



EFFICACY OF ZINCODERM G CREAM AGAINST WOUND INFECTION BY METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN SPRAGUE-DAWLEY RATS

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ABSTRACT

A fixed drug combinations (FDC) of clobetasol, gentamicin and Zinc namely Zincoderm G cream is available in the market. There are studies which shows the efficacy of each component in bacterial infections, but as FDC including Zinc has not been reported yet. A study was undertaken to evaluate the efficacy of Zincoderm G cream in burn wound infected with methicillin-resistant *Staphylococcus aureus*. Methods: A total of 18 Sprague-Dawley rats (male, pathogen free, 6-8 weeks old) were used in this study. The rats were divided into 3 groups of 6 rats each. 25-30 mg of test drugs (Zincoderm G cream with or without Zinc) was applied on *S.aureus* infected burn wound affected area of back of rats for two weeks. Degree of bacterial infection was assessed by quantification of bacteria. There was 30% mortality seen in both MRSA toxic control (cream base) and MRSA positive control (Zincoderm G cream without Zinc) groups. Only 10% mortality was observed in the MRSA group which was treated with Zincoderm G cream with Zinc. But, there was no significant difference found in MRSA bacterial concentration (number of CFU/ml wound fluid; after treatment) for both Zincoderm G with/without Zinc when compared with the MRSA toxic control (cream base) group. Addition of Zinc to Zincoderm G did not exhibit distinct killing profile against MRSA. We found that Zincoderm G cream with Zinc could not exhibit the distinct killing profiles against MRSA.

KEYWORDS: Zincoderm G cream, MRSA, Infection, Burn-wound

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INTRODUCTION

Burns may be caused by scalds, building fires and inflammable liquids and gases.¹ Life threatening infection is one of the complications of burn injury with potential risk of burn wound sepsis and septicaemia.² The risk of infection is further potentiated due to immune disturbances caused by thermal injury.³⁻⁵ The aim of management of burn wound is early healing without infection. Several bacterial microorganisms can infect the skin and soft tissue, but the most common agents are *S. aureus* and *S. pyogenes*.^{6,7} In general, the selection of topical antibiotic agent will be dependent on the probable microorganism causing the infection. Patients who have compromised epidermis, poor hygiene, live in crowded conditions, have comorbidities, and have close contact with people having skin and soft tissue infections are at high risk of acquiring a skin and soft tissue infection themselves. Zincoderm G skin cream is a fixed dose combination of clobetasol, gentamicin and zinc. There are reports on safety efficacy of each component like clobetasol and gentamicin but as a fixed dose combination including zinc has not reported so far. We planned to evaluate the efficacy of Zincoderm G cream, against methicillin-resistant *S. aureus* wound infection in Sprague-Dawley rats.

METHODS

Animals

Eighteen adult male Sprague-Dawley rats (male, weighing 150–200 g) were housed in polypropylene cages, maintained under standard conditions with temperature (22–24°C), 12-h light/12-h dark cycle and relative air humidity 40–60%. Rats had continuous access to standard rat pellet diet (VRK Nutritional Solutions, Pune, India) and to tap water. Approval of the Institutional Animal Ethics Committee (IAEC/KMC/88/2013) was obtained before starting the experiment. Experiments were carried out according to Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Drugs

The test drugs (Zincoderm G cream with Zinc, Zincoderm G cream without Zinc and cream base) were supplied by Apex Laboratories Private Limited, Chennai (India).

Test micro-organism

MRSA was grown overnight in Muller Hinton (MH) broth. The resulting stationary-phase cultures was transfused into fresh MH medium and incubated at 37°C until reaching the mid-logarithmic phase. The subculture was centrifuged (10 min, 41°C, 880 X g), and the resulting bacterial pellet was washed once with phosphate-buffered saline (PBS), pH 7.4, and re-suspended in cold PBS. Optical density (OD) was measured at 600 nm (OD600). Bacterial concentration as number of colony forming unit (CFU)/ml was calculated using the following equation⁵:

$$\text{Number of CFU/ml} = \text{OD600} \times 2.5 \times 10^8.$$

A total of 10⁸ CFU was re-suspended in 250 µl PBS, and the bacterial suspension was kept on ice until further use.

