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IN VITRO AND *IN VIVO* ACTIVITY OF E-101 SOLUTION AGAINST *ACINETOBACTER BAUMANNII* STRAINS FROM U.S. MILITARY PERSONNEL

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ABSTRACT

Background: *Acinetobacter baumannii* (Ab) is an important pathogen among military personnel injured while deployed overseas. These isolates are highly resistant, with limited treatment options. E-101 Solution (E-101) is a novel drug product containing porcine myeloperoxidase (pMPO), glucose oxidase, and their respective substrates, and represents a new class of broad-spectrum antimicrobial for the prevention and treatment of localized infections. Its unique mode of action relies on the local production of singlet oxygen. The aim of this study was to evaluate the *in vitro* activity and the *in vivo* efficacy of E-101 against Ab.
Methods: Five multi-drug resistant clinical isolates of Ab from wounded soldiers were provided by the US Army. The *in vitro* bactericidal activity of E-101 was determined in the absence and presence of 3% rat blood. To study the *in vivo* activity of E-101, a full-thickness excision rat model was performed. In this model, a 1 cm x 1.5 cm area of skin was exposed to the dorsal fascia and inoculated with 10^7 CFU of Ab. Infected wounds were treated with E-101 at 150 GU pMPO/mL. Viable organisms were quantified at 15 and 30 minutes posttreatment.
Results: Time-kill studies showed bactericidal activity of E-101 against all 5 strains of Ab. Complete kill was achieved within 5 minutes with and without blood added, depending on the concentration used. The rate and extent of kill was concentration- and time-dependent. In the full-thickness excision rat model, E-101 demonstrated rapid bactericidal activity on contaminated fascia tissue. A reduction of >3 $\log_{10}$ CFU/tissue was achieved at 15 minutes compared to the initial inoculum for all strains tested.
Conclusion: E-101 was highly active *in vitro* and effective in a full-thickness excision wound model against Ab. The results support further study of E-101 for the decontamination and prevention of infection in surgical and traumatic wounds.

INTRODUCTION

Acinetobacter baumannii is an important nosocomial pathogen, and in recent years an increased number of military casualties from Iraq and Afghanistan have been infected with multi-drug resistant (MDR) *A. baumannii* (2). These isolates are highly resistant, with limited choices of agents available for treatment. E-101 Solution is a topical agent developed as an adjunct to the standard of care for the clinical management of wounds and prevention of surgical-site infections. E-101 Solution is a defined formulated cell-free oxidant-generating enzyme system containing porcine myeloperoxidase (pMPO) and glucose oxidase (GO) from *Aspergillus niger*, the respective substrates of the enzymes, sodium chloride and glucose, and specific antimicrobial activity enhancing agents. The mechanism of action of E-101 Solution involves the binding of MPO to the surface of target microorganisms and the generation of short-lived, highly reactive singlet oxygen. This restricts the kill radius to the width of the bacterial cell wall, thus avoiding collateral damage to surrounding host cells and tissue. Hydrogen peroxide and hyphochlorous acid are produced in sufficient amounts for singlet oxygen generation, but insufficient to cause host tissue toxicity. Recent studies demonstrated E-101 Solution is a potent, broad-spectrum, and rapid bactericidal product effective against MDR organisms *in vitro* (5) and efficacious in rat wound models (4). In this study, we determined the *in vitro* susceptibility and *in vivo* efficacy of E-101 Solution against MDR isolates of *A. baumannii* associated with combat injuries.

