Microbicidal Activity of the Myeloperoxidase Enzyme System Against Clinically Relevant Bacterial and Fungal Isolates Including Multidrug-Resistant Strains


1Rebecca Biosciences, Concord, OR; 2Clarion Health Partners, Inc., Indianapolis, IN; 3Duke University Medical Center, Durham, NC; 4ExOxEmis, Inc., Little Rock, AR

ABSTRACT

Background: A novel neutrophil system utilizing myeloperoxidase (MPO) as a potential therapeutic agent has been developed (ExOxEmis, Inc., Little Rock, AR) for topical/local use. The purpose of this study was to investigate the in vitro activity of the MPO enzyme system against a broad spectrum of bacterial and fungal isolates represented in various clinical categories.

Methods: A total of 196 clinical and reference ATCC isolates were tested including multidrug resistant mechanisms. Isolates included aerobic gram-positive (S. aureus, S. epidermidis, S. pneumoniae, 37 for each respectively), and S. pyogenes, 35 strains. Limiting ETU early to late stationary phase cultures were pooled and concentrated to 1 x 10^8 cells/ml. A 1:1 ratio of enzyme formulation system with organism suspension at a final concentration of 0.05 x 10^8 cells/ml was used. The enzyme reaction was then stopped by the addition of catalase. Colony counts were performed and compared to an enzyme diluent control. Time-kill studies were also performed against 3 aerobic ATCC isolates. The results were compared to the natural neutrophil host defense system.

Results: The microbicidal activity of the MPO enzyme system against bacterial isolates occurred within the first 15 min of exposure for 99.0% of the organisms tested. Complete kill of all bacteria and yeast occurred within 30 min and Aspergillus conidia suspensions within 60 min. Time-kill studies demonstrated that the rate and extent of inorganic H2O2 exposure increased with increasing exposure and concentration of MPO formulation system.

Conclusions: The MPO enzyme system demonstrated rapid and potent activity against a broad spectrum of bacterial and fungal isolates, and may have prophylactic and therapeutic applications.

INTRODUCTION

Myeloperoxidase (MPO) is a major neutrophil protein which is stored in azurophilic granules and released into the phagosome during phagocytosis. The enzyme superoxide anions and hydrogen peroxides generated by the neutrophil respiratory burst form reactive oxidized species that cause cell damage. A cell free oxidant generating enzyme system containing MPO has been developed which exploits this natural antimicrobial system (ExOxEmis, Inc., Little Rock, AR). The mechanism of activity of the MPO enzyme system (Figure 1) involves the use of hydrogen peroxide, generated by the neutrophil respiratory burst, and hydrogen peroxide, generated by the enzyme system. Excess hydrogen peroxide is rapidly destroyed by the MPO enzyme system, which is stored in azurophilic granules. The enzyme system is designed to be effective against both aerobic and anaerobic bacterial and fungal strains.

METHODS

Microorganisms: A total of 196 clinical (n=98) and reference ATCC strains (n=98) comprised of 44 strains of Enterobacteriaceae (including 9 multidrug resistant strains), 12 Pseudomonas aeruginosa (including 1 biofilm producing strain), 5 Acinetobacter baumannii, 2 Enterococcus faecalis, and 11 Enterococcus faecium (including 3 MRSE, 2 MRSE, 3 MRSE, and 1 biofilm producing strain), 12 aerobic gram-positive cocci (including 3 MRSA, 2 MRSA, and 1 biofilm producing strain), 12 aerobic gram-negative bacteria, 11 non-fermenting bacteria, 11 fusobacteria, and 12 clostridia strains. The organisms were used to challenge the MPO enzyme system to a broad range of clinically relevant bacterial and fungal strains.

Determination of In-Vitro Activity: Bacterial and Candida suspensions were prepared by the diluted stock method to achieve limiting ETU early stationary phase growth. For fungal cultures (e.g. S. aureus), suspensions were prepared by the direct colony method (stationary phase growth). Aspergillus conidia suspensions were prepared from in vivo strain cultures by agitation with buffer-20 suspensions were prepared by mixing equal volumes of cultures grown on agar and buffer. A 100 ul volume of enzyme formulation, containing 8 ug MPO, plus organism suspension was added to the final target concentration of ~10^8 to 10^9 cfu/ml. The enzyme reaction was stopped by the addition of catalase at 15 min and 30 minutes for bacteria and 30 and 60 minutes for fungal strains.

The contents of each vial were inoculated into incubation media for quantitative culture and incubated for 24 h at 37°C. After incubation, colonies were counted and compared to an enzyme diluent control.

Time-Kill Studies: The rate and extent of survival of S. aureus exposed to low concentrations of MPO containing formulations were determined over time. Cultures were incubated as described above to MPO formulations containing 8 ug MPO and time intervals of 30 min, 30, 45, 60, 150, 180, and 210 minutes. Quantitative colony counts were performed to determine the time point for measurement.

RESULTS

• The in vitro activity of the MPO enzyme system against gram-positive and gram-negative bacteria is presented in Figure 2. In 30 minutes or less of treatment with 8 ug of MPO in the formulation, there were no survivors.

• The microbicidal activity of the MPO enzyme system against both antimicrobial susceptible and resistant strains of enterococci, staphylococci and 3 pneumococci occurred within 30 minutes (Figure 2).

• Upon re-testing, there were no survivors within 15 minutes of treatment except for one biofilm producing strain of E. coli, which showed no survivors within 30 minutes.

• The MPO enzyme system demonstrated rapid and cidal activity against both enterococci and viridans streptococci (Figure 4). There were no survivors at 15 minutes or less of treatment.

• Among gram negative strains tested, there were no survivors at 15 and 30 minutes of treatment with the MPO enzyme system, regardless of the antimicrobial phenotype of the organism (Figure 5).

• The in vitro activity of the MPO enzyme system against Aspergillus and Candida spp. is presented in Figure 5. At 30 minutes for Candida spp. and within 60 minutes for Aspergillus (conidia suspension) spp., treatment with 8 ug of MPO in the formulation, there were no survivors.

• The results of time-kill studies against S. aureus are shown in Figure 6. The data is presented as log10 decrease in survivors relative to the initial inoculum at predetermined endpoints. The rate and extent of bacterial kill appeared to increase with increasing exposure and concentrations to MPO formulations.

CONCLUSION

• The MPO enzyme system is rapidly microbicidal, broad spectrum, and highly active even in the presence of antibiotic resistant strains.

• MPO enzyme system mechanism of action is similar to that of the natural neutrophil host defense system which makes emergence of resistance, as seen with traditional synthetic antimicrobials, potentially unlikely.

• MPO enzyme system is in vitro activity against clinically important pathogens and unique mechanisms of action warrant further investigations for the prevention and treatment of infections.

REFERENCES