Rat burn infection model

Twenty four hours prior to wounding, the rats were depilated on back. They were anesthetized using ketamine (60 mg/kg; i.p) and xylazine (10 mg/kg; i.p). Two defined skin areas on the back of each rat was immersed with water for 25 sec at 65°C. Both areas were dried, marked, and thoroughly disinfected. A bacterial solution of 250 µl, containing a definite number of either 1x10⁸ CFU of MRSA was added topically to both areas. An occlusive dressing (Tegaderm, 6x7 cm; 3 m) was applied immediately after the application of bacteria to prevent cross contamination and favourable condition for growth of bacteria. The rats were bandaged with Peha-haft for stabilization and protection clipped with VisiStat.⁵

Experimental design

A total of 18(3 groups of 6 rats each) Sprague-Dawley rats (male, pathogen free, 6-8 weeks old) were used in this study. They were treated daily for 2 weeks as follows- Group I: MRSA induced control rats- 25-30 mg of

cream base was applied at the affected area (negative control). Group II: MRSA induced treatment rats- 25-30 mg of Zincoderm G cream without Zinc was applied at the affected area (positive control). Group III: MRSA induced treatment rats- 25-30 mg of Zincoderm G cream with Zinc was applied at the affected area (test drug treatment group). Wounds were occlusively dressed and bandaged as described above after each application.

Quantification of bacteria

On third post infection day before the start of the treatment, bacterial counts were performed in wound fluid. At the end of experiment, biopsy of wound were taken and they individually weighed and homogenized in 2 ml of PBS using a Polytron homogenizer. The homogenates and the collected wound fluids of each wound were then serially diluted in PBS (1:10, 1:100, 1:1,000, and 1:10,000) and plated on mannitol agar plates in triplicates, MRSA selection agar and MH agar plates containing 5% sheep blood. Plates were then incubated for at least 18 h at 37°C

under a humidified atmosphere. All colony counts were expressed as log₁₀ CFU/ml wound fluid. Bacterial counts of >1x10⁵ were considered to indicate bacterial infection.⁸

Data analysis

Data were analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test using SPSS 20.0. P value less than 0.05 was considered as statistically significant.

RESULTS

There was 30% mortality seen in both MRSA toxic control (cream base) and MRSA positive control (Zincoderm G cream without Zinc) groups. Only 10% mortality was observed in the MRSA group which was treated with Zincoderm G cream with Zinc. But, there was no significant difference found in MRSA bacterial concentration (number of CFU/ml wound fluid; after treatment) for both Zincoderm G with/without Zinc when compared with the MRSA toxic control (cream base) group (Table 1).

Table 1

Effect of Zincoderm G cream with/without Zinc on MRSA bacterial concentration (Number of CFU/ml wound fluid):[MRSA bacterial concentration before treatment - MRSA bacterial concentration after treatment]

Groups	Dose	Mean±SEM
I-	MRSA induced toxic control group- 25-30 mg cream base	10 ² ±10 ^{0.63}
II-	MRSA + Zincoderm G cream without Zinc treated positive control group- 25-30 mg Zincoderm G cream without Zinc	10 ^{4.33} ±10 ^{0.95} #a
III-	MRSA + Zincoderm G cream with Zinc treated test group- 25-30 mg Zincoderm G cream with Zinc	10 ^{4.66} ±10 ^{0.84} #a, b

^a compared to MRSA toxic control group, ^b compared to MRSA positive control group,

p > 0.05 (not significant)

DISCUSSION

Data generated shows that Zincoderm G cream with Zinc could not prevent the MRSA infection when used topically in burn wound infection. But, this formulation has decreased the mortality in this condition which might be attributed to the anti-microbial effect of gentamicin and zinc as well as anti-inflammatory property of clobetasol which are the components of this formulation. One of the studies suggests that MRSA is not sensitive to

gentamicin.⁹ This might be the reason for its failure in decreasing the MRSA bacterial count after the treatment. MRSA does not appear to be more virulent than methicillin-sensitive *Staphylococcus aureus*, but certainly poses a greater treatment challenge. Management of infection with MRSA has been associated with higher hospital costs and carries high mortality. MRSA strains generally are now resistant to other antimicrobial classes

including aminoglycosides, beta-lactams, carbapenems, cephalosporins, fluoroquinolones and macrolides.⁹ The present study supports the previous studies which suggest the resistance of aminoglycosides for MRSA infections. In conclusion, Zincoderm G was not effective in controlling MRSA infection of experimental burn wounds.

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