Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(6):265-268

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Justification of concentration of active substances in a new ointment for the wound healing process treatment

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ABSTRACT

The article presents the results of the optimal concentration of active substances as part of the new combined ointment intended for use in the first phase of wound healing. Based on the results of microbiological screening in vitro conditions as part of a new ointments compositions administered for topical treatment of purulent wounds, the optimal concentration of the antibiotic azithromycin was established in the amount of 4% and the antiseptic decamethoxine – 1%. A new ointment composition showed a strong antimicrobial activity in relation to cultures P. aeruginosa and B. subtilis (growth retardation zones are 26.6 and 30.3 mm respectively), slightly less activity – in relation to cultures S. aureus, P. vulgaris, E. coli (from 20.6 to 23.6 mm) and low activity – in relation to fungus C. albicans (16,3 mm).

Keywords: ointment, wound healing, microbiological research, concentration.

INTRODUCTION

Prevention and treatment of pyoinflammatory diseases and infectious postoperative complications continue to be one of the important problems of surgery. In Ukraine patients with purulent pathology constitute 35-60% of the total number of patients in surgical departments [1, 10]. The main risk factors for postoperative infection is low immunity in patients, the degree of microbial contamination of surgical wounds, surgery duration and resistance of microorganisms to current antimicrobial agents [7, 12, 13]. The most frequent complication of postoperative period is the wound abscess that is from 5 to 30%, which increases the duration of healing of wounds, causing a number of inconveniences both for the patient and the doctor [6, 9]. Late appeals for medical care and improper treatment of the wound healing process complicate its course, worsen the patient's condition and can lead to serious consequences [8]. In connection with the foregoing, the problem of treatment of purulent wounds is at the level of key socio-economic problems that require immediate solution.

Currently, in the pharmaceutical market of Ukraine there is an insufficient number of topical drugs for Ist phase of the wound healing process presented and those available drugs usually do not meet modern requirements for the treatment of purulent wounds due to development of resistance of the wound infection, narrow spectrum of pharmacological action and disadvantages of the ointment base [4]. To satisfy this need it is possible to develop and implement new efficient combined ointments containing active ingredients with multidirectional mechanisms of antimicrobial activity and those which exhibit joint potential action.

Given the situation in the pharmaceutical market of drugs for topical treatment of purulent wounds, the creation of complex products based on the combination of active substances with antibacterial action was declared as a promising area: a known antibiotic with a broad spectrum of antimicrobial activity – azithromycin with a known antiseptic agent – decamethoxine.

The antibacterial effect of azithromycin is caused by violation of ribosomal protein synthesis in microbial cells and inhibition of the process of reproduction of the pathogen. Azithromycin reveals bacteriostatic effect, which requires its administration in the first phase of the wound healing process, which is accompanied by bacterial contamination of the wound. Along with antimicrobial activity this antibiotic currently shows a moderate anti-inflammatory activity [2]. The uniqueness of azithromycin lies in the fact that it has expressed postantibiotic and immunomodulatory effects. Azithromycin does not bind to enzymes of cytochrome P_{450} complex, thus showing no drug interactions with other drugs metabolized in this way. This property is very important because in the real clinical practice most patients have chronic comorbidities and a weakened immune system, and in this respect they receive the appropriate treatment. Also, it must be emphasized that, along with good tolerability and lack of significant side reactions, azithromycin may be administered to pregnant women and children. That is why azithromycin is considered not only as a highly effective, but also as one of the safest antibiotics with a minimum number of contraindications [2, 10]. An important argument for the choice of this antibiotic as the ointment component is the fact that it is rarely used in ointments.

The mechanism of action of the other active component of the new combined ointment, antiseptic with a broad spectrum of activity – decamethoxine, lies in violation of the permeability of the cytoplasmic membrane of bacteria and fungi by connecting with phosphatidic groups of the membrane lipids. Along with high antibacterial activity decamethoxine positively affects nonspecific and specific immunity, which is implemented by humoral and cellular factors. The presence of anti-inflammatory action of decamethoxine which is caused by inhibition of serotonin production has been proven. It is known that decamethoxine increases the sensitivity of microorganisms to antibiotics [5].

Thus, the combination of azithromycin and decamethoxine in a dosage form will not only contribute to joint potential antimicrobial activity, but also prevent the target microbes to form mono or multiple antibiotic resistance, and expand the range of pharmacological actions by immunomodulatory effects of azithromycin and anti-inflammatory effect of decamethoxine that will allow improving effectiveness of treatment of purulent wounds.

The purpose of this study was to determine the optimal concentration of active substances in the composition of a new ointment administered for use in the Ist phase of the wound healing process.

EXPERIMENTAL SECTION

In the paper, ointment compositions with different content of azithromycin and decamethoxine were used. Samples of ointments were prepared on polyethylene oxide basis. Two sets of screening experiments were conducted. In the first set of experiments six ointment compositions containing the same amount of antiseptic, decamethoxine (1%) were used, but they differed in the amount of antibiotic azithromycin: 0.5 %, 1 %, 2 %, 3 %, 4 % and 5 %, respectively. After selecting the optimal concentration of azithromycin, the second set of screening experiments was conducted to select the optimal number decamethoxine as part of a new ointment. For this purpose four ointment compositions with the selected concentration of azithromycin (4%), one of which did not contain an antiseptic, and the other three contained 0.5%, 1% and 1.5% of decamethoxine, respectively, were used.

