Efficacy of zincoderm G cream against wound infection by *Pseudomonas aeruginosa* in Sprague-Dawley rats

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ABSTRACT

Background: Zincoderm G cream is a fixed dose combination of clobetasol, gentamicin and zinc. Studies have been carried out for efficacy of each component such as clobetasol and gentamicin in bacterial infections, but as fixed dose combination including zinc has not been reported yet. Hence, a study was planned to assess the efficacy of Zincoderm G cream in experimental wound infection by *Pseudomonas aeruginosa*.

Methods: In the experiment a total of 18 Sprague-Dawley rats (male, pathogen free, 6-8 weeks old) were used. The rats were divided into three groups of six rats each. 25-30 mg of test drugs (Zincoderm G cream with or without zinc) was applied on *Pseudomonas aeruginosa* infected burn wound affected area of back of rats for 2 weeks. Bacterial infection was assessed by quantification of bacteria.

Results: There was 80% mortality observed in *P. aeruginosa* infected toxic control (cream base) group. Whereas, only 40% mortality was seen in both Zincoderm G cream with/without zinc groups, which were inoculated with *P. aeruginosa*. Bacterial concentration (Number of colony forming unit/ml wound fluid) was significantly decreased (p<0.001) in *P. aeruginosa* infected rats treated with Zincoderm G cream with zinc when compared with *P. aeruginosa* infected control (untreated) rats.

Conclusions: We found that Zincoderm G cream with zinc exhibited distinct killing profiles against *P. aeruginosa*.

Keywords: Zincoderm G cream, *Pseudomonas aeruginosa*, Infection, Burn-wound

INTRODUCTION

A burn injury causes tissue necrosis and the raw area with serous exudation. The devitalized tissues and moist burn wound is favorable for the colonization and proliferation of microorganisms and subsequent infection. Therefore the potential risk of burn wound sepsis and septicemia persists until complete wound healing. The risk of infection is further potentiated due to immune disturbances caused by thermal injury. The aim of management of burn wound is early healing without infection. Most infections can be treated outpatient although physicians should be on alert for signs and symptoms of more severe infections. Therefore, clinical assessment of the severity of the infection, diagnosis, and knowledge of pathogen specific antibiotic resistance is important. *Pseudomonas aeruginosa* is an important life-threatening nosocomial pathogen and plays a prominent role in serious infections in burn patients. *P. aeruginosa* thrives on the moist burn wound surface and it is highly pathogenic in thermo-labile immunosuppressed patients. These bacteria usually gain access to burn patients through cross-contamination of burn wounds.

*Pseudomonas* infection is a common complication in burn patients, and it contributes to morbidity and mortality among them. 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 co-morbidities, 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. Studies have been done for safety and efficacy of each component such as clobetasol and gentamicin, but as fixed dose combination including zinc has not yet reported. Hence, a study was planned to investigate the efficacy of Zincoderm G cream, against P. aeruginosa wound infection in Sprague-Dawley Rats.

**METHODS**

**Animals**

A total of 18 adult male Sprague-Dawley rats (male, weighing 150-200 g) were housed in polycarbonate cages, maintained under standard conditions with temperature (22-24°C), 12 hrs light/12 hrs dark cycle and relative air humidity 40-60%. Rats had continuous access to norm calorific standard rat pellet diet (VRK Nutritional Solutions, Pune, India Ltd.) and to tap water. The animals were acclimatized to the laboratory conditions for 1 week before the start of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC/KMC/88/2013) and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment (Government of India), Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines.

**Drugs**

Zincoderm G cream with zinc, Zincoderm G cream without zinc and cream base were obtained from Apex Laboratories Private Limited, Chennai, Tamil Nadu, India.

**Test micro-organism**

P. aeruginosa was grown overnight in standard Luria Bertani (LB) broth medium. The resulting stationary-phase cultures was transfused into fresh LB medium and incubated at 37°C until reaching the mid-logarithmic phase. The subculture was centrifuged (10 mins, 41°C, 880 ×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 equation:

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

A total of \(10^8\) 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 hrs after depilation, the rats were anesthetized using ketamine (60 mg/kg; i.p) and xylazine (10 mg/kg; i.p). After immersing two defined skin areas on the back of each rat for 25 s in 65°C water, both areas were dried, marked, and thoroughly disinfected. A bacterial solution of 250 µl, containing a definite number of either \(1 \times 10^8\) CFU of P. aeruginosa, was added topically to both areas. To avoid cross-contamination and to improve growth conditions for the bacteria, an occlusive dressing (Tegaderm, 6 cm×7 cm; 3 m) was applied immediately after the application of bacteria. The rats were bandaged with Peha-haft for stabilization and protection clipped with VisiStat.

**Experimental design**

In the experiment, a total of 18 Sprague-Dawley rats (male, pathogen free, 6-8 weeks old) were used. The rats were divided into three groups of six rats each. Treatment was carried out every day for the duration of 2 weeks as follows:

1. **Group I:** P. aeruginosa induced control rats - 25-30 mg of cream base was applied at the affected area (negative control).
2. **Group II:** P. aeruginosa induced rats - 25-30 mg of Zincoderm G cream without zinc was applied at the affected area (positive control).
3. **Group III:** P. aeruginosa induced rats - 25-30 mg of Zincoderm G cream with zinc was applied at the affected area (test drug treatment group).

After application, the wounds were again occlusively dressed and bandaged as described above.

**Quantification of bacteria**

After 2 days of post infection before start of the treatment, bacterial counts were performed in wound fluid. At the end of experiment, to determine the bacterial counts in the tissue samples, biopsy specimens were 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, Pseudomonas isolation agar plates containing 5% sheep blood. Plates were then incubated for at least 18 hrs at 37°C under a humidified atmosphere. All colony counts were expressed as log10 CFU/ml wound fluid. Bacterial counts of >1×10^6 was considered to indicate bacterial infection.

**Data analysis**

Using SPSS 20.0, data were expressed as mean±standard error of mean and analyzed by one-way analysis of variance followed by *post-hoc* Tukey test. p<0.05 was considered to be statistically significant.
Table 1: Effect of Zincoderm G cream with/without zinc on P. aeruginosa bacterial concentration (Number of CFU/ml wound fluid): (P. aeruginosa bacterial concentration before treatment – P. aeruginosa bacterial concentration after treatment).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>P. aeruginosa toxic control group - 25-30 mg cream base</td>
<td>10^5±10^5</td>
</tr>
<tr>
<td>II</td>
<td>P. aeruginosa+Zincoderm G cream without zinc treated positive control group - 25-30 mg Zincoderm G cream without zinc</td>
<td>10^5±10^5</td>
</tr>
<tr>
<td>III</td>
<td>P. aeruginosa+Zincoderm G cream with zinc treated test group - 25-30 mg Zincoderm G cream with zinc</td>
<td>10^5±10^5</td>
</tr>
</tbody>
</table>

*Compared to P. aeruginosa toxic control group. **p<0.01.

REFERENCES


