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# DRUGS

## COMPARATIVE STUDY OF THE ANTIMICROBIAL ACTIVITY OF COMBINED TOPICAL MEDICINAL FORMULATIONS OF BETAMETHASONE, GENTAMICIN, AND CLOTRIMAZOLE *IN VITRO*

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We present here the results of an experimental study of the antimicrobial activity of combined topical medicinal formulations of betamethasone, gentamicin, and clotrimazole (Triderm® and Akriderm® GK as cream and ointment for external application). The minimum inhibitory concentrations (MIC) of the formulations Triderm® and Akriderm® GL against 20 of 25 bacterial and fungal strains were 2 – 4 times lower for Triderm® ointment and cream than for Akriderm® GK. As there were significant differences in the activities of the original and generic formulations in *in vitro* experiments, differences in the clinical efficacies should be expected on use in the treatment of allergic dermatoses complicated by bacterial and fungal infections.

**Keywords**: original formulations, generic medicines, antimicrobial activity, topical medicinal formulations, *in vitro*.

Many skin diseases (eczema, acne, atopic dermatitis, etc.) are accompanied by severe local dysbiosis. In particular, increases in colonization or infection of the skin with *Staphylococcus spp.* are seen in atopic dermatitis. The main role in the structure of staphylococcal skin infections is played by strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are seen in 54.76% and 38.69% of cases respectively, as well as associations of these microorganisms, seen in 6.55% of patients [4]. *S. aureus* produces various toxins and enzymes which damage corneocytes, which impair the formation of lipid plates in the epidermis, exacerbating defects in the epidermal barrier and aiding colonization by the pathogen [5, 6]. The presence of toxins induces mast cell degranulation, which becomes the cause of local allergic reactions, worsening the symptoms of atopic dermatitis [1, 3].

The undoubted involvement of microorganisms in the pathogenesis of allergic dermatitis dictates the need to use combined formulations containing both topical corticosteroids known to suppress the development of allergic inflammation and antimicrobial agents allowing pathogenic microorganisms to be eliminated. Local antimicrobial medicines have a number of advantages resulting from their direct contact with the pathogenic microorganisms and decreases in the risks of developing systemic side effects while having a wide spectrum of antimicrobial activity and high concentrations of active ingredient at the application site [4, 6 – 8].

A number of clinical studies using combined formulations containing antibacterial substances have not demonstrated clear advantages as compared with the use of topical steroids [9]. This may be due primarily to the addition of strains of *Candida spp.* and increases in their species diversity occurring as a result of dysbiotic changes [2]. Thus, treatment with topical steroids combined with antibacterial and antifungal drugs, particularly combined formulations containing betamethasone (a synthetic glucocorticosteroid), gentamicin (an aminoglycoside antibiotic), and clotrimazole (an imidazole derivative antifungal) is pathogenetically based [6].

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Three combinations of betamethasone + gentamicin + clotrimazole with trade names as ointments or creams for external application are currently registered in the Russian Federation for use in skin infections: Triderm® (reference formulation, Schering-Plough Labo N. V., Belgium), Akriderm® GK (generic, OAO Akrikhin, Russia), and Canison® Plus (generic, Agio Pharmaceuticals Ltd., India) [10]. When addressing the substitutability of the original and generic formulations it is critical to avoid lack of complete information on the quality of the active ingredients and excipients used for preparation of combined medicines [11], and the question of the intersubstitutability from the point of view of pharmacodynamic efficacy remains open. At the same time, both doctor and patient must be confident in the therapeutic equivalence of these drugs, which is determined not only by having comparable pharmacokinetics and compositions, but also in their pharmacodynamic properties, namely, activity against the target pathogens, which can be assessed in *in vitro* experimental conditions [12]. Differences between the main topical formulations of generic and proprietary medicines, as well as differences in their physicochemical properties, define the need for comparative studies predicting possible differences in efficacy in real clinical practice. Thus, the aim of the present work was to undertake a comparative evaluation of the antimicrobial activity of Akriderm® GK and Triderm® in cream and ointment formulations in *in vitro* conditions.

