E-101 solution, a novel myeloperoxidase mediated topical antimicrobial, demonstrates in vivo efficacy in whole animal models of surgical infection prevention


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Abbreviations:
E-101: E-101 solution, MPO: Myeloperoxidase, SSIs: Surgical site infections

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Abstract
There is an unmet need for new and effective antiseptics to reduce the risk of surgical site infections (SSIs). E-101 solution (E-101) represents the first-in-class topical myeloperoxidase-based microbicidal formulation applied directly into surgical wounds to prevent SSIs. Three infection prevention wound models in rats were used to assess the efficacy of E-101. Topical application of E-101 at 150 GU p-MPO/ml into full thickness rat excision wounds rapidly killed greater than 10^7 CFU/ml of Staphylococcus aureus and Escherichia coli after 15 min and 5 min, respectively. Time-kill was concentration-dependent. In the partial thickness wound model, application of E-101 at 150 GU p-MPO/ml resulted in a 2 log reduction of methicillin-resistant S. aureus (MRSA) within 3 hours. Single treatment with E-101 at 75, 150, and 300 GU p-MPO/ml showed statistically significant dose responses at 24 hours post-inoculation and treatment. E-101 at 300 GU p-MPO/ml produced a sustained antimicrobial effect against MRSA and Pseudomonas aeruginosa after four days post-inoculation and treatment in the deep thigh incision model. These results support the use of E-101 as a topical antimicrobial agent for direct wound site application. No other antiseptic can make this claim. E-101 should prove valuable in reducing the rate of SSIs.

Citation:
al., 1999; Miyasaki et al., 1987; Selvaraj et al., 1978; Winterbourn, 2002; Allen, 1994). Preliminary ultrastructural studies correlated with microbiology time kill assays (Denys et al., 2011) demonstrated that E-101 killed all microbes tested within minutes of direct exposure, and that this microbicidal action occurred prior to any observable ultrastructural disruption of the microbial plasma membrane suggesting oxidative inhibition of vital microbial membrane enzyme systems.

**Figure 1:** Mechanism of action of E 101 generating singlet oxygen (\(^{1}O_2\))*. Hydrogen peroxide (H\(_2\)O\(_2\)) is produced *in situ* through a glucose oxidase reaction. The myeloperoxidase catalyzed oxidation of chloride ion (Cl\(^-\)) by H\(_2\)O\(_2\) generates hypochlorous acid (HOCI). Once generated, HOCI reacts in a diffusion controlled reaction with a second H\(_2\)O\(_2\) molecule to yield \(^{1}O_2\). Singlet oxygen is a potent electrophilic oxygenating agent capable of reacting with a broad spectrum of electron rich compounds. Its microsecond lifetime restricts reactivity to within a 0.2 microns radius of its point of generation.

1.2 Clinical uses and biological activities

E-101 is currently undergoing clinical testing for the prevention of surgical site infections (SSIs). E-101 generates the reactive species; hydrogen peroxide (H\(_2\)O\(_2\)), hypochlorous acid (HOCI), and singlet oxygen (\(^{1}O_2\)) where topically applied. Although H\(_2\)O\(_2\) -dependent p-MPO microbicidal action is decreased at blood pH, inhibited by plasma ceruloplasmin, and competitively inhibited by erythrocyte catalase and hemoglobin, the formulation is active when applied directly to the surgical incisional wound (Allen, 1975; Segelmark et al., 1997). E-101 is effective even in bloody (e.g., Hematocrit as high as 24%), contaminated, viscous, and acidic wound environments. E-101 is primarily intended to be used as a topical supplement to current standard-of-care measures including the use of prophylactic antibiotics and is not intended for parenteral administration. E-101 formulation is expected to be effective against any kind of epithelial infection that can be topically contacted (e.g., skin, eyes, ear canals, vagina, bronchial tree, and bladder).

