

Pharmaceutical Composition Based on Two Innovative Chitosan-Containing Substances – The Chitosan-Chymopsin Complex and the Chitosan-Miramistin Complex – for Production of a Finished Dosage Form for the Treatment of Infected Wounds of Various Origins

Liliana Brkich
 Dept. Of Industrial Pharmacy
 Sechenov First Moscow State Medical
 University (Sechenov University)
 Moscow, Russian Federation
 brkich.lily@yandex.ru

Tatiana Salnikova
 Center for Pharmaceutical Technology
 Sechenov First Moscow State Medical
 University (Sechenov University)
 Moscow, Russian Federation
 mgmufarm@gmail.com

Natalia Pyatigorskaya
 Dept. Of Industrial Pharmacy
 Sechenov First Moscow State Medical
 University (Sechenov University)
 Moscow, Russian Federation
 osipova-mma@list.ru

Galina Brkich
 Center for Pharmaceutical Technology
 Sechenov First Moscow State Medical University (Sechenov
 University)
 Moscow, Russian Federation
 brkich@yandex.ru

Vasily Belyaev
 Dept. Of Industrial Pharmacy
 Sechenov First Moscow State Medical University (Sechenov
 University)
 Moscow, Russian Federation
 belyaev-mma@yandex.ru

Abstract — Developed pharmaceutical composition based on two innovative chitosan-containing substances: the chitosan-chymopsin complex and the chitosan-miramistin complex, for the treatment of infected wounds in gel dosage form for external use has four types of pharmacological effects - necrolytic, antimicrobial, wound healing and analgesic. The chitosan-chymopsin complex provides prolonged proteolytic action of the enzyme, restores microcirculation in wound walls, improves metabolic processes and relieves local inflammation. The chitosan-miramistin complex has a pronounced bactericidal effect against aerobic and anaerobic bacteria, gram-positive and gram-negative microorganisms, both in the form of monocultures and in the form of associations, including hospital strains with antibiotic resistance. Gel as a dosage form provides indirect anaesthetic effect due to cooling effect when applied to the damaged surface.

Keywords — *chitosan-miramistin complex, chitosan-chymopsin complex, treatment of infected wounds of various origins, lysozyme, chlorhexidine, miramistin, hydroxypropyl methylcellulose (HPMC), polyacrylamide (PAM)*

I. INTRODUCTION

One of the search directions for an effective treatment of infected wounds is the development of combined multifunctional drugs, containing active substances that have complex therapeutic activity against main pathophysiological processes of complex long-standing non-healing wounds, in particular, capable of cleaning the wound surface and providing antimicrobial action, but at the same time be free from side effects of antibiotics [1, 2, 3].

Since it is currently shown that the wound repair process is enzymatic in nature and needs presence of humid environment, the development of non-adhesive polymer gel

coatings with immobilized proteolytic enzymes is very relevant today. The latter are able to soften and lyse necrotic formations, have antimicrobial activity and cooling effect, are well-modeled, do not injure the wound, and allow visual monitoring of its condition [4, 5, 6].

The unique complex of chitosan properties, its biocompatibility, biodegradability, non-toxicity on the background of high biological and sorption activity, allows to attribute this aminopolysaccharide to a small group of industrially available, environmentally friendly polymers and, in the future, to potentially new biomaterials based on it, exclusively suitable for medical use. Chitosan has anti-inflammatory, antibacterial, immunostimulating and sorbing effects, so creation of new drugs based on chitosan is promising for the development of drugs with wound healing effects. Low toxicity of chitosan and its derivatives, compatibility with tissues of living organisms, and biodegradability make it promising as a carrier of enzyme and various drugs with the aim of prolonging their action and controlled release [7, 8].

Benzyltrimethyl-myristoylamino-propylammonium was used as an antimicrobial component, which, in addition to antimicrobial, also has regenerating effect, significant hyperosmolar activity, does not damage granulation and viable skin cells, does not inhibit edge epithelization, does not have local irritating and allergenic properties [9, 10].

Trypsin was used as a proteolytic enzyme to obtain new drugs. It has a pronounced anti-inflammatory and decongestant effect, is capable of cleaving dead tissue areas, viscous secretions and exudates. In relation to healthy tissues it is inactive and safe due to the presence of trypsin inhibitors in them [11, 12, 13].

