Efficacy of E-101 Solution, a Myeloperoxidase-Based Antimicrobial, in Different Rat Localized Wound Infection Models

Paper#: F1-3956
48th Annual ICAAC®/IDSA 46th Annual Meeting
Washington, DC
October 25-28, 2008

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ABSTRACT

Background: E-101 Solution (E-101), a novel broad-spectrum drug product containing porcine myeloperoxidase (MPO) and glucose oxidase (GO), was evaluated in rat wound infection models. **Methods:** A) In a full-thickness wound model, a 2-cm^2 area of skin was excised to expose the dorsal fascia and inoculated with 10^7 CFU of *Staphylococcus aureus* or *Escherichia coli*. Infected wounds were untreated or treated with 0.8 mL of E-101 at 150 guaiacol units (GU) MPO/mL. Viable organisms were quantified at selected times post treatment. B) In an incision model, a deep 3-cm incision was made into the thigh. Wounds were inoculated with 10^9 CFU of a methicillin-resistant *Staphylococcus aureus* (MRSA) isolate and treated 60 minutes later with 2 x 10 mL of either E-101 at 300 GU MPO/mL or saline. C) In the same incision model, wounds were inoculated with 10^8 CFU *Pseudomonas aeruginosa* and treated 60 minutes later with 2 x 5 mL of either E-101 at 300 GU MPO/mL or saline. For B and C models, incisions were closed and evaluated at 4 days based on area of induration and presence of purulence. The conversion of µg to GU of MPO is based on 0.375 GU/µg of MPO/mL. **Results:** A) In the full-thickness excision model, 10^1 CFU were recovered 15 minutes after E-101 treatment compared to 10^7 CFU from untreated sites for both organisms. B) In the incision model using MRSA, E-101 treatment of wounds 60 minutes after inoculation showed an average score of 1.4 (0/10 purulence) compared to 2.3 (5/10 purulence) for saline treated and 3.5 (2/4 purulence) for infection controls. C) Wounds inoculated with *P. aeruginosa* showed infectivity scores of 4.0 (purulence 2/4), 4.0 (purulence 1/4), and 1.5 (purulence 0/4) for the untreated, saline-treated, and E-101 Solution-treated rats, respectively. **Conclusion:** E-101 reduced bacterial loads in inoculated tissue in these wound models. Rapid microbicidal activity in the full-thickness excision model and extended effects in the 4-day incision model were demonstrated against both Gram-positive and Gram-negative bacteria. The results support the proposed use of E-101 Solution (Exoxemis, Inc., Little Rock, AR) for the decontamination and prevention of infection in surgical and traumatic wounds.

INTRODUCTION

E-101 Solution contains 2 highly purified therapeutic enzymes, porcine myeloperoxidase (MPO) and glucose oxidase (GO) from *Aspergillus niger*, and is prepared from 2 different aqueous solutions, an enzyme solution and a substrate solution, which are packaged in separate vials. The enzyme solution contains the enzymes MPO and GO, with selected amino acids formulated in a phosphate buffer. The substrate solution contains glucose (dextrose, USP) in the same phosphate buffer. The enzyme solution and substrate solution are combined in appropriate proportions prior to use. The objective of these studies was to demonstrate the effectiveness of E-101 in rat localized wound infection models against relevant microorganisms involved in skin and soft tissue infections.

E-101 solution contains MPO and GO, but for ease of presentation for this poster, only the MPO content is provided; the MPO to GO ratio was the same in all experiments. Additional information describing the potent and broad-spectrum in vitro activity, in vivo activity, and mode of action of E-101 can be found in posters C1-3844 and F1-3957.[1,2]

METHODS I

A) Rat full-thickness excision model. Wound sites were prepared on the back of each anesthetized rat by exposing ~2 cm^2 of fascia (see Figure 1). Three adult male Sprague-Dawley rats with 2 wound sites were used for each treatment group. A polystyrene cylinder was glued to the skin around each excised site as described by Breuing et al[3] and Saymen.[4] The exposed fascia was inoculated with a 100-µL suspension of 10^7 CFU/mL of either *S. aureus* ATCC 6538 (MSSA), *S. aureus* R136 (MRSA), or *E. coli* ATCC 25922. After 15 minutes, the wounds were treated with 800 µL of E-101 (18.75, 37.5, 75, or 150 GU MPO/mL) or with sterile saline (recovery controls). Both wound sites on a single rat received the identical treatment. Following treatment at 5, 15, 30, and 60 minutes, a catalase solution was added to neutralize E-101 treatment. The liquid in the cylinder was recovered and the underlying fascia was aseptically excised, weighed, and homogenized. Quantitative cultures were prepared on tryptic soy agar, incubated overnight at 35°C, and colonies were counted to determine organism survival. Treatment performance was calculated as the sum counts from the liquid and tissue for each wound, and reported as the average of all samples in each treatment group.

METHODS II

B) Rat deep thigh incision model 1. Each wound site consisted of a deep 3-cm-long incision in the thigh (see Figure 2). Two wounds were created in each of 20 rats; 1 leg was treated with E-101 and the other with saline. Ten animals were treated once with 2.5 mL per wound, and 10 animals were treated twice, 15 minutes apart, with 10 mL per wound. Four additional rats with 2 wound sites each were used as untreated controls. Each wound was inoculated with 100 µl of a 10^9 CFU/mL suspension of *S. aureus* R136 (MRSA). Wounds were treated, using a syringe with a gavage needle, 1 hour after inoculation. E-101 treatments of 2.5 mL contained MPO at 75 GU MPO/mL, and 10-mL treatments contained MPO at 300 GU/mL. Treated wounds were closed with 2 skin clips after the second treatment and untreated controls were closed following bacterial inoculation. After 4 days, wounds were evaluated and assigned an infectivity score. Infectivity scores were assigned by measuring the area of induration, and the presence or absence of purulence was recorded.