2. Objective of Research

Improved antiseptics for wound management are needed. At microbicidal strength, the topical antiseptics on the market are associated with untoward local tissue injury and impairment of wound healing when applied directly into a clean or infected wound. The most frequent causative pathogens of SSIs, including aerobic Gram positive (e.g., staphylococci, streptococci, and enterococci) and/ or aerobic Gram negative (e.g., Enterobacteriaceae [Escherichia coli] and non-Enterobacteriaceae (Pseudomonas aeruginosa) bacteria (Engemann et al., 2003; Kirkland et al., 1999; Whitehouse et al., 2002). Despite significant improvement in infection control practices (e.g., prophylactic systemic antibiotics), prevention of SSIs remains an important medical need for certain high-risk surgical patient populations, including colorectal surgery patients, transplant patients, and patients receiving implant devices. There is an unmet need for a safe and effective antiseptics to reduce the incidence of SSIs. This work was undertaken to demonstrate the antimicrobial potential of E-101 in established surgical wound rat models. These whole animal bacterial infection prevention models are relevant to E-101 intended use for direct application into wounds.

3. Experimental

3.1 Preparation of E-101 solution

Different concentrations of E-101 were tested for their antimicrobial effects in whole animal infection prevention wound models. E-101 is packaged as two vials. Vial #1 (enzyme solution) contains porcine myeloperoxidase (pMPO), glucose oxidase (GO) derived from Aspergillus niger, specific amino acids (viz., L-alanine, L-proline, and glycine) in an aqueous vehicle. The aqueous vehicle is clear and consists of sodium chloride, polysorbate 80 (Tween-80®, Corda International PLC) and sodium phosphate buffer in Water for Injection (WFI). The concentration of pMPO in vial #1 is expressed as Guaiacol Units per millilitre (GU/ml). A GU is the amount of myeloperoxidase enzyme that catalyses the conversion of one micromole of H\(_2\)O\(_2\) per minute at 25° C (Chance and Maehly, 1972).
The relationship of GU activity to the weight of purified p-MPO is 375 GU/mg. The amount of GO in vial #1 is expressed in Units per milliliter (U/ml). One U is the amount of GO enzyme that catalyses the oxidation of one micromole of glucose (producing one micromole of H₂O₂) per minute at 25°C. The ratio of p-MPO: GO in vial #1 is fixed at 5.6:1 ratio, based on enzymatic activity. As such, only GU/ml concentration is used to express the amount of E-101 tested. Vial #2 (substrate solution) contains a clear solution of 300 mM glucose (dextrose, USP) in the identical aqueous vehicle as the enzyme solution. The two vials are mixed together in equal volumes, thereby, creating and activating E-101. Once activated, E-101 retains its antimicrobial activity up to 6 h when stored at 5-8°C and 90 min at room temperature.

3.2 Experimental animals tested
Adult male Sprague-Dawley rats (approximately 250 grams) obtained from Charles River Laboratories, Portage, Michigan were used in all studies. Animals were housed in individual cages and given food and water ad libitum throughout the study. Prior to experimental wounding, the hair of the relevant target site was shaved with electric clippers. All animals were anesthetized using isoflurane (1-5% in O₂) and 10 strong passes. This produced a late log growth suspension, and colonies were counted to determine organism survival. Thirty-six rats with two wounds each, three rats for each treatment group and treatment time, were used in this experiment. Treatment performances were calculated as the surviving bacterial counts from the recovered liquid and tissue homogenate for each wound (log₁₀ CFU+1). CFU +1 represents the lowest level of detection being 1 CFU/ml.

