

ABSTRACT

**Background:** E-101 solution (E-101) is a novel cell-free myeloperoxidase-mediated antimicrobial developed for topical application directly into surgical wounds. It is composed of 1) porcine myeloperoxidase (pMPO) and glucose oxidase (GO), 2) glucose, 3) sodium chloride, and 4) specific amino acids. Once activated, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is produced *in situ* by GO dehydrogenation of glucose and reduction of oxygen. The pMPO-catalyzed oxidation of chloride by H<sub>2</sub>O<sub>2</sub> generates hypochlorous acid (HOCl). Once generated, HOCl reacts in a diffusion-controlled reaction with a second H<sub>2</sub>O<sub>2</sub> molecule to yield singlet oxygen. The *in vitro* fungicidal activity of E-101 against *Candida* spp and other yeast-like organisms was investigated.

**Methods:** Ten clinical isolates of *Candida* spp. (*C. albicans*-1, *C. dubliniensis*-1, *C. glabrata*-2, *C. guilliermondii*-1, *C. krusei*-1, *C. parapsilosis*-2, *C. tropicalis*-2), *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* 6258, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* were evaluated. Time kill studies were performed using a modified suspension-neutralization method. Two concentrations of E-101 (150 and 300 GU pMPO/ml), were tested and represent the proposed therapeutic doses for a phase 3 clinical study.

**Results:** E-101 was highly active against all *Candida* spp, *C. neoformans* and *S. cerevisiae* isolates. E-101 exhibited time-dependant *in vitro* activity. When the results of all 13 *Candida* spp were pooled, approximately 0.5 log<sub>10</sub> reductions in CFUs were observed within 30 min with 1.5 to 2.0 log<sub>10</sub> reductions in CFUs after 60 min at 150 and 300 GU pMPO/ml. Greater than 6.0 log<sub>10</sub> reductions in CFUs were observed within 120 min with both 150 and 300 GU pMPO/ml. No appreciable differences in the rate of kill between 150 and 300 GU pMPO/ml were observed. Increasing the starting inoculum of *C. albicans* showed a marginal effect on the time-kill of E-101 at 150 GU pMPO/ml.

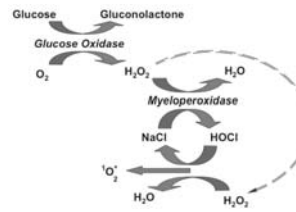
**Conclusion:** Based on the *in vitro* results observed, E-101 has potent activity against common pathogenic *Candida* species and related yeast. There is no apparent impact with regard to the species of *Candida* or the inherent resistance mechanisms common among these species on the activity of E-101. These data illustrate the potential of E-101 for the prevention of *Candida* infection in surgical patients.

INTRODUCTION

E-101 Solution (E-101) is a cell-free coupled enzyme system with multiple mechanisms of oxidative action against a broad spectrum of Gram positive and Gram-negative bacteria (1). It is developed as a microbicide for application directly into wound or surgical incision sites. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is produced *in situ* by glucose oxidase (GO) dehydrogenation of glucose resulting in the two equivalent reduction of oxygen. The acid (H<sup>+</sup>) optimum myeloperoxidase catalyzed oxidation of chloride ion (the Cl<sup>-</sup> of NaCl) by H<sub>2</sub>O<sub>2</sub> generates hypochlorous acid (HOCl). Once generated, HOCl (or its conjugate base OCl<sup>-</sup>) participates in a diffusion controlled reaction with a second H<sub>2</sub>O<sub>2</sub> molecule to yield singlet oxygen (<sup>1</sup>O<sub>2</sub>). Singlet oxygen is a potent electrophilic oxygenating agent capable of reacting with a broad spectrum of electron rich compounds (Figure 1). The mechanism of action of E-101 is enhanced by the binding of pMPO to the surface of target microorganisms (2).

The microbicidal combustive action of E-101 against target microorganisms is directed to a variety of molecular and enzymatic sites that are essential for metabolism or for the integrity of the microorganism. This study was undertaken to examine the *in vitro* activity of E-101 and its oxidative products on *Candida* species.