## EXPERIMENTAL SECTION

The study evaluated the antimicrobial activity of Triderm® (cream, lots 6MCEA20112 and 7MCEA29003; ointment lots 5NJDA08001 and 7NJDA03004) from Bayer (Russia) and Akriderm® (cream lots 491216 and 170416; ointment lots 180717 and 60217), Akrikhin (Russia). The

**TABLE 1.** Comparative Evaluation of the Formulations Triderm® and Akriderm® in Cream Formulations by Serial Dilutions

| Formulation, lot No., microorganism | MIC, μg/ml | | | |
|---|---|---|---|---|
| | Triderm® | | Akriderm® | |
| | 6MCEA21002 | 7MCEA29003 | 491216 | 170416 |
| *P. aeruginosa* ATCC 27853 | 1 | 1 | 1 | 1 |
| *S. aureus* ATCC 29213 | 0.125 | 0.25 | 0.5 | 0.5 |
| *E. coli* ATCC 25922 | 0.5 | 0.25 | 1 | 1 |
| *E. faecalis* ATCC 29212 | 1 | 2 | 16 | 16 |
| *S. epidermidis* 004T1 | 0.5 | 0.5 | 16 | 4 |
| *S. aureus* 1138 | 0.125 | 0.125 | 0.5 | 0.5 |
| *S. aureus* 13385 | 0.125 | 0.06 | 0.25 | 0.25 |
| *S. aureus* 1378 | 0.125 | 0.125 | 0.25 | 0.25 |
| *S. haemolyticus* 64-1099 | 0.03 | 0.125 | 0.25 | 0.125 |
| *S. haemolyticus* 61-3 | 0.03 | 0.03 | 0.125 | 0.125 |
| *S. saccharolyticus* 20222 | 0.03 | 0.03 | 0.125 | 0.125 |
| *S. epidermidis* 20637 | 0.03 | 0.03 | 0.125 | 0.125 |
| *S. epidermidis* 21555 | 0.03 | 0.03 | 0.125 | 0.125 |
| *S. epidermidis* 21457 | 0.03 | 0.06 | 0.25 | 0.25 |
| *E. faecalis* 23 | 0.25 | 0.25 | 0.25 | 0.25 |
| *E. faecium* 130 | 0.5 | 1 | 2 | 2 |
| *E. faecalis* Jh 2-2 | 0.5 | 1 | 2 | 4 |
| *E. faecalis* 583 | 2 | 4 | 16 | 16 |
| *Ñ. parapsilosis* ATCC 22019 | 0.03 | 0.03 | 0.125 | 0.125 |
| *C. albicans* ATCC 24433 | 0.06 | 0.06 | 0.25 | 0.25 |
| *C. tropicalis* 4205 | 0.5 | 1.0 | 2.0 | 1.0 |
| *C. albicans* 8P | 0.06 | 0.06 | 0.125 | 0.25 |
| *C. krusei* 432M | 0.125 | 0.25 | 0.5 | 0.25 |
| *C. krusei* 72-05 | 0.5 | 1.0 | 1.0 | 0.5 |
| *C. tropicalis* 5605 | 0.25 | 0.25 | 1.0 | 0.5 |

test system consisted of microbial strains obtained from the working museum of the G. F. Gauze Science Research Institute for the Search for New Antibiotics and clinical laboratories.

The sensitivities of bacterial strains to study formulations were determined by an agar dilution method as specified in CLSI M07-A10 [13]. The sensitivity of *Candida spp.* was analyzed using a micromethod by broth dilutions as specified in state standard GOST R ISO 16256-2015 [14].

The gentamicin and clotrimazole concentrations for stock solutions were calculated from their compositions in the ready formulations. Stock solutions were prepared by serial dilutions in broth using accurately weighed formulations dissolved in dimethylsulfoxide (DMSO). Analysis by serial dilutions in agar using stock solutions prepared in Muller-Hinton broth [13]. Prepared samples in DMSO and agar-containing medium were held in an incubator at 45°C for 30 – 40 min with periodic thorough shaking to obtain uniform drug distributions. Sequential two-fold dilutions were then prepared using a range of clotrimazole concentrations of 0.015 – 8 μg/ml and a range of gentamicin concentrations of 0.06 – 32 μg/ml.

Dilutions in agar-containing medium were inoculated using a 33-point replicator with points of diameter 2 mm. The final titer of inoculate contained $10^5$ cfu/ml. Microorganism growth was monitored using the same growth medium but not containing samples.

All experiments were run in three repeats.

MIC values were determined as the lowest concentration of antibiotic suppressing visible microbial growth as compared with monitoring of growth without drug.

Results were analyzed in Microsoft Excel 2010. The numerical MIC value in experiments using serial dilutions was expressed as the median. MIC values for each formulation were compared by unifactorial analysis of variance (ANOVA) in SPSS Statistics v. 23. Significant differences were identified using the Scheffe test at $p < 0.05$ [15].