3.3 Determination of antimicrobial activity
3.3.1 Full thickness excision wound model used
Full thickness experimental wounds were produced by a modification of the method reported by Saymen et al (1972). Staphylococcus aureus ATCC 6538 (MSSA) and E. coli ATCC 25922 were used as challenge organisms. The bacteria grown in Trypticase Soy Broth (TSB) were prepared as a late log growth suspension, and harvested from a shake flask, centrifuged and resuspended in buffered saline to yield a 10⁹ CFU/ml suspension. The stock was subsequently diluted in buffer to give a working suspension of approximately 10⁷.5 CFU/ml and used as the inoculum. Wound sites were prepared on the back of each rat by lifting loose skin and excising an elliptical area of skin with scissors using sterile technique and exposing approximately 1 x 1.5 cm of fascia. An open 2.5 cm diameter polystyrene cylinder was glued to the skin around each excised site with Quick Tite® (Loctite Corp.) cement as described by Breuing, et. al. (2003). 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 10⁷ CFU of the bacterial suspension. This volume of inoculum was sufficient to completely cover the exposed fascia without excessive pooling. After application, the inoculum was allowed to remain on the fascia for 15 minutes before treatment. A volume of 800 μL of E-101 (18.75, 75, or 150 GU p-MPC/ml) was added to the site resulting in a total volume of 1 ml per test site. Control sites, where 800 μL of 0.9% sterile saline (recovery control) was added, had no E-101 administered. Both wound sites on a single rat received the identical treatment. Following a 5, 15, 30, or 60-minute treatment time with E-101, 100 μL of a 10% solution of catalase was added to each site to neutralize any remaining generated hydrogen peroxide, thereby immediately inhibiting further microbicidal activity of E-101. The liquid in the cylinder was recovered and the underlying fascia aseptically excised, weighed, and homogenized. Quantitative cultures of liquid sample and tissue homogenate using serial 10-fold dilutions were prepared on Trypticase Soy Agar (TSA), incubated at 35°C overnight, and colonies were counted to determine organism survival. Thirty-six rats with two wounds each, three rats for each treatment group and treatment time, were used in this experiment. Treatment performances were calculated as the surviving bacterial counts from the recovered liquid and tissue homogenate for each wound (log₁₀ CFU+1). CFU +1 represents the lowest level of detection being 1 CFU/ml.

3.3.2 Partial thickness wound model used
Staphylococcus aureus ATCC 6538 was used as the challenge organism for partial thickness wounds. The inoculum was prepared as described above for the full-thickness excision wound. Experimental wounds consisted of two 10 mm x 7 mm sites midline on the back of each rat, one site being forward near the shoulders and one site being caudal. The wounds were achieved by controlled abrasion of the skin. The skin was pinched using fingers to form a fold and rubbed with a grater using 10 strong passes. This produced a
wound with a slight depression (i.e., about 1/3 to 1/2 the thickness of the skin) with some minor bleeding, which was blotted dry. The test animals were organized into 4 to 5 treatment groups per experiment with each treatment administered to 3 rats. Each of the rats had two wounds giving 6 wound sites per experimental group. Twenty-five microliters of a 10⁶ CFU/ml inoculum was dispensed in the center of the exposed wound and rubbed for 10 seconds over the entire wound area using a sterile polypropylene spatula. In a preliminary experiment, treatment with E-101 at 150 GU/ml was compared to a saline placebo group to determine the inhibitory effect of a single E-101 treatment applied after 3 hours post-inoculation. Twenty minutes after application of the inoculum, 1 ml of either E-101 or saline (placebo) was applied with a cotton swab for 30 seconds on the wound site. A saturated gauze containing 3 ml of E-101 (75, 150, or 300 GU p-MPO/ml) or saline (placebo) was then applied to each wound and the wound covered with Tegaderm. All treated sites were harvested at 3 or 24 hours post-inoculation and cultured for viable organisms. The Tegaderm and gauze were removed and the entire wound was excised down to the fascia, placed in a tared sterile tube, weighed, and 1 ml of sterile cold 0.9% saline added to each tube. The tissue was then homogenized and surviving bacterial counts in the recovered tissue homogenate for each site were assessed by quantitative culture using serial 10-fold dilutions, plated on Trypticase Soy Agar (TSA) and incubated at 37° C overnight. The Tegaderm and gauze were removed and the entire wound was excised down to the fascia, placed in a tared sterile tube, weighed and 1 ml of sterile cold 0.9% saline added to each tube. The tissue was then homogenized and surviving bacterial counts in the recovered tissue homogenate for each site were assessed by quantitative culture using serial 10-fold dilutions, plated on Trypticase Soy Agar (TSA) and incubated at 37° C overnight. The outcome of interest for the full and partial thickness wound models was the surviving bacteria collected and counted from the recovered liquid and tissue homogenate for each wound (log₁₀ (CFU+1)).