II. EXPERIMENTAL

We used chitosan (Xt) produced by R&D company «Bioprogress» (Schelkovo, Moscow Region, Russian Federation) (drug humidity 10%, TU 9289-067-00472124-03, deacylation rate 80.0%; kinematic viscosity not less than 383,7 centistokes; molecular weight about 500 kDa); chymopsin (HMP) (FSP 42-0179-5944-04, SamsonMed, Russia, casein activity 9.0 PE/mg); miramistin (Mir) (FSP 42-0414-2768-02, Infamed LLC, Vidnoe, Moscow Region, Russia). Lidocaine hydrochloride was used as local anesthetic (G. Amphray Laboratories, India, reg. No. JICP-008000/10). As auxiliary substances were used: hydroxypropyl methylcellulose (HPMC) (Methocel K4M Premium CR, ID34680, manufactured by Colorcon Limited, England), polyacrylamide (PAA) for medical use (science-technical company «Atombiotekh»), glycerin (OFS.1.3.0001.15) to give system elasticity and purified water (FS.2.2.0020.15).

Rheological properties of the finished dosage form «Wound healing gel» were determined on a rotary viscometer Rheotest RN4.1 with software in the measuring system of "cylinder in cylinder" type, ms din 33 and ms din 11 (cell volume 17 and 32 ml, respectively), at a temperature (20±1) °C, in the range of shear rate from 5 to 300 sec⁻¹. The effective viscosity of the wound healing gel should be in the range from 0.7 to 1.2 Pa·sec. Dynamic viscosity was studied using the "small shear - large shear - small shear" system in two ranges of shear rates from 0 to 10 sec⁻¹ and from 0 to 300 sec⁻¹.

Identification test for chymopsin in finished dosage form "Wound healing gel" was determined by the curdling effect on milk solutions. Main equipment: thermostat with transparent walls TIIC (Medlabortekhnika). The curdling time should be not more than 50 seconds. The proteolytic activity of finished dosage form "Wound healing gel" was determined by hydrolysis of casein by enzyme preparation (according to Hammarsten's method) to peptides and amino acids and their subsequent determination. Main equipment: UV-Vis spectrophotometer SF 2000, thermostatically controlled kinetic cell (TSS-240A), IR-Fourier spectrometer Nicolet 380 with ATR attachment (Thermo Scientific, USA). Proteolytic activity of the wound healing gel should not be less than (2.0 ± 0.5) PE/g of gel.

Microbiological studies were performed by analyzing the inhibitory effect of drugs on a microbial culture after inoculation on an agarized medium (L agar) using the well method, followed by measuring the growth inhibition zone. An experiment was also performed using a 96-well plate. Culture inoculum was added to nutrient medium (L broth) and incubated on a thermostatic shaker Thermo-Shaker PST-60HI-4, BioSan, at 350 rpm, at 37 °C for 24 hours. The resulting cell culture was diluted 1000 times. 20 µl of culture, 80 µl of sterile culture medium (L broth), and 100 µl of preparations were added to the wells of the plate. Every 2 hours optical density was measured at a wavelength of 505 nm on an iMark photometer for microplates, manufactured by Bio-Rad Lab. Inc., USA, within 48 hours.

To study the surface characteristics, an Ntegra Prima atomic-force microscope (NT-MDT, Russia, Zelenograd) was used, equipped with a scanner and a silicon cantilever HA_NC Etalon (console length 124 µm, force constant 3.5

N/m, resonant frequency 140 kHz). Bending radius of the needle is less than 10 nm in accordance with the production specification.

Qualitative and quantitative determination of miramistin and lidocaine in the finished dosage form was carried out by HPLC. Main equipment: Agilent liquid chromatograph equipped with UV detector (MWD, serial No. DE64256455; DAD, serial No. DE64262550); stainless steel column 150 mm x 4.6 mm filled with C18 sorbent (Zorbax Eclipse XDB-C18, Agilent, USA), with 5 µm particle size or similar. Content of miramistin in the finished dosage form is from 95 to 105% of labeled amount. Content of lidocaine in the finished dosage form is from 95 to 105% of labeled amount. During analytical validation the following characteristics were determined: specificity, range, linearity, accuracy, repeatability.

