E-101 Solution Effectively Reduces *Pseudomonas aeruginosa* in a Rat Heat-Induced Necrosis Model

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ABSTRACT

Background: E-101 Solution (E-101) is a novel drug product containing porcine myeloperoxidase (MPO) and glucose oxidase (GO) that relies on the local production of singlet oxygen for microbicidal activity and represents a new class of broad-spectrum antimicrobial for the prevention and treatment of localized infections. E-101 has potent and broad-spectrum bactericidal activity in vitro. In this study, the efficacy of E-101 was determined in a quantitative heat-induced necrosis (HIN) model against *Pseudomonas aeruginosa,* a major cause of infection in burn patients that is associated with increased morbidity and mortality.

Methods: Rats were anesthetized and their backs shaved. Wounds were created on the dorsum by exposing a 10-cm x 4-cm area to 100°C water for 10 seconds, creating a full-thickness burn covering approximately 20% of the total body surface. Sixty minutes later, the burn area was inoculated with 1 mL containing 7.5 Log_{10} CFU of *P. aeruginosa* ATCC 27317. Sixty minutes after colonization, E-101 was administered with a fine mist spray and swabbed into the wound. At designated time points, the wounds were excised and homogenized in sterile saline, and viable bacteria were quantified by plating dilutions of the homogenates on blood agar.

Results: Bactericidal activity of E-101 was concentration- and time-dependent. For example, after treatment with 3 mL of E-101 containing 150 guaiacol units (GU) MPO/mL, the Log_{10} reduction in CFU was 1.2 Log_{10} at 15 minutes, 2.5 Log_{10} at 30 minutes, and 3.0 Log_{10} at 4 hours compared to untreated controls. The 4-hour data represent an average of less than 150 CFU recovered from the wound. Treatment using 3 mL of E-101 containing 300 GU MPO/mL resulted in a Log_{10} reduction in CFU of 2.1 Log_{10} at 15 minutes and 3.2 Log_{10} at 30 minutes compared to untreated controls.

Conclusion: E-101 Solution was effective in rapidly reducing the bacterial burden in a rat burn model providing a high level of control of organisms for a 4-hour test period.

INTRODUCTION

A heat-induced necrosis (HIN) model was adapted based on the Walker-Mason Burn Model[1,2] to evaluate E-101 Solution for the control and management of contaminated burns. Experimental burn wounds were produced by scalding approximately 20% of the body surface area on the backs of anesthetized rats, creating a full-thickness burn, and subsequently inoculating the wound with a clinical strain of *P. aeruginosa* isolated from a burn patient. 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 amino acids, in a phosphate buffer. The substrate solution contains glucose (dextrose, USP) in the same aqueous vehicle as the enzyme solution. The enzyme solution and substrate solution are combined in appropriate proportions prior to use. The mechanism of action of E-101 is shown in Figure 1 and includes the selective binding of MPO to bacteria, like *P. aeruginosa*, and the generation of short-lived, highly reactive singlet oxygen at the bacterial cell wall. The hydrogen peroxide and hypochlorous acid produced is sufficient for singlet oxygen generation, but not substantial enough to cause host tissue toxicity. In this study, the amount of bacterial burden in contaminated burns was followed for 4 hours after treatment with E-101 to determine its potential to control infection. 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 and in vivo activity of E-101 can be found in posters C1-3844[3] and F1-3956.[4]

METHODS

Animals and burned (HIN) animal model
Adult male Sprague-Dawley rats (~250 g body weight) were used (Charles River Laboratories, Portage, MI). The animals were kept under conventional housing conditions. The animals were anesthetized with isoflurane and their backs shaved. Wounds were created on the dorsum by exposing a 10-cm x 4-cm area to 100°C water for 10 seconds, creating a full-thickness burn, covering about 20% of the total body surface (Figure 2). Animals received buprenorphine (0.05-0.25 mg/kg IP) for postoperative pain management. After scalding, the animals were returned to their cages for 1 hour.

Wound inoculation
Sixty minutes after scalding, wounds were inoculated and infection was initiated by spreading 1 mL of a suspension containing 7.5 Log_{10} CFU/mL of *P. aeruginosa* ATCC 27317 onto the burned surface using a pipette and swab technique. The inoculum was allowed to remain on the wound for an additional 1 hour before treatment. An inoculum recovery control group was included at 75 and 300 minutes postinoculation with no treatment applied.

