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THE JUSTIFICATION OF THE CREAM FOR THE THERAPY OF DERMATOLOGICAL DISEASES, COMPLICATED BY DEMODICOSIS

Antimicrobial activity of the cream was investigated depending on the concentration of the metronidazole and salicylic acid. The concentration of active pharmaceutical ingredients in the cream was justified on the basis of the results. It was experimentally found that the antimicrobial activity of test samples is dependent on the composition of a chosen substrate. A more complete drug release was observed for the gel and emulsion base of oil/water. The results were used in the development of the cream for the treatment of dermatological diseases, complicated by demodex.

Keywords: dermatological diseases, demodicosis, rosacea, microbiological researches.

INTRODUCTION. The most common parasitic diseases that lead to skin lesions, is demodicosis, scabies and pediculosis. Demodicosis - is widespread, invasive dermatozoonosis that is caused by a pathological multiplication of the mite genus the Demodex, which permanently lives in the skin. The Demodex Mites are conditionally pathogenic parasites, because for a long time in a small amount of them can parasite on human skin, mainly on the face, and do not show any symptoms and skin lesions [12,13]. However, under favorable conditions, in which the parasites multiply rapidly and increase their activity, there are clinical signs of the demodicosis as skin inflammations [4,8,12,13]. The presence of the mite Demodex folliculorum worsen the course of several dermatological diseases, primarily as to the rosacea

[1,3]. Besides the usual symptoms of rosacea, there are micropapules and follicular micropostules, where some mites can be found [4,7].

The treatment of the demodicosis is not really easy aim, because the disease can occur on the background of the lowered immunity, and combine with other diseases, so the treatment involves both systemic and local therapy, in which the principal place holds acaricidal (anti-parasitic) therapy [5,6,8]. Pretty effective way of treatment is the use of metronidazole, birch tar, benzyl benzoate. The regimen also includes the medications that contain salicylic acid, resorcinol [5,6].

Metronidazole has established itself as an effective remedy in the treatment of demodicosis and rosacea. Metronidazole enhances the protective and regenerative functions of the skin and mucous membranes and possesses a strong anti-edema effect [10, 11]. It also shows a bacteriostatic effect, and antiparasitic properties on the *Demodex folliculorum* [10, 11]. Also metronidazole, acting on the autonomic nervous system stimulates its adrenergic structures, reduces congestions, erythema and even teleangiectatic changes [10]. It was found that metronidazole in the synergy with palmitic acid, which is found in a human skin, dejects the functional activity of neutrophils, thereby reduces the production of the inflammatory mediators [10]. Duration of the treatment by the metronidazole per os is 4-6 weeks but for some patients - up to 8 weeks at a daily dose of 1-1.5 g [10, 11]. Abroad the metronidazole is used primarily as local forms. When being applied topically 0.75 and 1% metronidazole gel or cream is not inferior to oral administration [10,11].

In the pharmaceutical market of Ukraine dermatological medicines with metronidazole are presented with 1% by the creams "Rozamet» (Jadran Galenski Lab., Croatia), "Stop demodex", further comprising birch tar, extracts of chamomile, calendula, witch hazel, willow bark, oak leaves apple roses), hyaluronic acid, D-panthenol, Vitamin A and E (Fitobiotekhnolohiyi, Ukraine), 1% by the gel "Metrogyl» (Unique Pharma Lab. India), 0.75% by the gel "Rozex» (Galderma Lab. France), 2 % by the cream "Demalan" (NEP LLC eye Microsurgery, Russia), 5 and 7% by the cream-gel and cream "Hlikodem" (SPC Ltd. AS-COM, Russia) [9].

We have suggested the cream for the treatment of dermatological diseases being complicated by Demodex, which includes the metronidazole and salicylic acid. It should be mentioned that the rosacea is characterized by vascular lesions, leading to the cyanosis skin, venous stasis in the skin, varicosity. That's why it should be noticed that of the high importance there is the use in the medicine of the combined action of the troxerutin which shows veinsrestorative, angioprotective, antioxidant and anti-inflammatory effects [6].

