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

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Abstract:
As part of the normal inflammatory process, human phagocytes employ myeloperoxidase (MPO) to eliminate bacteria from wounds.1-7 Myeloperoxidase has been shown to be effective in killing bacteria in vitro and in vivo.8,9 Myeloperoxidase is produced by phagocytic cells and is one of several reactive oxygen species produced during inflammation.10 Myeloperoxidase catalyzes the production of hypochlorous acid from hydrogen peroxide and chloramine-T, a reaction that is used in clinical practice for wound disinfection.11 Myeloperoxidase is also known to inhibit bacterial biofilm formation.12 However, little is known about the effects of MPO on the healing of infected wounds in vivo. Therefore, we hypothesized that MPO might be 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 heme superfamily, a family of enzymes central in mammalian antimicrobial defense. MPO is present in granulocytes and plays a major role in the killing activity of these cells. These enzymes function primarily by generating various oxidized species from hydrogen peroxide and oxygen, which produces destructive action on pathogens. Several MPO-based systems have demonstrated antimicrobial activity in vitro.

Staphylococcus aureus is a biofilm forming pathogenic to the human skin. Therefore, infection with the bacterium is common. Biofilms are bacterial colonies which have become encased in an adhesive and protective biofilmic matrix (EPS); bacterial or host origin. The biofilm facilitates intercellular communication (quorum sensing), gene transfer and adherence resistance due to the adhesion and acquisition of resistance mechanisms. Due to its mode of action, MPO is extremely unlikely to result in resistance after long-term usage. Therefore, its use is a promising tool in the prevention and treatment of infections associated with biofilms.13

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.

Methods and Materials:

1. Experimental Animals and Wounding:
Three juvenile female SPF pigs were used. The pigs were anesthetized and sedated with ketamine to allow easy access to the skin. The skin was incised with a scalpel, and the subcutaneous tissue was exposed. A sterile measuring tape was used to determine wound size. The wounds were inoculated with S. aureus (ATCC 6538) directly into the area of the incision using a sterile loop. The wounds were then closed using a sterile suture and sterile saline was applied to the incision. The wounds were allowed to heal for 24 hours before the first treatment.

2. Wound Inoculation and Treatments:
Wounds were inoculated with 2.5x10^5 of Log CFU/ml Staphylococcus aureus (ATCC 6538). Wounds were treated twice, 20 minutes, 4 hours, and 24 hours after inoculation. Wounds were treated with an additional 20μl of treatment and individually covered with a polyurethane film dressing after each treatment.

3. Wound Inoculation
Wound Treatment
A: G-STH+High Concentration (MPO-GX MPO-100) B: Theophylline E: Placebo (Delivery Vehicle) P: Placebo Control G: Positive (Delivery Vehicle) F: Untreated Control

Treatments were randomized
Triplicate sterile colonies per wound, see Gill for the amount of enzyme required to deplete wound of S. aureus of 1 Log unit of CFU/ml.

4. Bacterial Quantification:
Bacterial suspensions were diluted 10-fold. Dilution series were performed on Mueller Hinton agar and plated with a sterile loop. After 24 hours of incubation at 37°C, colonies were counted and plated on a new plate. The plates were incubated at 37°C overnight and the Log of the colony forming units per ml (CFU/ml) determined.

5. Wound Recovery
Wound Treatment
Wound Recovery
There was no significant difference between the placebo, saline, and untreated controls
Wound treatment with mupirocin (positive control) resulted in no detectable bacteria (0 Log CFU/ml). Other treatments showed significant differences in bacterial reduction compared to placebo saline, or untreated controls. The results indicate that treatment with MPO-based drug products may be beneficial in the promotion of treatment of bacterial infections in wounds.

References:

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