C) Rat deep thigh incision model 2. The incision model described in model 1 (above) was modified by using 4 animals per treatment group and a single leg per animal. Wounds were inoculated with 100 µl of a 10^8 CFU/mL suspension of *P. aeruginosa* ATCC 27317. The model was modified due to the propensity of *P. aeruginosa* to become systemic. One hour after inoculation, wounds were treated twice, 15 minutes apart with 5 mL of E-101 at 300 GU MPO/mL or saline. Quantitative wound cultures were also performed on tissue samples.

Data analysis. Mean Log_{10} (CFU+1) survivors were compared using ANOVA. Confidence intervals were calculated from pooled estimates of standard error.

Figure 1. Full-thickness excision is prepared by lifting loose skin and excising a ~2-cm^2 elliptical area, exposing fascia.

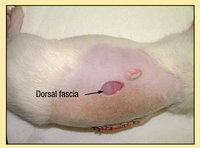
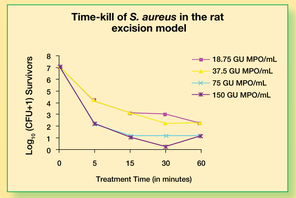


Figure 2. Deep thigh incision 3 cm in length through the gluteal muscles and down to the level of the femur.

RESULTS I

Figure 3. E-101 Solution exhibited rapid bactericidal activity within minutes on contaminated fascia tissue. Time-kill studies showed a concentration- and time-dependent activity against *S. aureus* and *E. coli*. E-101 Solution (150 GU MPO/mL) caused a 5 Log_{10} kill against both organisms at 5 minutes.



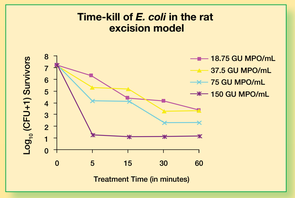
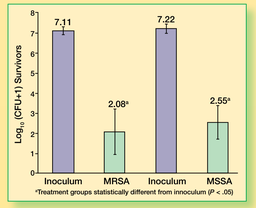


Figure 4. No significant difference in E-101 activity was seen against MRSA and MSSA in the rat excision model. The graph presents the mean Log_{10} (CFU+1) survivors isolated 15 minutes after treatment with 150 GU MPO/mL.



RESULTS II

Table 1. Infectivity scores and purulence were obtained 4 days after MRSA inoculation and treatment with E-101 or saline, or no treatment. E-101 resulted in significantly better infectivity scores compared to saline irrigation and untreated controls.

Rat deep thigh incision model: MRSA			
Treatment	Number[a]	Infectivity Score[b]	Purulence
Untreated	8	3.4	5/8
Saline 2 x 10 mL	10	2.3	5/10
Saline 1 x 2.5 mL	10	2.7[c,d]	1/10
E-101 300 GU MPO/mL 2 x 10 mL	10	1.4[c,d]	0/10
E-101 75 GU MPO/mL 1 x 2.5 mL	10	1.3[c,d]	1/10

[a]Number of legs per treatment group.
[b]Based on area of induration in mm^2 (1 = <100, 2 = 100-200, 3 = 200-300, 4 = >300)
[c]Treatment groups statistically different from from infection control ($P < .05$)
[d]Treatment groups statistically different from saline ($P < .05$)

Table 2. Infectivity scores and purulence were obtained 4 days after *P. aeruginosa* inoculation and treatment with E-101 or saline, or no treatment. E-101 resulted in significantly better infectivity scores compared to saline irrigation and untreated controls.

Rat deep thigh incision model: *P. aeruginosa*			
Treatment	Number[a]	Infectivity Score[b]	Purulence
Untreated	4	4.0	2/4
Saline 2 x 5 mL	4	4.0	1/4
E-101 300 GU MPO/mL 2 x 10 mL	4	1.5[c,d]	0/4

[a]Number of legs per treatment group.
[b]Based on area of induration in mm^2 (1 = <100, 2 = 100-200, 3 = 200-300, 4 = >300)
[c]Treatment groups statistically different from from infection control ($P < .05$)
[d]Treatment groups statistically different from saline ($P < .05$)

CONCLUSIONS

- E-101 Solution, a potent and broad-spectrum therapeutic enzyme-containing solution, effectively reduced infection against Gram Positive and Gram Negative organisms in all 3 animal models.
- In the full-thickness excision model, the activity of E-101 Solution is concentration- and time-dependent.
- In the deep thigh incision models, E-101 Solution is effective in reducing the bacterial load.
- E-101 Solution is equally effective against organism strains that are resistant or susceptible to standard antibiotics.
- These data support the safety and efficacy evaluation of E-101 Solution in current ongoing clinical trials.

REFERENCES

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The authors recognize John Davis, Kathy Bell, Sherry Parker, and Ann O'Leary (Ricerca Biosciences, LLC, Concord, OH) for their technical assistance.