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EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME COMPOUNDS FOR PURULENT WOUNDS TREATMENT

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Abstract

Results of *in vitro* studies of antimicrobial compounds effect on clinical strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*, causing purulent inflammation of open wounds are considered in the article. Activity of the compounds was determined according to the inhibition of the microorganisms growth by means of agar well diffusion method. Nisin and silver nitrate are shown to exhibit antimicrobial properties, while such classical antiseptics as ectericide and colloidal silver were inactive.

Key words: **infections; silver; ectericide; nisin**

Introduction

Purulent-inflammatory infections of open wounds are one of the urgent problems of surgery. Despite the sanitary and hygienic measures taken, the probability of surgical infections remains quite high and can pose a threat to the life and health of patients [1]. Contamination is possible through instruments, catheters, dressings, and household items. The number of patients with purulent surgical infection tends to increase year by year [2]. Contamination of open skin areas with bacteria *Staphylococcus epidermidis*, *Staphylococcus*

saprophiticus, some *Klebsiella* species, etc. leads to the developing of concomitant severe pyoinflammatory diseases, which, as a rule, do not respond well to standard treatment [3].

Choosing the proper medication plays a key role in treating patients with wound infections. The fate of the entire process of treating a patient largely depends on the correct determination of the wound process stage and the choice of a medicinal preparation for treating the wound surface [4].

High antibiotic resistance is the main problem in the treatment of diseases caused by staphylococcal infections. Natural adaptability and uncontrolled use of antibiotics have led to the emergence of methicillin-resistant Staphylococcus aureus, Klebsiella and Escherichia coli. Arising of micro-organisms producing beta lactamases of extended spectrum, vancomycin-resistant enterococci, etc. has forced researchers to pay special attention to this problem [5].

Nowadays, two main techniques for purulent wounds treatment are used in practical surgery: wound-closing technique – by active drainage [6] and the technique using topical agents [7]. Various ointments usually containing antiseptics and/or antibiotics are used in wound-closing technique.

Although the application of antibiotics in some cases is quite effective and can lead to a complete cure from wound infection, they have significant disadvantages due to the large number of side effects. The use of a certain type of antibiotics can also be accompanied by increasing resistance of infectious agent. Some authors [8] mention that such drugs as erythromycin, tetracycline, doxycycline and streptomycin have a limited application field and are not recommended for the routine treatment of purulent wounds.

Thus, despite the wide range of available antibiotics, their topical use is rather limited. Developing of resistance in pathogens, and, accordingly, decrease in the effectiveness of antibiotics, makes surgeons increasingly opt for antiseptics.

Antiseptics, as well as antibiotics, also have advantages and disadvantages. Although their positive effect predominates over the cytotoxic effect, an incorrect use of antiseptics can also have a negative impact on the wound healing process [9, 10]. The disadvantage of using antiseptics is the complexity of dosage which can lead to the development of hypersensitivity and dermatitis. Also, antiseptic treatment requires repeated use which can lead to slow tissue regeneration. Antiseptics cannot cause resistance in microorganisms but they can be contaminated in case of prolonged use from the same container.

Application of topical agents based on silver compounds can serve as a possible method to overcome resistance of bacteria to antibiotics and to improve effect on wound infection.

Silver compounds are known to be good antimicrobial drugs and have been used in medicine for a long time [11, 12]. Protargol (silver proteinate) and collargol are used in case of eye inflammation, various infections, colds, epilepsy, gonorrhea, etc. Silver sulfadiazine cream which is the standard antibacterial agent for severe wounds treatment is still used topically [13].

At the same time, despite significant clinical experience in application of silver compounds in medical practice, the results of scientific studies of their effectiveness and safety are regularly subjected to critical assessment. This is due to the fact that effect of application of silver containing drugs often depends on individual experience of the treating physician, i.e. when, for how long and in which clinical cases these drugs should be used [14]. There are also data about possible toxicity or adverse effects of silver nanoparticles on living organisms [15]. There is evidence that materials converted into nanoparticles radically change their physicochemical properties which may affect their physiological effects [16]. Compared to metallic silver macroscopic particles, nanoparticles can exhibit much higher toxicity which may be associated with oxidative stress, dysfunction of mitochondria, and increased membrane permeability [17].

Objective

Based on the data presented above, it was decided to investigate *in vitro* antimicrobial activity of various types of compounds. The main purpose of these studies was to determine the maximal antimicrobial activity of the compounds. Solution of colloidal silver (GreenPower (Ukraine), the manufacturer's declared concentration is 600 mg/L), $AgNO_3$ solution (C = 600 mg/L), and pharmacological mixtures of nisin and ectericide were tested.

Materials and methods

The antimicrobial activity of the compounds was studied against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Pure daily cultures of microorganisms grown on a solid nutrient medium were used. *In vitro* studies of the antimicrobial effect were carried out by agar well diffusion method [18]. A small amount of microorganisms was carefully transferred with a loop from the surface of the colonies into a test tube with sterile beef extract broth. The suspension of the test material was prepared according to the turbidity standard in such a way as its concentration corresponded to the indices of each specific microorganism expressed in CFU/ ml (Table 1).

