**Antibiotic and photo-porphyrin combination therapies against S. aureus**

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**ABSTRACT INTRODUCTION:**
Orthopaedic infection is a devastating problem whose treatment is complicated by antimicrobial-resistant pathogens and limited by the current armamentarium of antimicrobial agents. In looking at therapies, we have been examining porphyrins, compounds used in the field of photodynamic antimicrobial therapy. Upon illumination, porphyrins release singlet oxygen species (1O2) that result in cell death (Xing et al.). This process, porphyrins can be used in cancer treatments (Kolarova et al.) and as antimicrobial agents in light-illuminable areas such as skin and spinal infections (Komagoe et al.). In this study, we have asked if the known antimicrobial effects of porphyrins would combine with those of antibiotics important for treatment of orthopaedic infection.

**METHODS:**
*S. aureus* strain ATCC 25923 was cultured overnight, 37°C, in trypticase soy broth (TSB). Based on preliminary experiments, ~10⁶ bacteria/well (200 µl total volume) were exposed to meso-tetra (4-amino-phenyl) phorhine (TAPP) in the dark or in white light. To do this, a humidified chamber was created with transparent plastic so that the plate sat 5 inches from a 100 W, 120V Sylvania white light, with the chamber temperature calibrated to 37°C. Growth inhibition was determined using a microbroth dilution technique, and antibacterial efficacy determined visually after a 24 h incubation with the test agent. Time dependence of killing with illuminated TAPP was determined with ~10⁶ bacteria/well in the presence of 0, 5 or 50 µM TAPP. After illumination for 0, 1, 2, 3, 4, or 5 h, bacteria were serially diluted, plated, and counted. Sensitivity to antibiotics was determined at ~0.5X and 5X MIC for tobramycin (0.45 & 4.5 µg/ml), chloramphenicol (2 & 20 µg/ml), Ceftriaxone (0.5 & 5 µg/ml), and vancomycin (0.5 & 5 µg/ml) for 5 h, 37°C, in the light. Additivity of TAPP with antibiotics was determined by incubating ~10⁶ bacteria/well with 5 µM TAPP. 0.5 µg/ml Ceftriaxone, 0.5 µg/ml vancomycin, 2 µg/ml chloramphenicol, or 0.45 µg/ml tobramycin or combinations thereof for 5 h, 37°C, either in the light or dark. At 5 h, bacteria were harvested, serially diluted, and CFU determined by direct counting.

**RESULTS SECTION:**
Using visual assessment, the minimum inhibitory concentration of TAPP towards *S. aureus* 25923 was determined to be ~10 µM (under illumination); no apparent cell death was observed over the same concentration range in the dark. Under illumination, incubation of *S. aureus* with TAPP (5 µM, 50 µM) resulted in a time-dependent decrease in bacterial numbers (Fig. 1). Normal bacterial growth is observed in light without TAPP. With 5 µM TAPP, bacterial numbers dropped by ~1 log by 5 h; at 50 µM, viable bacteria are not detectable after 4 h. No apparent cell death was observed for the same treatment in the dark.

We next asked the effects of sub-MIC (0.5X) and above MIC (5X) concentrations of membrane active (Ceftriaxone, vancomycin) or protein synthesis inhibitory (tobramycin, chloramphenicol) antibiotics (Fig. 2). At MIC or higher concentrations, 3 to 4 log decreases in CFU were measured for all except tobramycin, which caused complete killing. At sub-MIC levels, tobramycin caused an ~2.5 log decrease in CFU; the other three antibiotics showed little if any growth inhibition. We next asked if the effects of these antibiotics could be enhanced by the presence of TAPP (Fig. 3). The porphyrin TAPP caused complete killing above its MIC, and at sub-MIC concentrations caused an ~1 log decrease in CFU. When TAPP was added to cultures containing antibiotics, the activity of membrane-active Ceftriaxone or vancomycin with TAPP were equivalent to that seen with TAPP alone. The addition of TAPP to tobramycin or chloramphenicol (protein synthesis inhibitors) resulted in an additional 2 log inhibition of bacterial growth over either agent alone, suggesting that TAPP and the protein synthesis inhibitors have complementary mechanisms.

**DISCUSSION:**
Orthopaedic infection remains difficult to cure because of poor antibiotic penetration and limited activity against adherent bacteria. As a first step towards developing new therapies to target these problems, we have been examining means to enhance the activity of important classes of antibiotics. Because antibiotic action correlates with release of reactive oxygen species, we asked whether the porphyrin TAPP, which is thought to be antimicrobial through release of these reactive oxygen species, would enhance the activity of antibiotics. It is interesting that both membrane-active antibiotics showed little enhancement of activity in the presence of TAPP, as the first target for reactive oxygen species is the cell wall. However, the additivity of TAPP with the protein synthesis inhibitors suggests that reactive oxygen species activate bacterial signaling pathways that may be sensitive to blocks in protein synthesis. Use of such combination therapies may allow the design of new potent systems that can target recalcitrant infections through local release and activation of antibiotics and complementary agents.

**SIGNIFICANCE:**
A very successful means to treat orthopaedic infection is through local delivery of antibiotics. This report demonstrates that use of an agent that produces reactive oxygen species can boost the activity of waning antibiotics to maintain an antimicrobial profile. Such coordinated systems would have the potential to allow for an effective long-term eradication of established infection.

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**REFERENCES:**