## RESULTS AND DISCUSSION

Results of comparative analysis of MIC values for Akriderm® GK and Triderm® against control strains and clinical isolates of the major bacterial and fungal pathogens are shown in Tables 1 and 2. It follows from these data that the activity levels of the study agents in both medicinal formulations were within the acceptable range [14], with the exception of Akriderm® GK cream against *S. aureus* ATCC 29213, where the MIC was one dilution greater than the control value. Despite the fact that the level of activity corresponded to the acceptable, the MIC values for Triderm® cream and ointment were greater than those of both drugs in Akriderm® GK against control strains by an average of 2 – 4 dilutions.

It follows from the results presented here that Triderm® (Bayer, Russia) and Akriderm® GK Akrikhin, Russia) as creams and ointments had similar spectra of actions against clinically important pathogens independently of their antibiotic sensitivities. However, the activity levels of gentamicin and clotrimazole in the combined formulations Triderm® and Akriderm® GK were different: the MIC values of Triderm® ointment and cream against 20 of the 25 bacterial and fungal strains were 2 – 4 dilutions lower than the MIC of Akriderm® GK. MIC values differed by six dilutions for some microbial species.

Akriderm® GK as the cream formulation (lot 60217) was more active against Gram-negative microorganisms than the other lot of the same substance (180717): MIC values against *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were 0.125 and 1 μg/ml respectively ($p < 0.01$). Similar results were obtained with *E. faecalis* ATCC 29212: MIC values for Akriderm® GK (ointment and cream) were 4 – 16 μg/ml, while those for Triderm® (ointment and cream) were 1 – 2 μg/ml ($p < 0.01$).

Akriderm® GK (cream and ointment) had weak activity against methicillin-resistant clinical isolate *S. epidermidis* 004T1, in contrast to Triderm® (cream and ointment), whose MIC values were 4 – 16 and 0.5 – 1 μg/ml respectively ($p < 0.003$). Thus, the activity of Triderm® formulations against individual strains was six dilutions greater than that of both Akriderm® formulations. *E. faecium* 130, *E. faecalis* Jh 2 – 2, and vancomycin-resistant *E. faecalis* 385 were also more sensitive to Triderm® (cream and ointment) than Akriderm® (cream and ointment) ($p < 0.001$). The acceptable variation in MIC for this method is no more than 1 – 2 dilutions, though 16 of the 18 clinical bacterial isolates were more sensitive to Triderm® than Akriderm® GK, where MIC values differed by 2 – 4 dilutions.

Similar data were obtained from comparison on drug activity against *Candida spp.* MIC values for study formulations for four of the seven *Candida spp.* strains used in the study differed by 2 – 4 dilutions with lower MIC values for Triderm cream and ointment.

Selection of combined topical formulations containing glucocorticosteroid, antibacterial, and antifungal components for successful and rapid resolution of the inflammatory process in dermatoses of mixed etiology is justified, as use of such combinations can provide more effective treatment of this type of disease [4, 6 – 9]. However, assessment of the comparability of the original and generic formulations was ambiguous, as the manufacturing conditions for the pharmaceutical substances and the excipient components used in them could differ and might have significant influences on their biological activities [11].

Data identifying significant differences in the antibacterial activity of generic formulations as compared with the proprietary formulations not infrequently come from *in vitro* studies [15]. This raises the question of the possible cause of such differences and their significance for real clinical practice. Particular attention is paid to the quality of the starting substances, which can often vary over wide ranges without

going beyond the limits of the officially determined norms. The specially selected base composition base may contribute to the activity of the proprietary formulations [11, 16]. The bases of generic formulations often use the most inexpensive components without consideration of their influences on the chemical activity of the active ingredients. Gentamicin is known to display its activity at particular pH values [16]. Triderm® cream contains a two-component buffer, in contrast to the generic, whose buffer system has a single component. The composition of the base not only influences the allergenic potential of the formulation (for example, Triderm cream and ointment do not contain parabens, which are used in the generics), but also determine such important properties as solubility, homogeneity, and stability temperature [16, 17]. Differences in the solubility profiles of the generic and original medicines have been noted [16]. Thus, comparison of the rheological properties of Triderm® cream showed stable structural-mechanical properties with variation of temperature over a wide range (21 – 30°C), which is relevant for cutaneous application, in contrast to the generic analogs [18]. The proprietary and generic formulations tested in the present studies are produced in compliance with Good Manufacturing Practice guidelines (GMP). However, this does not exclude the possibility of significant differences in the technical processes, which may influence the final result.

