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Novel bio-inspired chloramine for infection eradication in a full thickness porcine wound model

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Abstract

Introduction:

The wound environment is a fertile breeding ground for pathogens such as *Pseudomonas aeruginosa*.[1] Topical antiseptics are vital for managing these infections.[2] Chloramines have recently emerged as promising broad-spectrum antimicrobials.[3]

Methods:

Forty-five full-thickness wounds were created on 3 pigs with a sterile 10mm punch biopsy[4]. Wounds were inoculated with 25 µL of *P. aeruginosa* (ATCC 27312). After 24 hours, baseline wounds were recovered. Six wounds per group were treated daily with: 1) 0.3% liquid+Gel, 2) 0.6% liquid+Gel, 3) 1.2% liquid+Gel, 4) 0.6%+additives+Gel, 5) Silver Sulfadiazine (SSD), 6) Sterile PBS+unchlorinated Vehicle, or 7) Untreated Control. Wounds were covered with polyurethane film until assessment on days 3 and 7.

Results:

On day 3, all chlorinated biocides produced significant reductions ($p \leq 0.05$) in bacterial counts vs. Untreated Control. Formulations with 0.3% and 0.6% (with/without additives) significantly reduced ($p \leq 0.05$) bioburden vs. Baseline. By day 7, 0.6% liquid+additives+Gel had the lowest counts (3.97±0.23 Log CFU/g), showing a significant 99.8% and 99.5% reduction ($p \leq 0.05$) vs. Untreated Control and Baseline, respectively. This treatment reduced *P. aeruginosa* significantly more than SSD ($p \leq 0.05$) by 1.20±0.04 Log CFU/g. Bioburden in all treated wounds decreased significantly ($p \leq 0.05$) from day 3 to 7. No irritation was observed with experimental formulations.

Conclusions:

The chloramine formulations demonstrated significant antimicrobial efficacy against established *P. aeruginosa* infections, resulting in better efficacy compared to SSD. These bio-inspired chloramines have high therapeutic potential for infection prevention and management.

Introduction

Wound infections are often difficult to address due to the presence of biofilm, a protective matrix produced by bacteria that leads to delayed healing. *Pseudomonas aeruginosa* is a common biofilm-producing pathogen and is frequently responsible for wound infections.[5] A novel and promising therapeutic for these infections is the use of topical chloramines due to the potent antimicrobial and anti-biofilm properties these compounds have demonstrated.[6] This study evaluated the efficacy of multiple formulations of chloramines using an established porcine wound infection model.[7]

References

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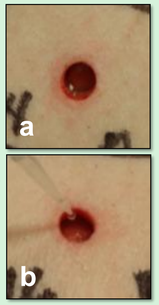
Materials and Methods

1. Experimental Animals:

- Three female specific-pathogen-free swine.

2. Wounding Technique:

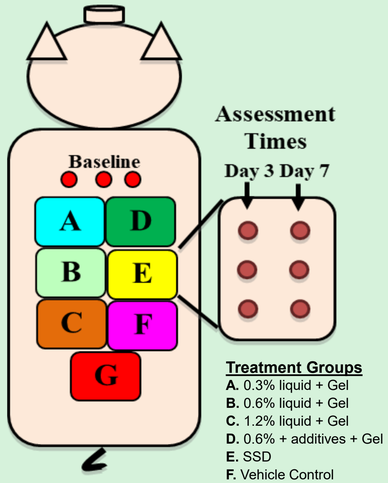
- Full thickness wounds (a) were created with a sterile 10mm punch biopsy. 45 wounds were created per animal.



3. Inoculation:

- Wounds were each inoculated with 25 µL of a 10^6 CFU/mL suspension of *P. aeruginosa* ATCC 27312 (b) Wounds were then covered with polyurethane film dressing for 24 hours to allow for biofilm formation before treatment application.[7]

4. Experimental Design

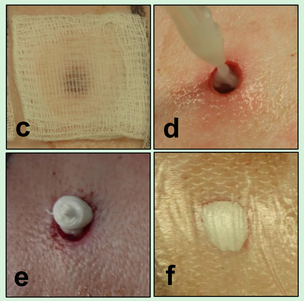


Treatment Groups

A. 0.3% liquid + Gel
B. 0.6% liquid + Gel
C. 1.2% liquid + Gel
D. 0.6% + additives + Gel
E. SSD
F. Vehicle Control
G. Untreated Control

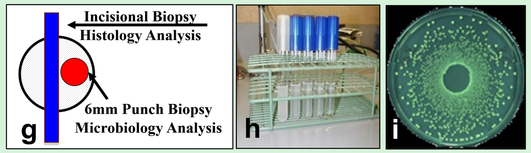
4. Treatment Regimen

- Wounds were treated daily during the study duration.
- Liquid treatments was applied prior to gel if applicable by applying treatment-soaked gauze (c) for 10 minutes to wounds.
- For gel treatments (d) and SSD (e), 200 µL of treatment was applied via syringe.
- Polyurethane film dressings were replaced at each treatment application (f).