The purpose of research - the microbiological justification of the concentrations of active pharmaceutical ingredients and the type of foundation for flexible dosage form.

Methods of research

Microbiological studies were conducted on the basis of the microorganisms laboratory and nutrient mediums DZ "IMI named after I.I. Mechnikov Medical Sciences of Ukraine " under the leadership by the head of laboratory, Ph.D. in biological sciences Osolodchenko T.P.

In accordance with the World Health Organization for the recommendations to assess the activity of drugs we used the test strains 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. The preparation of the bacterial suspensions of microorganisms were performed by using the device Densi-La-Meter (production PLIVA-Lachema, Czech Republic, wavelength 540 nm). The suspension was prepared according to the instructions that came with the device and a newsletter on the innovation in the Health care system № 163-2006 "The Standardization of the preparation of microbial suspensions", Kyiv.

The determination of antimicrobial activity of the test samples was performed by agar diffusion on two layers of a dense nutrient medium, which was spilled into some Petri cups. [2] In assessing the antibacterial activity we used the following

criteria: the absence of inhibition zones of a microbial growth around the hole, and also some areas of delay up to 10 mm indicate that the organism is not sensitive to the medicine which was put into the hole or its concentration, the growth inhibition zones of the diameter 10-15 mm indicate the small sensitivity of the culture to the researching concentration of antibacterial substances; the growth inhibition zones of 15-25 mm are regarded as an indicator of the sensitivity of the microorganism to the given medicinal product, and the growth inhibition zone of the diameter more than 25 mm, indicate a high sensitivity of microorganisms to the researching means. There was made the microbiological examination of the cream samples containing 1, 2 and 3% metronidazole (1, 2, 3, respectively), and metronidazole 2.0 and 0.5 and 1.0 salicylic acid (4 and 5).

These findings of the antibacterial activity of the samples are presented in Table 1.

Table 1

Antibacterial activity of the test samples of cream

The test samples of the microorganisms	Diameter of the growth inhibition zones in mm, n = 3				
	Test sample 1	Test sample 2	Test sample 3	Test sample 4	Test sample 5
<i>Staphylococcus aureus</i> ATCC 25923	15.0±0.5	17.2±0.6	20.4±0.4	20.2±0.5	22.6±0.3
<i>Escherichia coli</i> ATCC 25922	15.3±1.0	17.5±0.6	19.8±0.7	20.1±0.2	20.7±0.2
<i>Pseudomonas aeruginosa</i> ATCC 27853	14.2±0.8	18.8±0.7	20.1±0.5	19.8±0.3	20.3±0.5
<i>Proteus vulgaris</i> ATCC 4636	13.5±0.6	16.7±0.4	18.2±0.4	17.3±0.5	18.0±0.2
<i>Basillus subtilis</i> ATCC 6633	18.0±0.5	20.4±0.6	22.6±0.2	20.8±0.6	21.3±0.7
<i>Candida albicans</i> ATCC 653/885	growth	growth	growth	11.1±0.2	13.2±0.4
<i>Streptococcus pyogenes</i> 2432	15.5±0.6	18.2±0.8	20±0.5	20.8±0.6	21.2±0.4
<i>Klebsiella pneumoniae</i> 6	16.4±0.7	19.4±0.5	20.6±0.5	20.4±0.6	20.8±0.3
<i>Bacteroides fragilis</i> ATCC13/83	21.0±0.8	25.8± 0.6	27.3±0.7	26.1±0.2	26.6±0.3
<i>Peptococcus niger</i> 1	23.0±0.5	25.3±0.8	27.2±0.3	26.8±0.4	28.2±0.3

As the table shows, all the test samples exhibit antibacterial properties against a broad spectrum of microorganisms. The samples 1-3 had antimicrobial activity against the studied test strains and clinical isolates, with the exception of the fungi of the genus *Candida*. The samples, which also contain salicylic acid, exhibit a little antifungal activity. According to the results of microbiological studies we decided to select 2% of metronidazole and 1% salicylic acid to the cream.

