STUDY OF ANTIMICROBIAL ACTIVITY OF "FUZIPAN-DERMA" GEL

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Introduction. Providing the population with effective medicines for the treatment of infectious skin diseases has at present not only medical but also socio-economic significance. Antibiotic resistance of pathogenic microorganisms can level out the results of treatment and contribute to delaying the pathological process - this is an acute problem not only in dermatology, but also in other spheres of medicine. For complex therapy of infectious skin diseases, medicinal products are prescribed for local application in the form of ointments, sprays, emulsion gels and gels. In particular, the use of a gel containing fusidic acid which shows the marked antimicrobial effect on the nidus of suppurative inflammations of the skin affected by acne disease has good prospects [1, 2, 3]. Fusidic acid is used for treatment of the complicated staphylococcus infections [4, 5].

Materials and methods

Empirical, theoretical and experimental methods, analysis of professional scientific publications, antimicrobial researches and analysis of statistical data. As a research object, 4 experimental samples of gel with fusidic acid containing various concentrations in the composition and gel-base without fusidic acid as a control sample were used. The gel "Fuzipan-derma" containing fusidic acid was developed at the Department of Commodity Research of NUPha under the direction of prof. I. Baranova. The subject of the study was the antimicrobial activity of the drug "Fuzipan-Derma", which was studied on the experimental samples with different concentrations of the active pharmaceutical ingredient (API) [3, 6].

The fusidic acid, introduced into the gel as the main active ingredient, is a natural antibiotic that shows a narrow spectrum of antimicrobial activity. Fusidic acid is primarily active in staphylococci, including those that are methicillin-resistant. Sensitive to fusidic acid are corynebacterium, clostridia (*Clostridium difficile*), anaerobic cocci (*Peptococcus niger*, *Peptostreptococcus spp.*) [1, 4, 7].

For the study of antimicrobial activity, the samples of gel containing fusidic acid (samples No.1, No.2, No.3) and the gel base without operating substance (sample No.4) were used.

Sample 1	Base + of 0.5% of fusidic acid
Sample 2	Base + of 2.0% of fusidic acid
Sample 3	Base + of 3.0% of fusidic acid
Sample 4	Base

The antimicrobial activity of the gel specimens was investigated in vitro by diffusion in agar [5, 7]. This method is based on the ability of active substances to diffuse into agar medium, previously inoculated with microorganism cultures.

Results and discussion

The results of the studies allow to characterize both the antimicrobial activity of the drug and the speed and completeness of the release of antimicrobial substances from the base, since the zones of growth impairment of microorganisms are formed due to the diffusion of these substances into a dense nutrient medium.

The samples were stored in a refrigerator (5 ± 3°C). Antimicrobial activity was determined immediately after the samples were prepared. All the studies were performed in aseptic conditions of laminar box (Bureau of Biological Safety AS2-4E1 "ESCO", Indonesia).

As test cultures, pure cultures of microorganisms were used: gram-positive bacteria *Staphylococcus aureus* ATCC 25293, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 12344, spore culture *Bacillus subtilis* ATCC 6633; Gram-negative – *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853. Antifungal effect was found on a yeast-like fungus of the genus Candida - *Candida albicans* ATCC 885-653 [1, 2, 7].

In the course of research, one-day suspensions of bacterial microorganisms in a physiological solution were used, and a two-day culture of a yeast-like fungus. The microbial load was 10^7 microbial cells in 1 ml of nutrient medium.

In Petri dishes, mounted on a horizontal surface, 10 ml of liquefied "hungry" agar medium inoculated by microorganisms were introduced. After hardening of the lower layer of agar 3-5 sterile steel thin-walled cylinders (diameter – 8.0±0.1 mm, height – 10.0±0.1 mm) were evenly placed on its surface and the upper layer of the inoculated nutrient medium was poured (14-15 ml of liquefied nutrient agar cooled to 40-45 ºC and mixed with the seed culture of the test microorganism. When working with bacterial cultures meat-peptone agar (MPA), while working with a yeast-like fungus – Agar Saburo were used for the second layer). After cooling the upper layer, the cylinders were pulled out by means of sterile tweezers, and the test gel samples were put into the wells until they were completely filled. Petri dishes were kept for 30-40 minutes at room temperature and placed in a thermostat – bacterial cultures at a temperature of 32.5 ± 2.5°C for 18-24 hours, culture of the yeast-like fungus *Candida albicans* at 22.5 ± 2.5°C for 48 hours.

