Determination of the effects of a myeloperoxidase (MPO) formulation on wounds inoculated with *Staphylococcus aureus* using a porcine model

Roberto Perez, PhD[1]; Yan Rivas[1]; Joel Gil[1]; Jose Valdes[1]; Sophie Becquerelle, PharmD[2]; Obsidiana Abril-Hörpel, PhD[2]; and Stephen C. Davis[1]*

1. University of Miami, Miller School of Medicine, Department of Dermatology and Cutaneous Surgery, Miami, FL

2. Exoxemis, Inc., Little Rock, AR

Abstract:

As part of the normal inflammatory process, human phagocytes employ myeloperoxidase (MPO) to eliminate bacteria from wounds.[1,2] Staphylococcal species have been estimated to affect over 70% of wounds due to the prevalence of the bacteria on the skin and the high incidence of resistance to antimicrobial treatments.[3] It has been suggested that chronicity of wounds can be associated with a persistent elevation in bacterial counts, resulting in a prolonged and more intense inflammatory response.[4] Herein, we present research conducted to investigate the ability of a MPO containing formulation to combat *Staphylococcus aureus* infection using a well established porcine partial thickness wound model.[5,6,7]

Swine were used in the study due to the similarities of porcine skin to human skin. Deep partial thickness wounds (10 mm x 7 mm x 0.5 mm) were made on each animal using a specialized dermatome. Wounds (6 per treatment) were inoculated with *Staphylococcus aureus*. Wounds were treated with high or low concentration of MPO formulation, placebo, saline, or mupirocin, which served as a positive control. Untreated wounds served as a negative control. Treatments were applied 20 minutes after inoculation, 4 hours after initial treatment, and 24 hours after initial treatment at which time the bacteria were recovered using a catalase solution for the MPO formulations and a neutralizing solution for mupirocin. Recovered bacteria were plated on mannitol salt agar using the Spiral Plater System and the Log CFU/mL determined after overnight incubation.

Treatment with the MPO formulations reduced the 8 Log CFU/mL of challenge pathogens recovered from the untreated wounds by 3 Log ($p<0.01$). There was no difference in the placebo, saline, or untreated groups. As expected mupirocin treatment resulted in no detectable wound bacteria. These results indicate that treatment with MPO formulation is effective in reducing the number of *S. aureus* in wounds, which may have important clinical implications.

Introduction and Objectives:

Myeloperoxidase (MPO) is a member of the haloperoxidases, a family of enzymes central in mammalian antimicrobial defense. MPO is present in granules of neutrophils and plays a major role in the killing activity of these cells. These enzymes function primarily by generating various oxidized species from hydrogen peroxide such as singlet oxygen which proves destructive to invading pathogens. Several MPO-based systems have demonstrated promising antimicrobial activity *in-vitro*.

Staphylococcus aureus is a biofilm forming pathogen native to the human skin. Therefore, infection with the bacterium is common. Biofilms are bacterial colonies which have become encased in an adhesive and protective exopolysaccharide matrix (EPS) bacterial or host origin. The biofilm facilitates intercellular communication (quorum sensing), gene transfer, and antimicrobial resistance due to the activation and acquisition of resistance mechanisms. Due to its mode of action, MPO is extremely unlikely to result in resistance after long term usage. Therefore, it is a promising system for the prevention and treatment of infections associated with biofilm forming organisms.

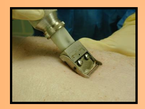
Our study set out to determine the efficacy of a new MPO-based drug product as an antimicrobial in a *S. aureus* inoculated deep partial thickness wounds in a porcine model.

References:

1. Hampton, MB, Kettle, AJ, and Winterbourn, CC. Inside the Neutrophil Phagosome: Oxidants, Myeloperoxidase and Bacterial Killing. *Blood 1998;92(9):3007-3017.*
2. Arisawa, F, Tatsuzawa H, Kambayashi, Y, Kuwano, H, Fujimori, K, and Nakano, M. MCLA-dependent chemiluminescence suggests that singlet oxygen plays a pivotal role in myeloperoxidase-catalysed bactericidal action in neutrophil phagosomes. *Luminescence 2003;18:229–238.*
3. Edwards R and Harding KG. Bacteria and wound healing. *Curr Opin Infect Dis.* 2004;17:91–96.
4. Konturek PC, Brzozowski T, Konturek SJ, Kwiecien S, Dembinski A, Hahn EG. Influence of bacterial lipopolysaccharide on healing of chronic experimental ulcer in rat. *Scand J Gastroenterol.* 2001 Dec;36(12):1239-47.
5. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Rep Regen* . 2001 Mar-Apr;9(2):66-76
6. Davis SC, Cazzaniga AL, Eaglstein WH, and Mertz PM. Over-The-Counter Antimicrobial Bandages and Proliferation of a Common Wound Pathogen. *Arch Dermatol Res.* 2005 Nov;297(5):190-5.
7. Davis SC, Ricotti C, Cazzaniga AL, Welch E, and Mertz PM. Microscopic and Physiological Evidence for Biofilm-Associated Wound Colonization in-vivo: *Wound Rep Reg* 2008 (16); 23–29

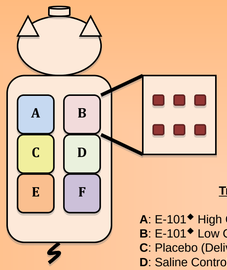
Methods and Materials:

1. Experimental Animals and Wounding:
- Three juvenile female SPF pigs were used
- Thirty-six deep partial thickness wounds (10x7x0.5mm) were made on the paravertebral area of each animal's back



Wounding

Wound

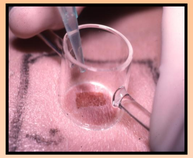


Treatment Groups*

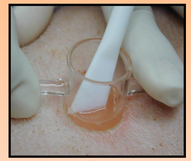
A: E-101◆ High Concentration (600 GU-MPO/ml)#
B: E-101◆ Low Concentration (300 GU-MPO/ml)#
C: Placebo (Delivery Vehicle)
D: Saline Control
E: Mupirocin
F: Untreated Control

**Treatments were randomized*
◆Provided by Exoxemis Inc.
#GU-MPO/ml = Guaiacol Units of MPO per milliliter, one GU is the amount of enzyme required to decompose 1μmol of substrate per minute at 25°C and pH 6.0.

2. Wound Inoculation and Treatment:
- Wounds were inoculated with 25μl of 8 Log CFU/ml *Staphylococcus aureus (ATCC 6538)*
- Wounds were treated 20 minutes, 4 hours, and 24 hours after inoculation (2ml of treatment was scrubbed for 30 seconds with a teflon spatula and aspirated)
- Wounds were moistened with an additional 200μl of treatment and individually covered with a polyurethane film dressing after each treatment



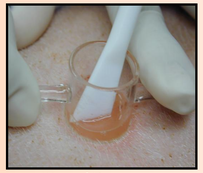
Wound Inoculation



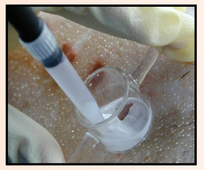
Wound Treatment

3. Treatment Recovery (24 hours after inoculation): After the last treatment, the solutions were aspirated and the neutralized with 1ml of 0.5% catalase solution and the bacteria quantified (#5)

4. Wound Recovery (24 hours after inoculation): After treatment recovery, bacteria were recovered from the wounds using 1 ml of 0.5% catalase solution or mupirocin neutralizing solution



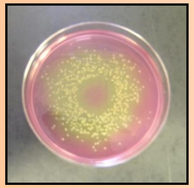
Wound Recovery



Treatment Recovery

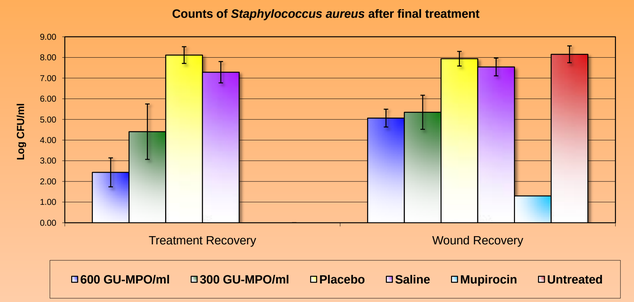
5. Bacterial Quantification:
- Bacterial suspensions were serially diluted 10-fold
- Dilution series was cultured on Mannitol Salt agar using the Spiral Plater System which deposits 50μl of suspension on a rotating agar plate
- Plates were incubated at 37°C overnight and the Log of the colony forming units per ml (CFU/ml) determined

Spiral Plater System



Selective Media

Results:



Note: Due to mupirocin treatments dissolving into wound area, a separate treatment count could not be performed. Total mupirocin counts were below the limit of quantification (LOQ = 1.3 Log CFU/ml)

Bacterial counts from recovered treatments:

Final treatments (24 hours after inoculation) were aspirated from the wounds and immediately neutralized. Quantification of the bacterial suspensions indicates that:

- 600 GU-MPO/ml MPO-based drug resulted in a near 6 Log CFU/ml reduction (>99.9999% reduction, $p<0.01$) as compared to controls
- 300 GU-MPO/ml MPO-based drug resulted in a near 4 Log CFU/ml reduction (>99.99% reduction, $p<0.01$) as compared to controls
- There was no significant difference between the placebo and saline

Bacterial counts from recovered wounds:

Immediately after final treatments (24 hours after inoculation), wounds were scrubbed using a sterile teflon spatula and the appropriate neutralizer. Quantification of the bacterial suspensions indicates that:

- Treatment with either concentration MPO-based drug product resulted in a near 3 Log CFU/ml reduction (>99.9% reduction, $p<0.01$) as compared to controls
- There was no significant difference between the placebo, saline, or the untreated controls
- Treatment with mupirocin (positive control) resulted in no detectable bacteria (limit of quantification 1.3 Log CFU/ml)

Conclusions:

Treatment with a new MPO-based drug product resulted in significant reductions of challenge pathogen in deep partial thickness wounds using a porcine model. Neither placebo nor saline treatments resulted in colony counts different from those determined for the untreated controls. These results indicate that treatment with MPO-based drug products may be beneficial in the prevention or treatment of bacterial infections in wounds.

Sponsorship Information:

This work was supported by the US Army Medical Research and Materiel Command under Award number W81XWH-05-2-0076. Opinions, interpretations, conclusions and recommendations are those of the authors and are not necessarily endorsed by the US Army. MPO-drug based product and supplementary support was provided by Exoxemis, Inc.

Contact Information:

Stephen C. Davis, Associate Professor
University of Miami, Miller School of Medicine
Department of Dermatology and Cutaneous Surgery
sdavis@med.miami.edu ***Ph: 305.243.4897***