SUBSTANTIATION OF THE COMPOSITION AND METHODS OF QUALITY CONTROL FOR “GENTA+” OINTMENT WITH THE SUCCINYL TANNIN ANTiresistant COMPONENT

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Control of wound infections that are resistant to the action of antibiotics has become currently important [7]. It is associated both with spreading of nosocomial microflora with multiple cross-resistance (multiresistant strains) in hospitals and with the weakening of the immune system of patients and, therefore, with a long-term “selection” of resistant strains in “aggressive” wound conditions [11]. Many ointments and gels are presented among drugs, which are used for treating the wound process of the II stage [13]. But the majority of antibacterial agents included in their composition are not effective long ago in relation to nosocomial infections. Wounds, which are infected with multiresistant forms of microorganisms, for example Pseudomonas aeruginosa, does not heal for a long time, and it often causes disability of patients. Lately there are tendencies concerning a considerable increase of the death rate from multiresistant nosocomial infections [12]. Especially this pattern is typical for post-operative rehabilitation period, which a patient spends exactly in the hospital. Therefore, control of nosocomial multiresistant wound infections is a very urgent task of modern medicine and pharmacy [9].

One of the areas of this control is deprivation of bacteria with resistance factors and as a consequence – recovery of their sensitivity to the action of classical antimicrobial drugs. An example of such successful synergistic composition for antimicrobial medicines is the composition of Amoxiclav, which contains amoxicillin and antiresistant substance – clavulanic acid. The latter blocks enzyme, which destroys the penicillin ring in the amoxicillin structure [13]. Unfortunately, penicillins in the ointment formulation are relatively unstable and less effective. There is a well-known Gentamicin ointment which still has not lost its popularity thanks to the wide spectrum of action, relative efficiency at the II stage of the wound process. The concentration of the main active substance is very low and is 0.1%, but imperfect formulation of the ointment composition do not allow to use it extensively in surgical practice: the vaseline-paraffin base harms the wound, causes embolism and removes badly from the wound when bandaging. In more than 60% of cases the resistant forms of bacteria make the treatment process with Gentamicin ointment noneffective [8].

As a result of our long-term research a drug, which can selectively interact with enzymes of bacteria resistance in the lowest doses and inhibit the activity of these enzymes without direct antimicrobial action in the absence of toxic effect on the human organism, has been developed. This drug belongs to chemically modified gallotannins. In previous studies we isolated the most active gallotannin – succinyl gallotannin, which was able to recover antibiotic sensitivity of multiresistant bacteria in the dose of 1/100 from the antibiotic concentration [1]. Acylation of phenolic hydroxyls of the gallotannin molecule blocked its quick interaction with wound components and its inactivation of the antibiotic, but did not deprive its ability to intercalation to the protein globule of penicillinase and other enzymes present in the wound and blockade of their activity. The effectiveness of native plant enzymes in treatment of purulent wounds is shown in the research [10].

Thus, the aim of our research was to substantiate the formulation and quality control methods for “Genta-plus” ointment, to select the most effective ointment composition with the maximal antimicrobial action in
relation to multiresistant forms (strains) of microorganisms.

**Materials and Methods**

The following reagents were used in the research: succinyl gallotannin (SGT) (SI “Institute of Microbiology and Immunology named after I.I.Mechnikov of the NAMS of Ukraine, Kharkiv) as an antiresistant component; Carbomer (carbopol) grade 934 (“BF Goodrich”, USA) as a gel former; gentamicin (“Shing-su chemical”, China) as an active substance; PEO-400 (Merck, USA) as a penetration enhancer of antibiotics and antiresistant agent; triethanolamine (Sigma, USA) as an activator of carbopol polymerization; suttocide (Garmill, USA) as a preservative. Glycerine, isopropanol, stearic acid, potassium hydroxide, vaseline oil, Tween-80, emulsifier No.1, propylene glycol, polymethylsiloxane 200, castor oil, distilled monoglycerides were used only for mathematical modeling and according to literary data (by variance analysis and Duncan rank test). The antimicrobial activity of the composition obtained was studied on multiresistant strains from the Clinical Microbiology laboratory and the laboratory of Biochemistry of Microorganisms and Culture Media of the State Institution “Institute of Microbiology and Immunology named after I.I.Mechnikov of the National Academy of Medical Sciences of Ukraine and such strains as S. aureus poly-2008, P. aeruginosa Port 293, C. albicans rts232, A. niger rs33. All strains mentioned were resistant to the action of the corresponding antibiotics and the preservative suttocide in the concentration of 0.2%. The study of the antimicrobial properties of the composition was conducted according to the standard methods. The use of the main active substances in the lowest concentrations caused the choice of the composition formulation. Planning (modeling) is based on the effect of “mechanical stability”, which is measured on a “Reotest-2” rotational viscometer and correlates with different formulations of the base compositions. In the concentrations of 0.1% of gentamicin and 0.001% of succinyl gallotannin the main active substances did not reveal any effect on rheological and other values of the composition.

**Results and Discussion**

Along with the active substance, excipients provide the pharmacological effect needed [6]. Thus, the first stage was to plan the experiment and to select the composition of the base. The second stage of our research was the study of the most stable composition in order to reveal the antimicrobial activity in comparison with pure gentamicin. The preservative suttocide in the concentration of 0.2% did not reveal the antimicrobial activity in relation to multiresistant strains.

As carriers for the ointment being developed the bases-carriers, which are widely used in manufacture of soft medicinal dosage forms and cosmetic products, do not cause allergic and sensitizing effects after application, they are available for producer and well described in literature. The formulation of the compositions is given in Tab. 1.