The analysis of the results was carried out by measuring the zone of inhibition of the growth of microorganisms, taking into account the diameter of the wells. Measurements were carried out with an accuracy of 1 mm, while focusing on the complete lack of visible growth. The diameter of the zone of growth impairment of microorganisms characterized the antimicrobial activity of experimental samples:

- absence of the zones of growth impairment of microorganisms around the well, as well as

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an impairment zone with a diameter of up to 10 mm, was assessed as insensitivity of microorganisms to the sample inserted into the well;

- growth impairment zones with a diameter of 11-15 mm were assessed as a weak sensitivity of the culture to a given concentration of the active substance;

- growth impairment zones with a diameter of 16-25 mm – as an indicator of the sensitivity of the strain of microorganisms to the test sample;

- growth impairment zones greater than 25 mm testify to the high sensitivity of microorganisms to the test sample.

As a result of the carried out research to study the antimicrobial properties of the gel containing different concentrations of fusidic acid in relation to different cultures of microorganisms, the data were obtained, given in Table 1.

Table 1. Results of antimicrobial activity of samples (n=5)

Cultures of microorganisms	Sample			
	No.1	No.2	No.3	No.4
	Diameters of impairment zones of microorganisms, mm			
Staphylococcus aureus ATCC 25293	25.6±0.5	33.8±0.4	35.4±0.5	_
Staphylococcus epidermidis ATCC 12228	23.4±0.5	29.6±0.5	31.8±0.4	_
Streptococcus pyogenes ATCC 12344	24.2±0.4	31.6±0.5	33.6±0.5	_
Bacillus subtilis ATCC 6633	20.8±0.4	23.6±0.5	25.4±0.5	_
Escherichia coli ATCC 25922	_	9.4±0.5	10.2±0.4	_
Proteus vulgaris ATCC 4636	_	_	_	_
Pseudomonas aeruginosa ATCC 27853	8.8±0.4	9.6±0.5	10.4±0.5	_
Candida albicans ATCC 885-653	_	_	_	_

Note: " - " - the impairment zone of microorganism is absent.

The data presented in Table 1 indicate that Sample No.4 (base of gel) does not show antimicrobial activity in relation to the used cultures of bacteria as well as to the fungus of the genus *Candida*.

The investigated samples No.1, No.2, No.3 do not show fungicidal activity against yeast-like fungus *Candida albicans* ATSC 885-653.

Samples (No.1, No.2, No.3) exhibit high antimicrobial activity (the diameter of growth impairment zones is 16-25 mm) to gram-positive bacterial cultures *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtiliss*. It should be noted that the most susceptible to the action of the samples containing fusidic acid is the culture of *Staphylococcus aureus* ATCC 25293. With the increase in the concentration of API (fusidic acid) in the gel, antibacterial activity increases: in relation to *Staphylococcus aureus* ATCC 25293, the diameters of growth impairment zones of the culture are: 25.6±0.5 mm (sample number 1), 33.8±0.4 mm (sample number 2), 35.4±0.5 (sample number 3); to the culture of *Staphylococcus epidermidis* ATCC 12228: 23.4±0.5, 29.6±0.5, 31.8±0.4 mm respectively 0.5%, 1.0% and 2.0%; to the culture of *Streptococcus pyogenes* ATCC 12344: 24.2±0.4, 31.6±0.531.6±0.5, 33.6±0.5, respectively, samples with a concentration of fusidic acid of 0.5%, 1.0%, and 2.0%. In relation to the gram-positive spore culture of *Bacillus subtilis* ATCC 6633, gel samples showed activity at the level of 20.8±0.4, 23.6±0.5, 25.4±0.5, depending on the content of fusidic acid.

The obtained results indicate that the used gram-negative cultures of *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853 are insensitive to the used gel samples under study – the diameter of the growth impairment zones of the test cultures did not exceed 10 mm. The research showed that gel samples with fusidic acid content of 3% had a higher antimicrobial activity than 2.0% gel, but in order to minimize the risk of local allergic reactions and reduce the resorptive action of the antibiotic and the development of microbial resistance, it is advisable to select a sample of fusidic acid of 2.0% as a sufficiently effective API concentration.