METHODS

Bacterial strains. Five clinical isolates of *A. baumannii* were provided by the US Army Institute of Surgical Research (Fort Sam Houston, TX) under CRADA #W81XWH-05-0153. Four of the 5 strains were isolated from wounds and the fifth strain from blood. All strains were multi-drug resistant. One reference clinical strain of *Pseudomonas aeruginosa* (R-463) with known activity to E-101 was included for comparison.
E-101 Solution. E-101 Solution is comprised of 2 aqueous solutions designated as enzyme solution and substrate solution, which are packaged in 2 separate vials. These 2 solutions were prepared by AAI International (Charleston, SC). The enzyme solution contains porcine myeloperoxidase (pMPO), glucose oxidase (GO) from *Aspergillus niger*, with selected amino acids formulated in a phosphate buffer. The substrate solution contains glucose (dextrose, USP) in the same phosphate buffer as the enzyme solution. The enzyme and substrate solutions are mixed together in varying proportions just prior to use to produce the drug product E-101 Solution at a desired concentration. The activity of E-101 Solution is expressed as guaiacol units (GU) of pMPO per mL (GU pMPO/mL), based on an adapted assay by Chance and Maehly (3) to determine peroxidase activity (6). Low and high concentrations of E-101 Solution containing pMPO used in the *in vitro* studies were 2.5, and 9.4 GU pMPO/mL in the absence of blood and 37.50, and 150 GU pMPO/mL in the presence of 3% rat blood. The concentration of E-101 Solution used in the *in vivo* studies was 150 GU pMPO/mL. The ratio of pMPO to GO was the same in all experiments.

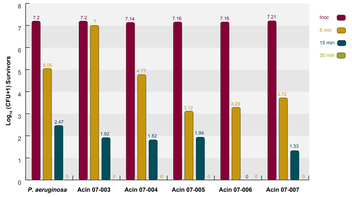
***In vitro* efficacy.** Time-kill studies were performed using a suspension-neutralization method (7) in the absence and presence of 3% rat blood. Bacterial suspensions were prepared to achieve late log to early stationary phase growth. A 1.0 mL volume of E-101 Solution, organism suspension at a final target concentration of approximately 10^7 CFU/mL followed by 30 µL of rat blood when necessary was tested. Reaction vials were incubated at room temperature and the enzyme activity was stopped by the addition of 100 µL of a sterile 1% catalase solution at 5, 15, 30, or 60 min. Samples were collected from the reaction vials and quantitative cultures were performed.

***In vivo* efficacy.** Experimental wounds were produced by a modification of the method reported by Saymen (6). Two full-thickness excision wound sites were prepared on the backs of anesthetized, adult male Sprague-Dawley rats by exposing a 1 cm x 1.5 cm area of fascia. Three rats with 2 wounds each were used for each treatment group. An open 2.5 cm diameter polystyrene cylinder was glued to the skin around each excised site with QuickTite® (Loctite Corp.) cement as described by Breuing (1). Each cylinder formed a liquid-tight test chamber, the base of which was the exposed fascia. The exposed fascia was inoculated by depositing 200 µL containing 10^7 CFU of the bacterial suspension. The inoculum was allowed to remain on the fascia for 15 minutes before treatment with E-101 Solution. A volume of 800 µL of E-101 Solution was added to the site-resulting in a total volume of 1 mL per test site. Recovery control sites were treated with 800 µL of 0.9% sterile saline. Both wound sites on a single rat received the identical treatment. Following 15- and 30-minute treatment times with E-101 Solution, 100 µL of a 10% solution of catalase was added to each site to neutralize any remaining enzyme and/or subsequently generated hydrogen peroxide, thereby inhibiting further microbicidal activity. The liquid in the cylinder was recovered and the underlying fascia was aseptically excised, weighed, and homogenized. Quantitative cultures of liquid sample and tissue homogenate were prepared and colonies were counted to determine organism survival.

Data analysis. In the *in vitro* studies, the mean $\log_{10}$ (CFU +1) survivors at each E-101 Solution concentration was determined versus time. In the *in vivo* studies, treatment performance was calculated as the sum of counts from the recovered liquid and tissue homogenate for each wound and reported as the average of all samples in each treatment group. The $\log_{10}$ reduction [$\log_{10}$ inoculum – $\log_{10}$ (CFU +1) survivors] was used for comparison.

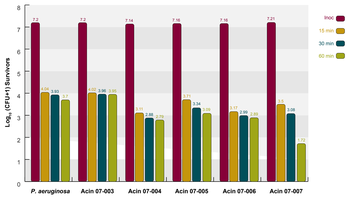
RESULTS

***In vitro* Efficacy (no blood added)**
Figure 1. In vitro activity of E-101 Solution at low concentration (2.5 GU pMPO/mL) against *A. baumannii*.