III. RESULTS AND DISCUSSION

Pharmaceutical substances of chitosan with chymopsin complex and chitosan with miramistin complex based on acid-soluble chitosan were prepared in advance. For this purpose, 1% chitosan solution with the addition of acetic acid was prepared, the solution was left to swelling and structuring for one day. Then a certain amount of chymopsin was added in an amount of 2.0g per 100 g of the drug. In order to incorporate the proteolytic enzyme into the structure of chitosan, gel-like solution was left for two hours at room temperature. Similarly, the substance of chitosan-miramistin complex was prepared by adding antiseptic miramistin to chitosan solution in an amount of 0.05 g per 100 g of the drug. The solution was left for two hours at room temperature.

The prepared gel-like solutions were freeze-dried. Lyophilization of gel-like solutions of enzymes and antiseptics in chitosan was carried out in a frozen state under vacuum, while water was removed from the frozen substance by sublimation of ice, i.e. turning it into steam, bypassing the liquid phase. The dried material retains its structural integrity and biological activity to a greater extent than films, dried in air. When moistened, the material restores its original properties. In addition to the listed advantages of this method, there is a removal of excess acetic acid used to dissolve chitosan.

In order to obtain a solution of lidocaine in polyacrylamide, PAA was added to the reactor with purified water heated to 60-65 °C, while stirring, then left it for swelling and structuring of the polymer mass, after which lidocaine was added. The resulting reacting mass was periodically stirred and kept at room temperature until lidocaine was completely dissolved.

HPMC was dispersed into the production tank with purified water, preheated to 60-65 °C, and left for swelling and disappearance of blobs, then the system was mixed with a propeller stirrer until homogeneous structure was obtained. After that, with constant stirring, PAA was combined with the previously dissolved anesthetic lidocaine.

The finished dosage form was produced by combining the obtained base with an aqueous solution of pharmaceutical substances of chitosan complexes, addition of glycerin, and the resulting mixture was homogenized for 10-15 minutes at 2000-3000 rpm. The reaction mass in the form of a thick

white foam was kept at room temperature for 24 hours to remove air bubbles. After degassing, the mixture took the form of a colorless, milky-white gel. Compliance with established technological regimes was ensured at production control points.

ABL tubes with a protective membrane and a screw-on polymer cap, which have significant chemical inertness, high strength and resistance to temperature changes, lack of deformation during use, when exposed to moisture, fats and acids, were chosen as the primary packaging for the finished dosage form "Wound-healing Gel". Table I presents composition of the finished dosage form per tube.

TABLE I. COMPOSITION OF FINISHED DOSAGE FORMS FOR ONE TUBE

Composition	Mass, g 30,0
Active substances	
Chitosan-chymopsin complex, including: chymopsin	0,45 0,06
Chitosan-miramistin complex, including: miramistin	0,42 0,015
Lidocaine	0,03
Excipients	
Hydroxypropylmethylcellulose (HPMC)	0,60
Polyacrylamide (PAA),	0,03
Glycerin	1,5
Water	up to 30,0

Quality assessment of the finished dosage form was carried out in accordance with the developed draft regulatory documentation.

IV. CONCLUSION

Based on the specifics of the course of the wound healing process and composition of the drugs used in the first stage of the wound healing process - inflammation stage, it was confirmed that complex gels with proteolytic, anti-inflammatory, antimicrobial, analgesic effects will help to clean and accelerate wound healing.

Pharmaceutical composition based on two substances for the treatment of infected wounds has four pharmacological effects - necrolytic, antimicrobial, wound healing and analgesic. Gel as a dosage form provides indirect anaesthetic effect due to cooling effect when applied to the damaged surface [14].

Therapeutic effect of the developed gel is achieved through a combination of biological activities of the constituent components. The choice of dosage form and the excipients for it play an important role in development of a topical drug for the treatment of wounds. This should be based on achieving the ultimate goal – maximum bioavailability of biologically active ingredient of the drug when applied to the damaged surface – the wound defect [15, 16].

Advantages of choosing gel base for the treatment of wound processes are explained by the fact that they provide mild effect on the wound, have an analgesic effect due to cooling, are not a nutrient medium for microorganisms, and are well absorbed.