3.3.3 Deep thigh incision wound model used

For deep thigh incision wounds S. aureus R136 (MRSA) was used as the challenge organism and the inoculum was prepared as described above. Wounds consisted of one incision in both thighs of each rat. The sites were delineated by marking the spine, the greater trochanter, and the knee. On each leg, a line from the knee through the greater trochanter, toward the spine was traced. The skin was then homogenized and surviving bacterial counts in the recovered tissue homogenate for each site were assessed by quantitative culture using serial 10-fold dilutions, plated on Trypticase Soy Agar (TSA) and incubated at 37° C overnight. Treatment outcomes are reported as the surviving bacteria from the recovered tissue sample for each treatment group (log₁₀ (CFU+1)).

3.4 Statistical analyses conducted

The outcome of interest for the full and partial thickness wound models was the surviving bacteria collected and counted from the recovered liquid and tissue homogenate for each wound (log₁₀ (CFU+1)). In the full thickness wound model the means of the log₁₀ (CFU+1) results for the two wounds per rat were computed and then averaged across the three rats assigned the same treatment and time of catalase application per challenge organism. Means, standard errors, and 95% confidence intervals were computed to compare log₁₀ (CFU+1) between treatments and challenge organisms.

In the partial thickness model, mean log₁₀ (CFU+1) results and 95% confidence intervals were computed using mixed models with random effects to account for correlation between the measurements for two wounds on the same rat. T-tests were performed to test...
for associations between wounds treated with E-101 and saline (placebo). Separate models were applied for different dosing regimens of E-101 and saline and time post-inoculation of wound evaluation.

The outcome of interest for the deep thigh wound model for both challenge organisms was wound area of induration (wound area). For the S. aureus experiment, mean and standard deviation of wound area were computed for wounds treated with E-101 and placebo using mixed models with random effects to account for correlation between the two wounds on each rat. Models were adjusted for the assigned treatment leg in order to account for any differences between legs. T-tests were performed to test for associations between wound areas and treatment.

For the P. aeruginosa experiment, mean and standard deviation of wound area were computed for wounds treated with E-101 and placebo. T-tests were performed to test for associations between wound area and treatment.

All statistical analyses were performed using SAS software, Version 9.2 of the System for Windows (SAS, SAS Institute Incl, Cary, NC). Rats that were untreated with either E-101 or saline or that expired prior to wound evaluation (1 rat in deep thigh incision model treated with 10 ml E-101) were excluded from the statistical models. All hypothesis tests were performed at the 0.05 significance level.