Antimicrobial properties of ointment samples were studied at the premises of I.I. Mechnikov Institute of Microbiology and Immunology of Academy of Medical Sciences of Ukraine SE under the guidance of Head of the Laboratory "Biochemistry of Microorganisms and Nutrient Media", Cand. Sc. Biology T.P. Osolodchenko by common microbiological practice of agar diffusion in the modification of the "wells", which is based on the ability of active substances to diffuse in the nutrient medium, which is previously inoculated with the strain of microorganisms studied.

Under the guidelines [7] to estimate the antimicrobial activity of ointment compositions the following test strains of microorganisms were used: *Staphylococcus aureus ATCC 25923*, *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 27853*, *Basillus subtilis ATCC 6633*, *Proteus vulgaris ATCC 4636*, *Candida albicans ATCC 885/653*. Microbial burden amounted to $1x10^7$ CFU per 1 ml of the medium. Germ cultures were grown on Mueller-Hinton agar and Sabouraud agar (Dagestan NGO of nutrient media). The level of antimicrobial activity of the studied objects was judged by the diameter of the zone of microbial growth retardation around the hole with applied ointments, estimating their activity according to the following scale: the growth retardation zone with a diameter up to 10 mm – the strain is *not sensitive* to the drug; 11–15 mm – the strain is *slightly sensitive* to the drug; 15–25 mm – the strain *is sensitive* to the drug; >25 mm – *high sensitivity* of the microorganism to the drug. Experimental ointment compositions were studied in sixth repetitions against strains of microorganisms. The diameter of the zones of microbial growth retardation was measured using the measuring bar with the measurement error of ±0.1 mm. The results were processed statistically using the Newman-Keuls method and the significance level $p<0.05$.

RESULTS AND DISCUSSION

The results of the first set of studies presented in Table 1 showed a strong antimicrobial activity of the ointment samples studied, the level of which varied depending on the test strain of the microorganism and concentration of azithromycin. Test samples showed the highest activity in relation to culture P. aeruginosa (growth retardation zone is 20-26 mm) and B. subtilis (growth retardation zone is 24-30 mm), as evidenced by more marked growth retardation zones of these cultures. In relation to other cultures diameters of growth retardation zones ranged from 16 to 24 mm. At the same time, test samples showed activity in relation to cultures of fungus C. albicans (15-16 mm).

Analysis of the data showed that with increasing concentration of azithromycin in the ointment compositions from 1% to 4% a gradual increase in the diameter of the growth retardation zones of test cultures of microorganisms occurred. The antimicrobial action of the ointment compositions containing 4% and 5% of azithromycin had the highest rates, which significantly outperformed the antimicrobial action rates of the ointment composition with 0.5% of azithromycin against all studied strains (except strain of the fungus culture C. albicans) and against most test cultures of microorganisms of the ointment composition with 1% of azithromycin.

Table 1 Antimicrobial activity of ointment samples on polyethylene oxide basis with different amount of azithromycin, $\overline{X} \pm S_{\overline{X}}$ **(set of experiments No 1)**

Azithromycin concentration, %	Diameter of the growth retardation zones of microorganisms, mm; n=6					
	S. aureus ATCC 25923	*E. coli ATCC 25922*	*P. vulga-ris ATCC 4636*	*P. aeru-ginosa ATCC 27853*	*B. subtilis ATCC 6633*	*C. albicans ATCC 653/885*
0.5	17.3±0.54	16.6±0.54	16.3±0.54	20.3±0.54	24.6±0.54	14.6±0.54

*Notes: 1. * – differences as to the ointment composition with 0.5 % of azithromycin are possible, p<0.05; 2. ** – differences as to the ointment composition with 1.0 % of azithromycin are possible, p<0,05.*

A further increase in antibiotic concentration from 4% to 5% did not increase the antibacterial activity that is why the chosen content of azithromycin 4% was optimal. The next stage of our work was to determine the optimal concentration of antiseptic decamethoxine at a constant concentration of antibiotic (4%). Research results in a set of experiments No 2 are presented in Table 2.

Table 2 Screening of antimicrobial activity of ointment samples on polyethylene oxide basis with different amount of decamethoxine, $\overline{X} \pm S_{\overline{X}}$ **(set of experiments No 2)**

Amount of decamethoxine, %	Diameter of the growth retardation zones of microorganisms, mm; n=6					
	S. aureus ATCC 25923	*E. coli ATCC 25922*	*P. vulgaris ATCC 4636*	*P. aeruginosa ATCC 27853*	*B.subtilis ATCC 6633*	*C. albicans ATCC 653/885*
Without antiseptic	19.0±0.54	19.0±0.93	20.0±0.93	20.3±0.54	22.0±0.54	11.6±0.54

The results showed that the introduction of antiseptic increases the antimicrobial activity of the ointment compositions as indicators of the diameter of growth retardation zones without antiseptic were less pronounced. Comparing the data with different concentration of decamethoxine, it was found that the ointment composition with 1% concentration exceeded the rates of the ointment composition with a concentration of decamethoxine 0.5% and a further increase in the concentration of antiseptic up to 1.5% did not increase the antimicrobial action. Thus, the optimal concentration of decamethoxine as part of a new ointment is 1%.

CONCLUSION

Based on the results of microbiological screening in vitro conditions as part of a new ointments compositions administered for topical treatment of purulent wounds, the optimal concentration of the antibiotic azithromycin was established in the amount of 4% and the antiseptic decamethoxine – 1%.

A new ointment composition showed a strong antimicrobial activity in relation to cultures P. aeruginosa and B. subtilis (growth retardation zones are 26.6 and 30.3 mm respectively), slightly less activity – in relation to cultures S. aureus, P. vulgaris, E. coli (from 20.6 to 23.6 mm) and low activity – in relation to fungus C. albicans (16,3 mm).

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