REFERENCES

- [1] V.K. Gostishchev, General surgery. Monograph. M.: Medicine, 1997, 672 p.
- [2] N.A. Efimenko, "Proteolytic enzymes in surgery: historical aspects and modern concepts of application", Russian medical journal, № 19 (5), pp. 368-372, 2011.
- [3] A.V. Blednov, "Promising directions in the development of new medical dressings", News of surgery, vol. 14, № 1., pp. 9-19, 2006.
- [4] L.L. Brkich, N.V. Pyatigorskaya, G.E. Brkich et al., "Development of composition and manufacturing method for combination drug product based on chitosan-containing pharmaceutical substances", International Journal of Pharmaceutical Research, vol. 10(4), pp. 292-296, 2018.
- [5] D.V. Shablin, S.G. Pavlenko, A.A. Evlevsky et al., "Modern wound coverings in local treatment of wounds of various origins", Basic research, 2 (part 2), pp. 361-365, 2013.
- [6] A.A. Belov, "Medical dressing containing protein drugs", Nanotechnology and Healthcare, vol. IV, № 2 (11), pp. 16-28, 2012.
- [7] K.V. Lipatov, "Etiopathogenetic features of surgical soft tissue infection", Surgery, № 5, pp. 48-54, 2013.
- [8] A.A. Belov, Textile materials containing immobilized hydrolases for medical and cosmetic purposes. Preparation. Properties. Application, LAP LAMBERT Acad. Pub., GmbH & Co. KG, Germany, 2012, 242 p.
- [9] Shurshina A.S., Kulish E.I., Kolesov S.V., Zakharov V.P., "Production of enzyme-containing chitosan films", Chemical Pharmaceutical Journal, vol. 3, pp. 43-45, 2015.
- [10] RF patent No. RU 2466720 C2. Method of treatment of purulent wounds in experiment. Authors: Oleg Valer'evich Bukharin, Valerij Nikolaevich Charushin, Oleg Nikolaevich Chupakhin, Jurij Ivanovich Skorobogatyh, Natal'ja Borisovna Perunova, Tat'jana Grigor'evna Khonina // Application: 2011107059/15, 2011.
- [11] L.L. Brkich, N.V. Pyatigorskaya, G.E. Brkich, A.A. Belov, I.I. Krasnyuk, "Miramistin as an antimicrobial component in the innovative substance of Chitosan-Miramistin Complex for the treatment of infected wounds of various genesis", Journal of Pharmaceutical Sciences and Research, vol. 10 (8), pp. 2027-2029, 2018.
- [12] E.E. Dosadina, A.A. Belov, "Possible mechanisms of interaction of chitosan, cellulose carriers and proteins of proteolytic complex from crab hepatopancreas", Successes in chemistry and chemical technology, № 8, vol. XXIX, pp. 82-84, 2015.
- [13] L.L. Brkich, T.S. Salnikova, G.E. Brkich et al., "Formulation and Production of a Novel Pharmaceutical Substance for Treatment of Infected Wounds - a Chitosan Chymopsin Complex", Journal of Pharmaceutical Sciences and Research, vol. 10 (6), pp. 1310-1313, 2018.
- [14] RF patent No. RU 2691144 C1. Combined composition for the treatment of infected wounds of various origins. Authors: Pyatigorskaya Natalya Valerievna, Brkich Liliana Lyubanovna, Medusheva Elena Olegovna, Brkich Galina Eduardovna, Belov Aleksey Alekseevich, Kulagina Alla Semenovna, Beregovy Valeriy Vasilievich, Svistunov Andrey Alekseevich, Salnikova Tatyana Sergeevna // Application: 2018116176, 2019.
- [15] RF patent No. RU 2687102 C1. Pharmaceutical substance for the treatment of infected wounds of various origins. Authors: Pyatigorskaya Natalya Valerievna, Brkich Liliana Lyubanovna, Medusheva Elena Olegovna, Brkich Galina Eduardovna, Belov Aleksey Alekseevich, Kulagina Alla Semenovna, Beregovy Valery Vasilyevich, Svistunov Andrey Alekseevich // Application: 2018116181, 2019.
- [16] RF patent No. RU 2697869 C1. Pharmaceutical substance for the treatment of infected wounds of various origins. Authors: Pyatigorskaya Natalia Valeryevna, Brkich Galina Eduardovna, Brkich Liliana Lyubanovna, Medusheva Elena Olegovna, Belov Aleksey Alekseevich, Kulagina Alla Semenovna, Beregovy Valery Vasilyevich, Svistunov Andrey Alekseevich // Application: 2018116179, 2019.