For all compositions obtained the “mechanical stability” was determined as an objective quantitative indicator of the ointment rheological evaluation. The “mechanical stability” as an indicator for mathematical modeling has been selected due to the fact that structure-mechanical properties of ointments are one of the most important for providing the form quality, ability to active substances release and ability to application [4]. The results are given in Tab. 2.

Modeling and variance analysis of the results showed a considerable effect of the type of the base on mechanical stability of the compositions (Tab. 3).

Variance analysis by Duncan rank test [3] gave a chance to outline the effect of the compositions’ formulation on the main factor – “mechanical stability”. In general, compositions No.1, 2, 3, 5, 6 were the most stable. Among them compositions 1, 2 and 6 can be referred to one aggregate by stability. Due to the fact that the presence of the penetration enhancer PEO-400

| The composition ingredients | The number of the composition |
|-----------------------------|-------------------------------|
| Sodium carboxymethylcellulose | 5 |  
| Glycerine                   | 10 | 5 | 13.5 | 5  |
| Carbomer                    | 0.5 | 0.5 | 0.5 |
| Sodium hydroxide, 10%       | 0.5 | 0.5 | 0.5 |
| Isopropyl alcohol           | 25  |
| Glycerol monostearate       | 25  |
| Potassium hydroxide, 10%    | 0.5 |
| Vaseline oil                | 22  |
| Tween-80                    | 8   |
| Polawax                     | 4   | 15 | 12 |
| Propylene glycol            | 4   |
| PEO-400                     | 25  |
| Castor oil                  | 25  |
| Distilled monoglycerides    | 8   |
| Purified water              | To 100 |

Table 1

Formulation of bases for compositions of local application for developing the gentamicin ointment with the antiresistant component GENTA+
Rheological properties of samples of the compositions with gentamicin-containing ointments

| The number of the composition | The value of “mechanical stability” | The average value | Standard deviation |
|------------------------------|-------------------------------------|-------------------|-------------------|
| 1                            | 1.00                                | 1.02              | 0.01              |
| 2                            | 1.09                                | 1.11              | 0.01              |
| 3                            | 1.20                                | 1.30              | 0.02              |
| 4                            | 4.50                                | 4.70              | 0.01              |
| 5                            | 1.41                                | 1.45              | 0.02              |
| 6                            | 2.08                                | 2.00              | 0.03              |
| 7                            | 1.80                                | 1.90              | 0.56              |

The results of statistical processing of results by the analysis-of-variance method according to Duncan rank test

| Variation | Value | Degrees of freedom | Variance estimate | F       |
|-----------|-------|--------------------|-------------------|---------|
| External  | 27.6  | 6                  | 4.6               | 88.189  |
| Internal  | 0.7302| 14                 | 0.05216           |         |
| General   | 28.33 | 20                 |                   |         |

The antimicrobial activity of the classic 0.1% gentamicin ointment on resistant forms of microorganisms (without suttocide in the composition)

| Exposure time in the presence of microorganisms (1 g of the ointment per 5 ml of the medium) | Requirements of Ph. Eur. – 2012 (Criterion B) | The number of microorganisms, CFU/ml |
|-------------------------------------------------------------------------------------------|---------------------------------------------|-------------------------------------|
| The number of bacteria CFU/ml | The number of fungi CFU/ml | S. aureus poly-2008 | P. aeruginosa Port 293 | C. albicans* Port 293 | A. niger* rs33 |
| 2 days                       | HP                             | 8.90·10⁴           | 5.90·10⁴          | 1.50·10⁵          | 1.80·10⁷ |
| 7 days                       | HP                             | 8.21·10⁴           | 5.50·10⁴          | 1.11·10⁵          | 1.11·10⁷ |
| 14 days                      | HP                             | 1.59·10⁴           | 1.47·10⁴          | 5.00·10⁴          | 1.70·10⁷ |
| 28 days                      | HP                             | 5.70·10⁵           | 1.15·10⁵          | 3.20·10⁵          | 2.70·10⁷ |

* – Given for control of the absence of antifungal properties in the composition.

The antimicrobial activity of 0.1% GENTA+ ointment containing succinyl gallotannin as an antiresistant component on the resistant forms of microorganisms (without suttocide in the composition)

| Exposure time in the presence of microorganisms (1 g of the ointment per 5 ml of the medium) | Requirements of Ph. Eur. – 2012 (Criterion B) | The number of microorganisms, CFU/ml |
|-------------------------------------------------------------------------------------------|---------------------------------------------|-------------------------------------|
| The number of bacteria CFU/ml | The number of fungi CFU/ml | S. aureus poly-2008 | P. aeruginosa Port 293 | C. albicans* Port 293 | A. niger* rs33 |
| Day of introduction             | –                              | 8.00·10⁵           | 5.00·10⁵          | 1.00·10⁵          | 1.11·10⁷ |
| 2 days                         | HP                             | HP                  | HP                | 2.70·10⁵          | 1.56·10⁷ |
| 7 days                         | HP                             | HP                  | HP                | 5.00·10⁴          | 1.70·10⁷ |
| 14 days                        | HP                             | HP                  | HP                | 6.50·10²          | 7.00·10² |
| 28 days                        | HP                             | HP                  | HP                | 3.20·10²          | 2.70·10² |

* – Given for control of the absence of antifungal properties in the composition.
CONCLUSIONS
The choice of a carbomer gel with the penetration enhancer PEO-400 in its composition is optimal for "Genta+" ointment as the base.

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