4. Results and discussion

4.1 Antimicrobial activity

4.1.1 Antimicrobial activity in full thickness excision wound model

E-101 exhibited effective microbicidal activity against S. aureus in a concentration (i.e., 18.75, 75, and 150 GU p-MPO/ ml) and time dependent fashion in the full thickness excision model. As shown in Fig. 2a, E-101 solution containing 75 GU p-MPO/ ml and 150 GU p-MPO/ ml yielded nearly complete kill of bacteria.
the inoculum of *S. aureus* ATCC 6538 (containing ~10^7 CFU) within 15 minutes. Treatment with E-101 solution at both concentrations resulted in a greater than 4 log_{10} CFU reduction within 5 minutes. When the concentration was decreased to 18.75 GU p-MPO/ml, an approximate 3 log_{10} CFU reduction was still achieved within 5 minutes. The log number of CFU per wound recovered from the E-101 treated wounds was statistically different (p< 0.05) from both the inoculum (7.3 log_{10}) and untreated saline controls (7.25 log_{10}) for all of the concentrations of E-101 and all time-points tested. The effect of E-101 treatment on wounds inoculated with *E. coli* ATCC 25922 is shown in Fig. 2b. As observed in the *S. aureus* model, the *E. coli* CFU counts decreased rapidly within 5 minutes after treatment with E-101. Near complete kill of the inoculum (7.2 log_{10}) was observed within 5 minutes in wounds treated with E-101 containing 150 GU p-MPO/ml. These results confirmed the rapid, concentration- and time dependent treatment activity of E-101 against *S. aureus* and *E. coli* in an environment comprising tissue and wound exudates.

### 3.1.2 Antimicrobial activity in partial thickness wound model

The extent of E-101 activity was assessed at 3 hours and at 24 hours post-treatment in the partial thickness wound model and the findings are presented in Table 1. In the first 3 hour experiment, after a single treatment of E-101 at 150 GU p-MPO/ml into the experimental infected wound, the mean number of organisms isolated from the wounds was approximately 2.0 log_{10} CFU at 3 hours. This represented a significant reduction compared to wounds treated with the placebo saline solution (Mean log_{10} CFU equals 4.2, p<0.0001).

Table 1: *In vivo* activity of E-101 at 3 hours post-inoculation and treatment against *S. aureus* ATCC 6538 in the partial thickness wound model

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>No. Rats</th>
<th>No. Wounds</th>
<th>Mean Log_{10} Survivors</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-101 (150 GU p-MPO/ml)</td>
<td>3</td>
<td>6</td>
<td>2.04</td>
<td>1.84-2.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Placebo (Saline)</td>
<td>3</td>
<td>6</td>
<td>4.20</td>
<td>3.99-4.40</td>
<td>--</td>
</tr>
</tbody>
</table>

In the second experiment, single treatments with E-101 containing p-MPO at 75, 150, and 300 GU p-MPO/ml were applied into the experimental infected wounds and the wounds were examined to determine dose response at 24 hours compared to the placebo control. E-101 treatment at 75 GU p-MPO/ml reduced the number of organisms recovered from 6.2 log_{10} CFU (placebo saline) to 5.3 log_{10} CFU (p=0.0719). After a single E-101 treatment at 150 GU p-MPO/ml, the number of organisms recovered from the experimental infected wounds was 0.5 log_{10} CFU less than the placebo control (p=0.0352). Experimental infected wounds treated with E-101 at 300 GU p-MPO/ml contained 4.4 log_{10} CFU, significantly fewer than the placebo group (p=0.0028).

Table 2: *In vivo* dose response of E-101 at 24 hours post-inoculation and treatment against *S. aureus* ATCC 6538 in the partial thickness wound model

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>No. Rats</th>
<th>No. Wounds</th>
<th>Mean Log_{10} Survivors</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-101 (75 GU p-MPO/ml)</td>
<td>3</td>
<td>6</td>
<td>5.31</td>
<td>4.59-6.02</td>
<td>0.0719</td>
</tr>
<tr>
<td>Placebo (Saline)</td>
<td>3</td>
<td>6</td>
<td>6.21</td>
<td>5.50-6.93</td>
<td>--</td>
</tr>
<tr>
<td>E-101 (150 GU p-MPO/ml)</td>
<td>3</td>
<td>6</td>
<td>5.73</td>
<td>5.43-6.03</td>
<td>0.0352</td>
</tr>
<tr>
<td>Placebo (Saline)</td>
<td>3</td>
<td>6</td>
<td>6.21</td>
<td>5.90-6.51</td>
<td>--</td>
</tr>
<tr>
<td>E-101 (300 GU p-MPO/ml)</td>
<td>3</td>
<td>6</td>
<td>4.36</td>
<td>3.64-5.07</td>
<td>0.0028</td>
</tr>
<tr>
<td>Placebo (Saline)</td>
<td>3</td>
<td>6</td>
<td>6.21</td>
<td>5.50-6.93</td>
<td>--</td>
</tr>
</tbody>
</table>