As it is known, the effectiveness of medicines, medicine substances release rate, duration of effect are largely determined by the type and composition of the base. In order to study the optimal composition of the base, we conducted the study based on the dependence of antimicrobial properties of the test samples on the nature of the base. We used an emulsion (1-st and 2-nd kind) and a gel base as the base. The fat base was not used in the researches, taking into account the specificity of the disease and the application field of the product. We injected 2% of the metranidazol, 1% of the salicylic acid and 2% of the troxerutin in the test samples. The base number 1 contained 20.0 of vaseline oil, 8.0 of emulsifier number 1 and some purified water up to 100.0; the base number 2 - 60.0 of vaseline, 1.5 of Cremophor A-6 (mixture tsetaret-6 and stearyl alcohol), 5.0 of cetostearyl alcohol and some purified water up to 100.0; the base number 3 - hydroxyethylcellulose - 1.5, propylene glycol - 1.0, some purified water - up to 100.0.

As the salicylic acid is not soluble in water, but is soluble in vaseline oil, and propylene glycol, in the samples 1 and 2, it was dissolved in the vaseline oil, in the sample 3 – in the propylene glycol.

Evaluation of the release of the active substances was performed by the microbiological approach of the agar diffusion to the growth zones inhibition of the test cultures of the microorganisms. The results of the growth zones inhibition of the test cultures of the microorganisms, which are depending on the composition of the base, are presented in Table 2.

According to the results of the research, the antimicrobial activity to some extent depends on the composition of the base. A more complete release of the

medicines occurs with the gel and emulsion type of the basis. The lowest level of the test-sample activity 2 may be associated with the hydrophobic dispersion medium of the given emulsion base and hydrophilic properties of the nutrient medium, in which the diffusion of medicines occurs to a less extent (Table 2).

Table 2

Antibacterial activity of the test samples depending on the composition of the base

The test strains of microorganisms	Diameter of the growth inhibition zones in mm, n = 3		
	Test sample 1	Test sample 2	Test sample 3
<i>Staphylococcus aureus</i> ATCC 25923	22.6±0.3	17.8±0.4	20.1±0.4
<i>Escherichia coli</i> ATCC 25922	20.7±0.2	16.3±0.2	17.2±0.5
<i>Pseudomonas aeruginosa</i> ATCC 27853	20.3±0.5	17.8±0.5	19.0±0.2
<i>Proteus vulgaris</i> ATCC 4636	18.0±0.2	16.2±0.3	17.0±0.1
<i>Basillus subtilis</i> ATCC 6633	21.3±0.7	19.8±0.4	18.6±0.3
<i>Candida aibicans</i> ATCC 653/885	13.2±0.4	13.0±0.2	14.8±0.2
<i>Streptococcus pyogenes</i> 2432	21.2±0.4	18.6±0.3	20.0±0.5
<i>Bacteroides fragilis</i> ATCC13/83	26.6±0.3	20.6±0.3	22.4±0.5

Taking the results of the research into consideration, and also the peculiarities of skin care during the treatment of the rosacea and the other dermatological diseases, complicated by the mite *Demodex folliculorum*, the form of a cream on the emulsion base of the type o/w (oil / water) was chosen for the further research.

CONCLUSIONS

We researched the antimicrobial activity of the cream depending on the concentration of the metronidazole and salicylic acid. According to the results we selected the concentration of the metronidazole and salicylic acid - 2 and 1%, respectively.

It's been established that the antimicrobial activity of the test samples depends on the composition of the chosen basis. A more complete release is observed for the emulsion-type base oil / water and gel base. The obtained results were used for developing the composition of the medicinal product for the dermatological diseases treatments, which are complicated by the demodicosis in the form of a cream.

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