Efficacy of the Myeloperoxidase Enzyme System in a Rat Dermal Model for the Prevention of Surgical Site Infection

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Objective

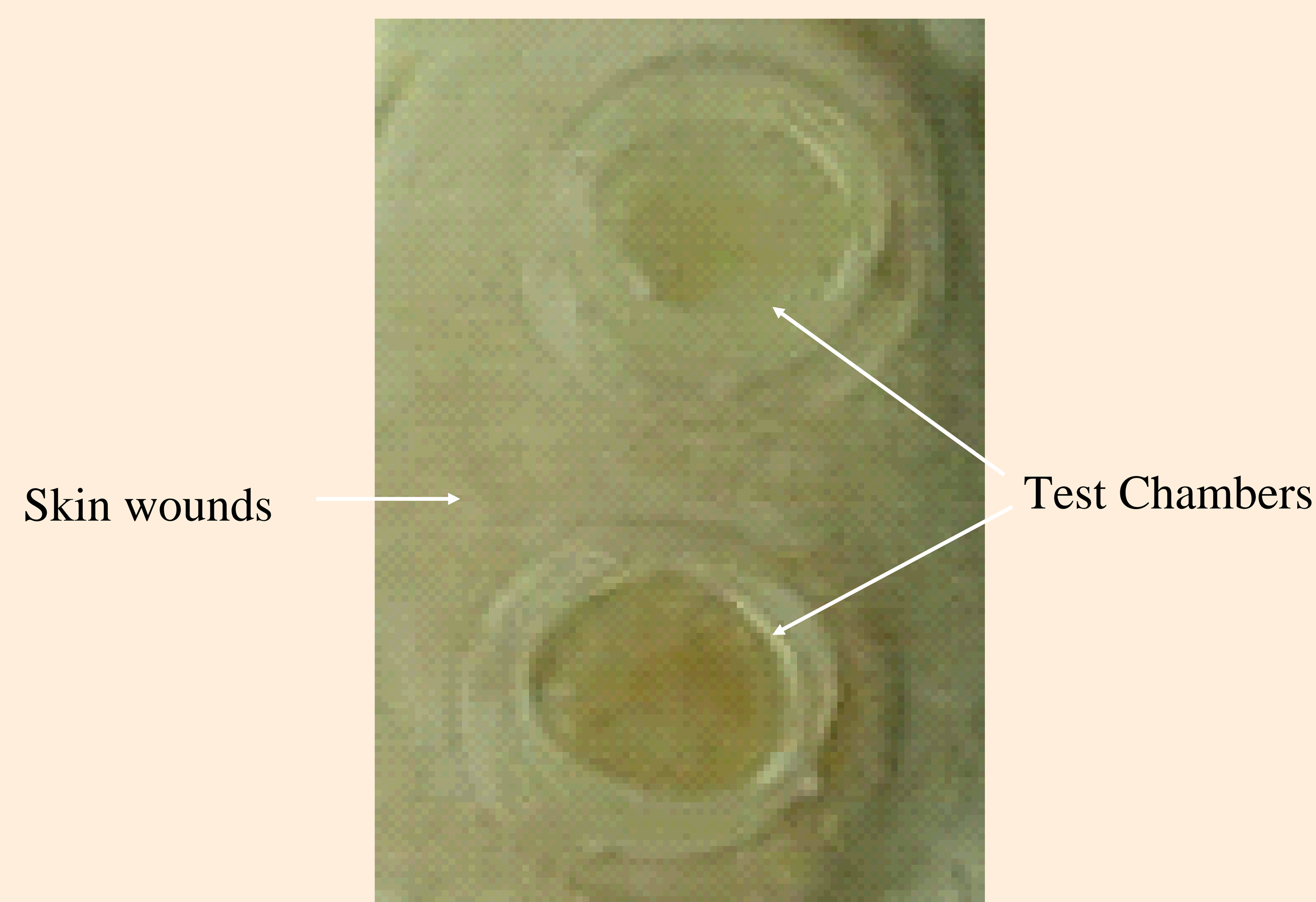
Surgical site infections (SSI) are a major source of morbidity and mortality, and add significant cost to healthcare. An oxidant generating enzyme system containing myeloperoxidase (MPO) has been developed as a new topical/local product to prevent SSI (Exoxemis, Inc., Little Rock, AR). *In vitro*, the MPO enzyme system is rapidly microbicidal against a broad range of microorganisms, even in the presence of metal implant material (1,2). The major source of SSI bacterial inoculation is believed to be dermal flora at wound margins (3). Even a very small inoculum of an indolent organism can result in delayed clinical infection. In this study, we developed a rat dermal model to evaluate the *in vivo* efficacy of the MPO enzyme system for the prevention of SSI.