Figure 1. Mechanism of action of E-101 Solution



METHODS

**Fungal strains.** Clinical strains of *Candida* species (10), *Cryptococcus neoformans* (1), and *Saccharomyces cerevisiae* (1) were selected based on their common frequency of isolation (Table 1) and resistance phenotype. *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, and *C. krusei* 6258 were also evaluated.

**Antimicrobial agent.** Stock solutions of E-101 enzyme solution and substrate solution were prepared at Exoxemis, Inc. (Little Rock, AR). E-101 contains pMPO, GO derived from *Aspergillus niger*, and proprietary amino acids in an aqueous formulation vehicle consisting of 150 mM sodium chloride and 0.02% w/v polysorbate 80 in pH 6.5, 20 mM sodium, phosphate buffer. The substrate solution contains 300 mM glucose in the same aqueous formulation as the enzyme solution. The enzyme and substrate solutions are packaged in two separate vials and mixed together to activate the system.

**Time-kill assay.** Time kill studies were performed using modified suspension-neutralization (3). Yeast suspensions were prepared from stationary phase growth on Sabouraud's dextrose agar. Reaction tubes were prepared to contain the appropriate inoculum (~5 x 10<sup>6</sup> CFU/ml), substrate and enzyme solution. The final concentrations of pMPO in the enzyme solution were 150, and 300 GU pMPO/ml. Reaction tubes were incubated at room temperature and samples were removed at 0, 5, 15, 30, 60, and 120 minutes for quantitative culture. The log<sub>10</sub> CFU survivors were determined at each time point.

RESULTS

Table 1. Antifungal activity of E-101 solution against 15 *Candida* species and other yeast-like organisms

| Concentration  | No. of strains killed after different treatment times <sup>b</sup> |        |        |         |
|----------------|--|--------|--------|---------|
|                | 15 min   | 30 min | 60 min | 120 min |
| 150 GU pMPO/ml | 0  | 0      | 3      | 15      |
| 300 GU pMPO/ml | 0  | 3      | 8      | 15      |

<sup>a</sup>*C. albicans*-1, *C. dubliniensis*-1, *C. glabrata*-2, *C. guilliermondii*-1, *C. krusei*-1, *C. parapsilosis*-2, *C. tropicalis*-2, *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019, *C. krusei* 6258, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae*

<sup>b</sup>Reduction in viability ≥10<sup>5</sup> CFU/ml

RESULTS (CONT)

Figure 2. Collective time-kill curve results of E-101 solution against 13 *Candida* spp. Approximately 0.5 log<sub>10</sub>, 1.5-2.0 log<sub>10</sub>, and > 6.0 log<sub>10</sub> reductions in CFU were observed after 30, 60 and 120 min, respectively at both 150 and 300 GU pMPO/ml.

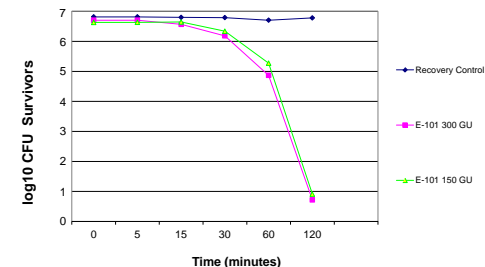
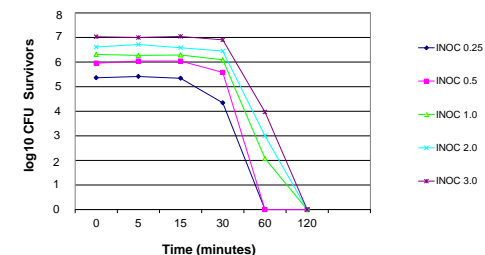


Figure 3. Time-kill results of E-101 solution at 150 GU pMPO/ml exposed to increasing inoculum on *Candida albicans* ATCC 90028. Increasing the *in vitro* inoculum had little effect on the rate of kill.



CONCLUSIONS

- Time-kill studies showed that E-101 solution is fungicidal against *Candida* species, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae* in a time-dependant manner.
- The rapid rate of killing induced by E-101 solution is consistent with its combustive oxygenation mode of action.
- Inoculum size did not have a major influence on *in vitro* activity of E-101 solution.
- These data further illustrates the broad spectrum of activity of E-101 solution and its potential fungicidal (candidicidal or yeast-killing activity) when used topically to prevent surgical site infections.

REFERENCES

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