### 3.1.3 Antimicrobial activity in deep thigh incision wound models

The effect of E-101 on the progression of infection by a MRSA isolate was determined at 4 days post-inoculation in the deep thigh incision wound model (Table 3). Experimental wounds inoculated with MRSA treated twice with 10 ml of E-101 at 300 GU p-MPO/ml had significantly smaller mean wound areas (Mean: 97.80, SD: 11.8) than wounds treated with the placebo (Mean: 200.1, SD: 16.1; p<0.0001), as did experimental wounds treated once with 2.5 ml of E-101 at 75 GU p-MPO/ml (E-101 Mean (SD): 80.2(22.3), Saline Mean (SD): 227.8(22.3); p<0.0001). There was no statistically significant difference between the mean wound sizes of the two E-101 treatment volumes (p=0.4902). Against the progression of infection by *P. aeruginosa* R463 (Table 3), the mean E-101 (300 GU p-
MPO/ml wound area was 85.75 (SD: 57.4), significantly smaller than the mean saline wound area of 681.8 (SD: 344.2; p=0.0386).

Table 3: In vivo effect of E-101 on the progression of infection by MRSA or P. aeruginosa at 4 days post-inoculation and treatment in the deep thigh incision wound model

<table>
<thead>
<tr>
<th>Treatment volume (E-101)</th>
<th>E-101 solution</th>
<th>Placebo</th>
<th>E-101 vs Saline p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Wounds</td>
<td>Mean Wound Area1 (SD)</td>
<td>No. Wounds</td>
</tr>
<tr>
<td>MRSA: 1 x 2.5 ml (75 GU p-MPO/ml)</td>
<td>10</td>
<td>80.20 (22.34)</td>
<td>10</td>
</tr>
<tr>
<td>MRSA: 2 x 10 ml (300 GU p-MPO/ml)</td>
<td>39</td>
<td>97.80 (11.76)</td>
<td>19</td>
</tr>
<tr>
<td>P. aeruginosa: 2 x 5 ml (300 GU p-MPO/ml)</td>
<td>4</td>
<td>85.75 (57.44)</td>
<td>4</td>
</tr>
</tbody>
</table>

Treatment of experimental infected wounds with E-101 effectively reduced Gram-positive and Gram-negative infections in all three animal models. In the full-thickness excision model, E-101 demonstrated dose- and time-dependent activity against S. aureus and E. coli wound infections. The microbicidal effect of E-101 was rapid e.g. within minutes. The partial thickness wound model demonstrated a dose response for E-101 against S. aureus that remained in effect even 24 hours after treatment. The reduction in the level of wound contamination after a single application of E-101 was significant 24 hours post-treatment. Notably, in the deep thigh incision model the inoculum of MRSA was 1.5 log_{10} CFU higher than that used in the full-thickness excision model. E-101 was effective in reducing the progression of infection of both MRSA and P. aeruginosa, providing sustained activity in a closed wound environment.

Haloperoxidase/ hydrogen peroxide/halide systems are microbicidal in vitro against a variety of bacteria (Olsen et al., 1985), yeast (Wright and Nelson, 1988), viruses (Belding et al., 1970), and bacterial and fungal spores (Washburn et al., 1987). Application of E-101 in the three different animal models of infection prevention described herein validates its antibacterial effects under in vivo conditions, and substantiates its previously described in vitro and in vivo potency against antibiotic susceptible and multi-drug resistant microorganisms (Denys et al., 2011) These models represent the spectrum of wounds in which E-101 might be used in humans. The selection of these models was based primarily on two major factors. First, the range in depth of the experimental wounds needed to vary from shallow to deep. Second, the post-treatment time interval needed to vary for assessing the effects of treatment. For these reasons, the full-thickness excision model was a prototype for short-duration evaluation time, and the partial thickness wound model and deep thigh incision wound model were prototype for extended-duration evaluation. Taken together, use of the three models validated the in vivo microbicidal action of E-101 using different wound scenarios and under conditions that could influence E-101 efficacy.