These tests provide evidence that the activity of gentamicin and clotrimazole in the combined formulations Triderm® and Akriderm® GK differ, including against *Staphylococcus spp.* Considering the predominant involvement of microorganisms of this species in the pathogenesis of atopic dermatitis [1 – 3], the clinical efficacy of the test preparations used in the treatment of skin infections due to *S. aureus* may differ significantly. In addition, *Candida spp.* and *Staphylococcus epidermidis* may play a significant role in the pathogenesis of atopic dermatitis and microbial eczema, as these may not only complicate the course of the infectious

**TABLE 2.** Comparative Evaluation of the Formulations Triderm® and Akriderm® in Ointment Formulations by Serial Dilutions

| Formulation, lot No., microorganism | MIC, μg/ml | | | |
|---|---|---|---|---|
| | Triderm® | | Akriderm® | |
| | 5NJDA08001 | 7NJDA03004 | 60217 | 180717 |
| *P. aeruginosa* ATCC 27853 | 0.125 | 0.125 | 0.125 | 1 |
| *S. aureus* ATCC 29213 | 0.25 | 0.25 | 1 | 2 |
| *E. coli* ATCC 25922 | 0.25 | 0.25 | 0.125 | 1 |
| *E. faecalis* ATCC 29212 | 2 | 1 | 4 | 4 |
| *S. epidermidis* 004T1 | 1 | 0.5 | 4 | 4 |
| *S. aureus* 1138 | 0.125 | 0.125 | 0.25 | 0.25 |
| *S. aureus* 13385 | 0.06 | 0.125 | 0.25 | 0.25 |
| *S. aureus* 1378 | 0.125 | 0.125 | 0.25 | 0.125 |
| *S. haemolyticus* 64-1099 | 0.06 | 0.06 | 0.25 | 0.25 |
| *S. haemolyticus* 61-3 | 0.03 | 0.03 | 0.25 | 0.25 |
| *S. saccharolyticus* 20222 | 0.03 | 0.03 | 0.25 | 0.25 |
| *S. epidermidis* 20637 | 0.03 | 0.03 | 0.25 | 0.25 |
| *S. epidermidis* 21555 | 0.03 | 0.03 | 0.25 | 0.25 |
| *S. epidermidis* 21457 | 0.06 | 0.03 | 0.25 | 0.25 |
| *E. faecalis* 23 | 0.125 | 0.25 | 0.25 | 0.125 |
| *E. faecium* 130 | 1 | 0.5 | 2 | 2 |
| *E. faecalis* Jh 2-2 | 0.5 | 0.5 | 2 | 2 |
| *E. faecalis* 583 | 2 | 2 | 16 | 16 |
| *Ñ. parapsilosis* ATCC 22019 | 0.03 | 0.03 | 0.125 | 0.125 |
| *C. albicans* ATCC 24433 | 0.06 | 0.06 | 0.25 | 0.125 |
| *C. tropicalis* 4205 | 0.5 | 0.5 | 2.0 | 2.0 |
| *C. albicans* 8P | 0.06 | 0.06 | 0.125 | 0.125 |
| *C. krusei* 432M | 0.25 | 0.25 | 0.25 | 0.25 |
| *C. krusei* 72-05 | 0.5 | 0.5 | 2.0 | 2.0 |
| *C. tropicalis* 5605 | 0.125 | 0.25 | 2.0 | 2.0 |

process due to *Staphylococcus aureus*, but may also be the cause of separate diseases [19 – 22]. These results suggest that the proprietary formulations of Triderm® have higher clinical efficacy in the treatment of infections due to *Staphylococcus aureus* associated with candidiasis and/or infection of the skin with other staphylococcal species.

Infection of the skin with *Enterococcus faecalis* is more characteristic of wound surfaces or areas located in the anogenital area; it is rarely seen in atopic dermatitis, though the distribution antibiotic-resistant strains of *Enterococcus faecalis* is quite high [23 – 25]. Thus, complete and partial elimination of organisms of this species can be justified from the point of view of preventing late complications and the production of strains with low sensitivity to antibacterial substances. From these positions, the greater efficacy of Triderm® cream and ointment against *Enterococcus faecalis* reduces the risk of selecting strains resistant to gentamicin.

Thus, the clinical efficacy of the original medicines Triderm® may be greater than that of the generics, as the activity of Triderm® cream and ointment in *in vitro* conditions against the most important microorganisms involved in the pathogenesis of cutaneous infections *Staphylococcus spp.*, *Enterococcus spp.*, and *Candida spp.*) was greater than that of the Akriderm® GK formulations. In addition, rational qualitative and quantitative assessment of therapeutic efficacy requires further comparative studies, including clinical trials.

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