Table 1 - The scale of correspondence of microbial suspensions optical density indices (McFarland scale) to the concentration of microbial cells, CFU/ ml

Groups	Microorganisms	McFarland Scale	Microbial cells concentration/ml (M ± m)
II	*Staphylococcus spp.*	0,5	$3,8 \pm 0,1 \cdot 10^8$
		1,0	$5,3 \pm 0,1 \cdot 10^8$
		2,0	$8,4 \pm 0,15 \cdot 10^8$
		3,0	$1,2 \pm 0,2 \cdot 10^9$
		4,0	$1,5 \pm 0,2 \cdot 10^9$
III	*Pseudomonas spp.*	0,5	$1,5 \pm 0,1 \cdot 10^8$
		1,0	$3,0 \pm 0,15 \cdot 10^8$
		2,0	$6,0 \pm 0,2 \cdot 10^8$
		3,0	$9,0 \pm 0,3 \cdot 10^8$
		4,0	$1,2 \pm 0,3 \cdot 10^9$

An automatic turbidimeter Densi-La-Meter was used to register the optical density of microbial suspensions.

The growth of microorganisms was carried out in Petri dishes on Mueller-Hinton agar. For this, 38 g of M173 powder (HIMEDIA (India)) was dissolved in 1000 ml of distilled water until complete dissolution followed by sterilization in an autoclave at 1.1 atm and 120°C for 15 min. After sterilization, stainless steel cylinders 6 mm in diameter and 10 mm in height were placed in a sterile Petri dish, and the culture medium prepared and cooled to 40°C was poured around the cylinders. Suspension of the microbial culture 1 ml per 100 ml of the medium was added in order to obtain a continuous lawn of the culture. This technique allows to obtain an optimal final concentration of microorganisms of 10^7 CFU/ ml.

After cooling the agar, the cylinders were removed with sterile tweezers, and 0.3 ml of antimicrobial solution was placed in the wells formed.

After 24 hours of incubation in a thermostat at 35°C, the results were analyzed by the zone of growth inhibition measured with an accuracy of 1 mm including the diameter of the wells. At the same time, too small colonies of test strains and plaque at the edge of the growth inhibition zone were neglected [19].

Results and discussion

The antimicrobial effect of the studied compounds was recorded by measuring the population growth inhibition around the well in mm. Fig. 1*a*, *b*. visualize experiment of antimicrobial activity investigation.

Fig 1*a*. Control samples of the *Pseudomonas aeruginosa* colony. Saline solution of sodium chloride is in the wells for the antimicrobial substance.

Fig 1*b*. Study of the *Pseudomonas aeruginosa* colony growth inhibition. Solution of silver nitrate, concentration is 600 mg/L is in the wells for the antimicrobial substance.

Figure 1a shows the complete absence of growth inhibition of the colony around the wells with saline solution. At the same time, clear growth inhibition of *Pseudomonas aeruginosa* can be observed in a Petri dish with $AgNO_3$ solution as an antimicrobial agent (Fig. 1*b*). The growth inhibition in this case is about 20 mm (average).

Investigation of other antimicrobial compounds effect on the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was carried out in a similar way. The ratio of the mixtures components and results of growth inhibition are presented in table. 2.

The greatest antimicrobial effect is shown by silver nitrate solution with concentration 600 mg/L. Slightly lower activity is shown by the samples 2,3 and 4, consisting of carbopol, ektericide and nisin. Samples 1, 5, as well as sample 6, which is a solution of colloidal silver, do not show antimicrobial action.

Ammonium hydroxide was used for gel polymerization with carbopol in the samples 1–4. Carboxymethyl cellulose (CMC) was used as a gel base in the samples 5–7.

Table 2 - Composition of antimicrobial compounds and results of inhibition of microorganisms growth

Sample number	Composition	Growth inhibition, mm	
		Pseudomonas aeruginosa	*Staphylococcus aureus*
1	Carbopol – 0,1 g; NH_4OH 10 % – 1 ml; Ectericide – 9,4 ml	–	–
2	Carbopol – 0,2 g; NH_4OH 10 % – 0,5ml; Ectericide – 4,3 ml; Nisin – 0,2 g	17	12
3	Carbopol – 0,2 g; NH_4OH 10 % – 0,4 ml; Distilled water – 9,5 ml; Ectericide – 4,3 ml; Nisin – 0,3g	15	13
4	Carbopol – 0,5 g; NH_4OH 10 % – 0,5 ml; Ectericide – 4,3 ml; Nisin – 0,2 g; Saline solution – 10 ml	18	12
5	CMC – 1 g; Ectericide – 50 ml; Distilled water – 50 ml;	–	–
6	Colloidal silver GreenPower (600 mg/L)	–	–
7	$AgNO_3$ solution (600 mg/L)	20	16

Conclusions

The investigation shows antimicrobial activity of compounds containing nisin and silver nitrate. Other studied compounds do not show bactericidal properties. This effect can be explained by the development of resistance in clinical strains of microorganisms to the active substances due to their long-term use in medical practice.

Silver nitrate and nisin possess antimicrobial properties but the mechanism of their influence on microorganisms is not entirely clear. The possibility of cytotoxic and allergic effects of $AgNO_3$ forces us to continue research in this field.

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