Conclusions. Thus, samples No. 1, No. 2, and No. 3 with different content of fusidic acid (0.5%, 1.0% and 2.0% respectively) showed high activity against gram-positive bacteria of *Staphylococcus aureus* ATCC 25293, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 12344; marked antibacterial activity against *Bacillus subtilis* ATCC 6633.

The gram-negative test microorganisms of *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853 are insensitive to the used sampless of gel. The fungicidal action of the gel on *Candida albicans* ATCC 885-653 in the test samples was not detected.

For further studies to develop the technology of gel with fusidic acid for the treatment of acne, it is advisable to select sample number 2 with a content of fusidic acid of 2.0%.

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Introduction. Providing the population with effective drugs for the treatment of infectious diseases of the skin has an important medical and socio-economic significance. Antibiotic resistance of pathogenic microorganisms can contribute to delaying the pathological process, which is an acute problem not only in dermatology, but also in other spheres of medicine. For the comprehensive treatment of infectious diseases of the skin prescribed medicines for local use. Fusidic acid, which is included in the experimental samples of the gel as an active ingredient, is a natural antibiotic that detects a narrow spectrum of antimicrobial activity. Fusidic acid is primarily active in staphylococcus, including those that are methicillin-resistant. Thus, the use of gel with fusidic acid, which exhibits a pronounced antimicrobial effect on the cells of purulent skin inflammations in acne [1, 2, 3] and used to treat complicated staphylococcus infections also [4, 5]. **Materials and methods.** Empirical, theoretical and experimental methods, analysis of professional scientific publications, antimicrobial researches and analysis of statistical data have been used. The subject of the study was to determine the antimicrobial activity of 4 experimental samples of the drug "Fuzipan-derma", with different concentrations of the active pharmaceutical ingredient (API), fusidic acid, in its composition [3, 6] and gel-base without fusidic acid as a control. **Results and discussion.** The results of the studies allow to characterize both the antimicrobial activity of the drug and the speed and completeness of the release of antimicrobial substances from the base, since the zones of growth impairment of microorganisms are formed due to the diffusion of these substances into a dense nutrient medium. In the course of research, one-day suspensions of bacterial microorganisms in a physiological solution were used, and a two-day culture of a yeast-like fungus. The microbial load was 10^7 microbial cells in 1 ml of nutrient medium. The investigated samples No.1, No.2, No.3 does not show fungicidal activity against yeast-like fungus *Candida albicans* ATSC 885-653. Sample No.4 (base of gel) does not show antimicrobial activity in relation to the used cultures of bacteria as well as to the fungus of the genus *Candida albicans*. Samples (No.1, No.2, No.3) exhibit high antimicrobial activity (the diameter of growth impairment zones is 16-25 mm) to gram-positive bacterial cultures *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtiliss*. It should be noted that the most susceptible to the action of the samples containing fusidic acid is the culture of *Staphylococcus aureus* ATCC 25293. With the increase in the concentration of API (fusidic acid) in the gel, antibacterial activity increases: in relation to *Staphylococcus aureus* ATCC 25293, the diameters of growth impairment zones of the culture are: 25.6±0.5 mm (sample number 1), 33.8±0.4 mm (sample number 2), 35.4±0.5 (sample number 3); to the culture of *Staphylococcus epidermidis* ATCC 12228: 23.4±0.5, 29.6±0.5, 31.8±0.4 mm respectively 0.5%, 1.0% and 2.0%; to the culture of *Streptococcus pyogenes* ATCC 12344: 24.2±0.4, 31.6±0.531.6±0.5, 33.6±0.5, respectively, samples with a concentration of fusidic acid of 0.5%, 1.0%, and 2.0%. In relation to the gram-positive spore culture of *Bacillus subtilis* ATCC 6633, gel samples showed activity at the level of 20.8±0.4, 23.6±0.5, 25.4±0.5, depending on the content of fusidic acid. The research showed that gel samples with fusidic acid content of 3% had a higher antimicrobial activity than 2.0% gel, but in order to minimize the risk of local allergic reactions and reduce the resorptive action of the antibiotic and the development of microbial resistance, it is advisable to select a sample of fusidic acid of 2.0% as a sufficiently effective API concentration. **Conclusions.** For further studies for development the technology of gel with fusidic acid for the treatment of acne, it is advisable to select sample number 2 with a content of fusidic acid of 2.0%.

Keywords: antimicrobial activity, biological researches, fusidic acid, dermatological medicines

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