Wound treatment
Sixty minutes after inoculation, E-101 at 2 concentrations (150 GU MPO/mL and 300 GU MPO/mL) was applied using a spray and swab technique. At 150 GU MPO/mL, rats were treated for 15, 30, and 240 minutes. At 300 GU MPO/mL, rats were treated for 15 and 30 minutes. At the designated times, the treatment area was then sprayed with 1% catalase to stop further microbicidal activity. Compared to inoculum recovery, untreated recovery controls at 15 and 240 minutes only were included for comparison.

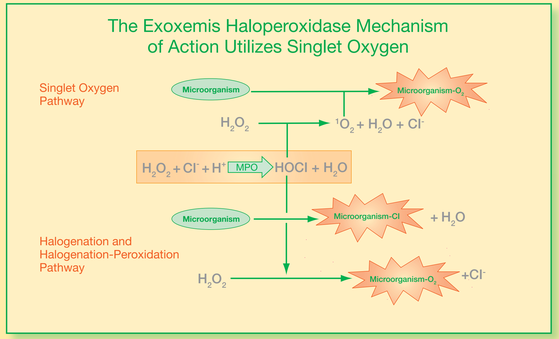
Quantitative culture
At the designated time, the rats were euthanized, the wounds excised, and homogenates prepared in sterile saline. Serial 10-fold dilutions of homogenates were plated on trypticase soy agar and incubated overnight at 35°C. Total survivors in the tissue samples were determined based on tissue weights and plate counts.

Statistical analysis
Analyses were conducted using SAS PROC MIXED and limited to comparisons within an experimental day. Upper and lower 95% confidence intervals were calculated along with least-square mean Log_{10} (CFU+1) survivors. Significance thresholds for differences were set at $P < .05$.

Figure 2. HIN model: full-thickness burn on shaved dorsum

MECHANISM OF ACTION

Figure 1. Hydrogen peroxide is produced in situ through a glucose-glucose oxidase reaction. The myeloperoxidase-catalyzed oxidation of chloride ion by hydrogen peroxide generates hypochlorous acid. Once hypochlorous acid is generated, it further participates in nonenzymatic reactions, leading to either the direct halogenation of target cell components or reaction with a second hydrogen peroxide to yield singlet oxygen. Singlet oxygen is a potent electrophilic oxygenating agent, capable of reacting with a broad spectrum of electron-rich compounds.



RESULTS

Table 1. In vivo microbicidal activity of E-101 against *P. aeruginosa* in the Quantitative HIN model

Treatment Time (Number of rats)	Untreated[a]	E-101 150 GU MPO/mL		E-101 300 GU MPO/mL	
	Total Log Survivors	Total Log Survivors	Total % Kill[b]	Total Log Survivors[a]	Total % Kill[b]
15 minutes (3;6)[c]	6.23[d]	5.09[d,e]	99.52559	4.09[d,e]	99.95265
30 minutes (6;3)[c]	—[f]	3.77[d]	99.97714	3.03[d]	99.99588
240 minutes (6;3)[c]	5.17[d]	2.17[d,e]	99.99943	NT	NT

GU = guaiacol units, NT = not tested
[a]Recoveries were calculated from 9 rats at 15 minutes and 8 rats at 240 minutes.
[b]Total % kill calculated based on a 7.4 Log_{10} inoculum of *P. aeruginosa* ATCC 27317.
[c]First number tested with 150 GU MPO/mL; second number treated with 300 GU MPO/mL.
[d]Difference from inoculum is significant at $P < .05$.
[e]Difference from recovery control is significant at $P < .05$
[f]No recovery controls were measured at 30 minutes.

RESULTS (cont)

- The results from two E-101 treatments are summarized in Table 1. The number of viable organisms (survivors) after a single application decreased with both E-101 concentration and treatment time.
- Treatment with 3 mL of E-101 150 GU MPO/mL resulted in a Log_{10} reduction in CFU of 1.2 Log_{10} at 15 minutes, 2.5 Log_{10} at 30 minutes, and 3.0 Log_{10} at 4 hours compared to untreated controls. The 4-hour data represent an average of <150 CFU recovered from the wound.
- Treatment with 3 mL of E-101 300 GU MPO/mL resulted in a Log_{10} reduction in CFU of 2.1 Log_{10} at 15 minutes and 3.2 Log_{10} at 30 minutes compared to untreated controls.
- E-101 treatments at 15 and 240 minutes were statistically superior to the untreated recovery controls.

CONCLUSIONS

- E-101 Solution rapidly reduces the bacterial burden in a quantitative rat heat-induced necrosis model and provides a highly effective level of control of *P. aeruginosa* for up to 4 hours.
- E-101 Solution demonstrates significant concentration- and time-dependent bactericidal activity.
- These preclinical data support the use of E-101 Solution for the potential treatment and management of contaminated burns in humans.

REFERENCES

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