Methods

Part 1
Dermal experimental sites were created on the dorsal surface of 36 male Sprague-Dawley rats. All rats had hair shaved with a 40 Oster blade 24 hours prior to testing. Under anesthesia, rats were positioned prone and wounds were created. Preparations included simple shaving (12 rats), abrasion into the dermis using 10 strokes with the 40 Oster blade along the dorsum with uniform pressure yielding reddened and streaked skin with no bleeding (6 rats), skin stripped into the dermis using repetitive application and removal of tape 20 times yielding glossy skin with oozing (6 rats), and full thickness skin wounds created by lifting loose skin and excising an elliptical area with scissors using sterile technique, thus exposing the fascia (12 rats). Two liquid-tight clear plastic cylinders (2.5 cm diameter) were glued directly onto the surface of each wound or skin surrounding exposed fascia. This formed test chambers, the base of which was the skin or wound (Fig. 1). Exposed skin or fascia was then inoculated with 10^6 cfu of *Staphylococcus aureus*, ATCC 13709, in 200 µl. Immediately 800 µl of MPO (400 µg/ml) formulation was added to the site, except for controls, which had no MPO administered. After 15 minutes of treatment, catalase was added to stop the MPO reaction, and the liquid content from each test site was removed. Surviving bacterial count (endpoint) of all liquid samples was assessed by quantitative culture.

Part 2
Bacterial recoveries from both the liquid and fascia tissue were determined on 6 rats using sham treatments. Then, MPO treatments using the fascia model above were applied to 8 additional rats. For each rat, 10^6 cfu of *Staphylococcus aureus*, ATCC 6538, in 200 µl, was added to each site. After 15 minutes of undisturbed exposure, 800 µl of the MPO formulation was added. At 5 or 30 minutes after the addition of MPO the liquid was removed, and a sample of the fascia was excised, weighed, and homogenized. All liquid and tissue samples were assayed for survivors by quantitative cultures. All animals were euthanized following collection of tissue and/or liquid test samples.

Figure 1. Rat dermal model using plastic test chambers glued over wounds created on skin or fascia to contain MPO formulation.



Results

Part 1
Within 15 minutes post-treatment, the liquid samples from all control sites with no MPO yielded no decrease in bacterial burden, i.e., no killing. All sites treated with MPO, however, showed complete kill of the entire ~6 log inoculum, with no survivors noted in quantitative culture (Table 1).

Table 1. Effect of MPO formulation on skin wounds inoculated with *S. aureus*

Wound Type (# rats)	Log Kill w/o MPO (Control)	Log (%) Kill w/MPO (15 min.Treatment)
Shaved (12)	0	5.9 (100%)
Abraded (6)	0	6.3 (100%)
Stripped (6)	0	6.3 (100%)
Fascia (12)	0	5.9 (100%)

Part 2
After 5 and 30 minutes the liquid supernatant yielded no survivors. At 5 minutes the combined tissue and liquid samples yielded an average log reduction of 2.6 from the original inoculum, a 99.5% reduction of bacterial load. At 30 minutes, the combined samples yielded an average log reduction of 6.2 from the original inoculum, a 100% reduction of bacterial load (Table 2). Controls with no MPO yielded no decrease in bacterial burden, i.e., no killing in liquid or tissue samples.

Table 2. Effect of MPO formulation on liquid and fascia tissue inoculated with *S. aureus*

MPO Treatment Time (# rats)	Total Log Reduction	Total Percent Kill
5 min (4)	2.6	99.75%
30 min (4)	6.2	100%

Conclusion

MPO is effective in eliminating bacteria in solution and at open surgical sites., even with an inoculum of 10^6 cfu, an extraordinary challenge for a wound.

All bacteria in solution were rapidly killed, and very few were recovered from quantitative tissue samples at early time points indicating that the MPO system is a rapid *in vivo* microbicidal agent.

These studies shows that the MPO enzyme system is a promising antimicrobial agent for the prevention of SSI.

References

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