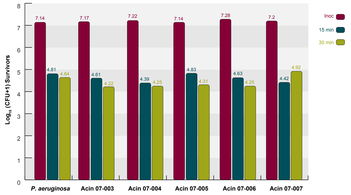
- *A. baumannii* was highly susceptible to E-101 Solution *in vitro*. Complete kill of a 7 $\log_{10}$ inoculum was achieved within 30 minutes at a low concentration (2.5 GU pMPO/mL).
- The extent of activity of E-101 Solution was time-dependent.

***In vitro* Efficacy (blood added)**
Figure 3. *In vitro* activity of E-101 Solution at low concentration (37.5 GU pMPO/mL) against *A. baumannii* in the presence of 3% rat blood.



- In the presence of 3% rat blood, a >3 $\log_{10}$ reduction of *A. baumannii* was achieved within 15 minutes at 37.5 GU pMPO/mL.

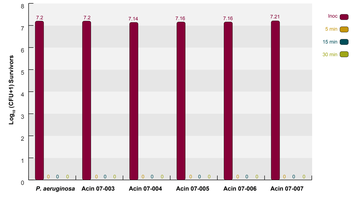
***In vivo* Efficacy**
Figure 5. *In vivo* activity of E-101 Solution at 150 GU pMPO/mL in the rat full-thickness excision model against *A. baumannii*.



- In the rat full-thickness excision model, the *in vitro* activity seen with E-101 Solution translated into good *in vivo* activity.
- For all strains tested, a >2.5 $\log_{10}$ reduction in CFUs was observed in 15 minutes at 150 GU pMPO/mL.

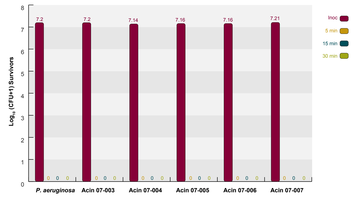
RESULTS (CONT)

Figure 2. *In vitro* activity of E-101 Solution at high concentration (9.4 GU pMPO/mL) against *A. baumannii*.



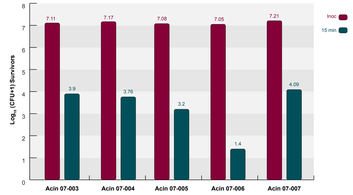
- Within 5 minutes at a high concentration (9.4 GU pMPO/mL) of E-101 Solution, complete kill of *A. baumannii* was achieved.
- The rate of kill was concentration-dependent.
- Activity of E-101 Solution against *A. baumannii* was comparable to the activity against *P. aeruginosa*.

Figure 4. *In vitro* activity of E-101 Solution at high concentration (150 GU pMPO/mL) against *A. baumannii* in the presence of 3% rat blood.



- In the presence of 3% rat blood, complete kill of *A. baumannii* was achieved within 15 minutes at 150 GU pMPO/mL.
- The inhibitory effect of whole blood on the activity of E-101 Solution was overcome by increasing the concentration of pMPO containing E-101 Solution.

Figure 6. Comparison of *in vivo* activity of E-101 Solution against 5 strains of *A. baumannii* in the rat full-thickness excision model. Studies were conducted on the same day using E-101 Solution at 150 GU pMPO/mL and treatment efficacy at 15 minutes posttreatment.



- The *in vivo* performance of E-101 Solution against all 5 strains of *A. baumannii* demonstrated similar patterns of susceptibility.
- One blood isolate of *A. baumannii* (07-006) appeared repeatedly more susceptible to E-101 Solution both *in vitro* and *in vivo* than the wound isolates.

CONCLUSION

E-101 Solution is potent and broad-spectrum antimicrobial for topical use. These properties, along with its efficacy exhibited against MDR *A. baumannii*, support further studies of E-101 Solution for the decontamination and prevention of infection in surgical and traumatic wounds.

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