Bactericidal Activity of the Myeloperoxidase Antimicrobial Enzyme System Against Vegetative and Spore Forms of Bacillus cereus, Bacillus thuringiensis, and Bacillus subtilis

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In vitro myeloperoxidase enzyme system

Mechanisms of action of the myeloperoxidase enzyme system

CONCLUSION

- This study demonstrated that the myeloperoxidase enzyme system is highly effective in vitro against vegetative cells and germinating spores of closely related strains of B. anthracis.
- The broad spectrum of activity and unique mechanism of action of the myeloperoxidase enzyme system against Gram-positive and Gram-negative bacteria and Bacillus spores make it a potential weapon against microorganisms used as a biological weapon.
- Additional studies are needed to assess the in vivo efficacy of the myeloperoxidase enzyme system for the prevention and treatment of infections as a new adjunct or alternative to traditional antibiotics.

METHODS

- **Organism**
  - Bacillus cereus ATCC 10876, Bacillus thuringiensis ATCC 35617, and Bacillus subtilis ATCC 6633 were used in this study. Strains of B. cereus and B. thuringiensis were selected because of their genetic relatedness to Bacillus anthracis.
  - Spore preparation: All strains were grown on loop-agar surfaces at 37°C for 24 h. Spore suspensions were pelleted and resuspended in buffer to ~10^7 spores/ml.
  - In vitro myeloperoxidase enzyme system activity determination:
    - Cell suspensions of vegetative growth were prepared by the shake flask method to achieve late log to early stationary phase growth. Spore and vegetative cell suspensions were pelleted and resuspended in buffer to 10^7 cells/ml.
    - A 1.0 ml volume of enzyme formulation plus vegetative growth or spore suspension at a final target concentration of ~10^8 cfu/ml was tested. Treatment vials were incubated at 37°C in a dry bath. The entire vial was treated by the addition of 1.0 ml enzyme solution plus vegetative cells or spores after 15, 30, 60, and 120 min. The entire mixture of each vial was inoculated onto nutrient agar for quantitative colonies and inoculated for 24 h at 37°C. Colonies were then counted and compared to an enzyme diluent control. Results: Time kill studies demonstrated that the extent of kill increased with time of exposure to enzyme formulations. Within 2 minutes of treatment, there was a 5-log reduction in cell numbers (>99.99% kill) in culture broth. By 120 minutes of treatment there were no living cells capable of forming colonies. The unique mechanism of action and broad spectrum of activity of the myeloperoxidase enzyme system compared favorably to the potential for antibiotic resistance as well as other infections, which may be caused by agents used as a biowarfare weapon.

- **Results**
  - The broad spectrum of activity and unique mechanism of action of the myeloperoxidase enzyme system was demonstrated with rapid bacterial killing (~6 log) within 30 min. (>99.99% kill) of exposure (Figure 1). The bactericidal activity of the myeloperoxidase enzyme system was also demonstrated with extended treatment time.

- Additional studies are needed to assess the in vivo efficacy of the myeloperoxidase enzyme system for the prevention and treatment of infections as a new adjunct or alternative to traditional antibiotics.

REFERENCES