The demonstrated efficacy of E-101 in decreasing bacterial inoculums illustrates its potential as a topical treatment for prevention of SSIs. The distinct advantages of using E-101 are its rapid microbicidal activity, broad spectrum of activity, and safety profile. No significant toxicological damage was observed at the concentrations of E-101 used in these surgical infection prevention models (data not presented). Previous experimental studies using the partial full-thickness pig wound model confirmed that topical application of E-101 into surgical wounds between 150 p-MPO GU/ml and 600 GU p-MPO/ml did not impair subsequent wound healing based on clinical criteria, histological evaluation, and the profiles of m-RNA growth factors (Sullivan et al., 2001).

Proof that E-101 is efficacious for prevention of SSIs in human trials, would support a paradigm shift with regard to topical antisepsis. No antiseptics on the market are currently recommended for direct wound application since they impede wound healing.

Research Highlights

E-101 is a myeloperoxidase-based microbicidal formulation that effectively reduced Gram-positive and Gram-negative organisms in three infection prevention wound models in rats. These results support previously in vitro and in vivo findings (Denys et al., 2011).

In the full-thickness excision wound model, E-101 demonstrated concentration- and time-
dependent bactericidal activity. The microbicidal effect of E-101 was within minutes.

In the partial thickness wound model, a dose response for E-101 against *S. aureus* was observed that remained in effect even 24 hours after treatment.

In the deep thigh incision wound model, E-101 was effective in reducing the progression of infection of both MRSA and *P. aeruginosa*.

**Limitations**

One of the inherent limitations of our study was the short time for measuring efficacy used in the animal models described. We do not think that this diminishes the finding of this study.

**Recommendations**

Further studies are needed to determine the long term effect of E-101 treatment on infection prevention. More stringent infection models were not used in this study since the intended use of E-101 is for surgical prophylaxis and not treatment of established infections.

**Justification of Research**

Antibiotics are the principle therapeutic (prophylactic) approach for preventing infections associated with surgical procedures. The factors illustrating the need for new and different formulations to reduce the risk of SSIs include: 1) a limited number of antimicrobial classes that can be used to prevent SSIs, 2) the potential for the emergence of resistance, 3) an increasing incidence of single-drug and multi-drug-resistant microorganisms, 4) the larger number of high-risk patient undergoing surgery, and 5) a paucity of adequate intraoperative measures to disinfect surgical wound directly before closure. Myeloperoxidase-based E-101 produces oxidative microbicidal action consistent by a mechanism homologous to neutrophil leukocyte phagolysosomal killing of microbes. E-101 represents the first active myeloperoxidase-mediated drug product developed to address the need for new agents to reduce the risk of SSIs, and has been formulated for topical application as a direct microbiocide onto the surgical wound.

**Conclusion**

Treatment with E-101 resulted in significant reductions of challenge pathogens in full thickness excision wounds, partial thickness wounds, and deep thigh incision wounds using a rat model. E-101 is equally effective against both susceptible- and antibiotic resistant-bacteria and should prove beneficial in the prevention of SSIs.

**Author’s Contribution and Competing Interests**

Gerald A Denys serves as a consultant for Exoxemis, Inc. John C Davis is an employee of Ricerca Biosciences, LLC, a Clinical Research Organization (CRO). Ricerca Biosciences LLC was paid by Exoxemis, Inc. for access to its animals, research facilities, and equipment for this study. Peter D. O’Hanley serves as Executive Vice-President of Clinical Development and Regulatory Affairs of Exoxemis, Inc. Jackson T Stephens, Jr. serves as President and CEO of Exoxemis, Inc.

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