Springer Science+Business Media B.V., 2012.