5. Microbiology Assessment

- After 24 hours of biofilm formation (day 0), Baseline wounds were recovered for microbiology.
- Wounds were recovered for microbiology and histology on days 3 and 7 after treatment initialization.
- 6 mm punch biopsies (g) of wounds were collected and homogenized with neutralizing solution. Serial dilutions (h) of these samples were plated using a Spiral Plater System onto Pseudomonas Agar base with CN supplement. (i). Following overnight growth, colonies from were counted and the colony forming units per g (CFU/g) were calculated.



6. Histology Assessment

- Incisional biopsies (g) were recovered from the center of the wound and stained with Hematoxylin and Eosin (H&E). Samples were then assessed for:
- Re-epithelization (%), or the measurement of the length of the wound surface that has been covered with epithelium.
- Epithelial thickness, the average thickness of the epithelium in µm measured at five equidistant points in the biopsy.
- White cell infiltrate, measured by the presence and amount of subepithelial mixed leukocytic infiltrates. Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant

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Results

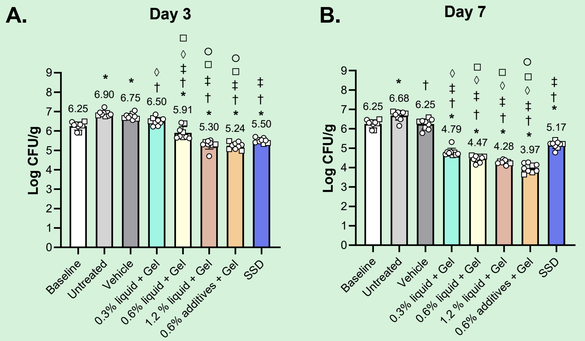

Figure 1. Reduction in bacterial burden. PA 27312 counts in wounds (Log CFU/g) prior to treatment (Baseline) and at days 3 (**A**) and 7 (**B**) after treatment initiation (n=9 samples plotted as means with error bars corresponding to standard deviation, One-way ANOVA followed by Tukey's multiple comparison's test).

**p≤0.05 compared to Baseline;*
†p≤0.05 compared to Untreated;
‡p≤0.05 compared to Vehicle;
◊ p≤0.05 compared to SSD;
□ p≤0.05 compared to 0.3% liquid + gel;
○ p≤0.05 compared to 0.6% liquid + gel.

- The greatest reduction in *P. aeruginosa* ATCC 27312 burden in wounds was observed for wounds treated with the chlorinated biocide, 0.6% + additives + Gel, resulting in 1.02±0.03 Log CFU/g reduction on day 3 and by 2.28±0.02 Log CFU/g reduction on day 7 compared to Baseline counts.
- The chlorinated treatments exhibited bacterial reductions in a dose dependent manner and the wounds treated with 0.6% or greater concentrations of treatments (with/without additives) demonstrating significantly lower *P. aeruginosa* counts compared to SSD (positive control) on day 7.

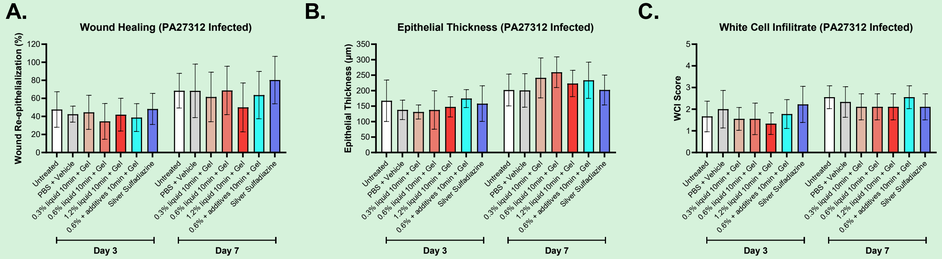

Figure 2. Histological assessment. PA 27312 infected wounds were assessed for changes in re-epithelialization (A), epithelial thickness (B) and white cell infiltrate (C), after H&E staining (n=9 samples plotted as means with error bars corresponding to standard deviation). No statistically significant differences between the different conditions on either Day 3 or Day 7 were observed by histological assessment.

Conclusions

- The novel chloramine formulations evaluated in this study demonstrated potent antimicrobial activity against *P. aeruginosa* infection and reduced bacterial burden significantly compared to current standards of treatment. The formulations did not adversely affect wound healing or produce irritation.
- Additional investigation into broad spectrum antimicrobial efficacy should confirm these findings and the clinical potential of chloramines as a topical therapeutic for the management of wound infections.