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Antimicrobial Photodynamic Therapy for S. Pyogenes Eradication

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Abstract:
Antimicrobial photodynamic treatment (aPDT) kills bacteria across the prokaryotic spectrum by combining a photosensitive chemical with an activating light source. High amounts of oxidative stress exerted on bacteria membranes cause disinfection, resulting in fast mortality without genomic exposure and subsequent induction of resistance. The electrostatic interaction between cationic photosensitizers and generally anionic bacteria membranes causes specificity, which is a side effect of soft tissue diseases like necrotizing fasciitis. The goal of this investigation was to see if the bacterium was susceptible to aPDT in both planktonic and biofilm cultures, as a prelude to using it in human clinical trials.

Introduction:
In circumstances of trauma, changed pH, or immunosuppression, Streptococcus pyogenes, a Gram-positive aerotolerant coccus, is a member of the skin microbiota and an opportunistic pathogen. This pathogen is one of the most common causes of skin and soft tissue infections[1]and can cause infections as minor as pharyngitis to severe invasive infections as necrotizing fasciitis, a soft-tissue infection that can cause sepsis, shock, organ failure, and death[2,3]. Severe S. pyogenes infections have a high reported mortality rate, ranging from 10% to 30%, with 650,000 deaths each year[4]. Skin invasion is mediated by erythrogenic exotoxins including streptolysins and streptococcal superantigens among others[5]. Antimicrobial photodynamic treatment (aPDT) kills bacteria and their innate virulence factors by combining a photosensitive substance with an activating light source[6]. The approach has been shown to eliminate multidrug-resistant organisms across the prokaryotic spectrum in a variety of in vitro and in vivo studies[7,8,9]. Disinfection is caused by oxidative stress on pathogen membranes, which inhibits activity without exposing the pathogen to genomic DNA[10]. This lethal mechanism prevents germ resistance from increasing [11]. In eukaryotic cells, there are no known adverse consequences, making the approach ideal for topical disinfection of patients[12]. The goal of this study was to demonstrate the efficiency of aPDT against Group A Streptococcus in both planktonic and biofilm forms utilising two distinct photosensitizer formulations.

Keywords: Antimicrobial photodynamic therapy • Streptococcus pyogenes • Microbiota • Soft tissue infections

Methods:
Bacterial culture :
Streptococcus pyogenes (ATCC® 700951TM) was obtained from ATCC and incubated for 18-24 hours at 35 2°C in tryptic soy broth (TSB) (Becton, Dickinson and Company, Franklin, NJ). After incubation, streak cultures were plated on tryptic soy agar (TSA) plates and cultivated aerobically at 35 2°C (Becton, Dickinson and Company, Franklin, NJ).

Test solutions and illumination system:
A commercial formulation (SteriwaveTM ND, Ondine Biomedical Inc., Vancouver BC) and a solution of 0.01 percent methylene blue USP in sterile water for injection were employed as photosensitizer formulations (PS). Phosphate buffered saline (PBS) at pH 7.4 was used as the control solution. A 670nm non-thermal diode laser (PW1100 system, Ondine Biomedical Inc.) attached to a light delivery handpiece through a 400 m diameter glass fiberoptic cable provided illumination for the photodynamic disinfection technique..

Planktonic culture aPDT: Test and control solutions were made by pouring 180 litres of (a) PBS, (b) 0.01 percent methylene blue in sterile water for injection, and (c) commercial PS into triply spaced wells of black 96-well plates (VWR, Tualatin, OR) with 0.25-2mm magnetic stir bars in a dark environment. Sterile aluminium foil was used to cover the wells. The plates were put on a magnetic stir plate (Corning PC420; Fisher Scientific, Pittsburgh, PA) set to 800 rpm, with the light delivery handpiece positioned above the first well as indicated above.

Biofilm culture aPDT:
200 mL of working inoculum was put to each well of a 96-well plate in a dark environment and incubated for 48 hours while shaking at 125 rpm at 37°C. After the first 24 hours of incubation, the media was changed, and the presence of biofilm was visually confirmed after 48 hours. PBS was used to wash the biofilm three times. To preserve biofilm hydration, plates were further prepared by adding 250 L of PBS to each well and then covering with sterile aluminium foil.

Result:
The results of S. pyogenes eradication in planktonic and biofilm models, respectively. In planktonic culture, aPDT was shown to reduce S. pyogenes populations by an average of (a) 0.1 log10[20.567 percent] when light alone was used vs. PBS control; (b) 4.19 log10[99.993 percent] when MB 0.01 percent plus light was used vs. PBS control; and (c) 5.5 log10[99.999 percent] when commercial photosensitizer solution plus light was In biofilm culture, aPDT resulted in average decreases of (a) 0.2 log10[36.904 percent] for light alone vs. PBS control; (b) 2.23 log10[99.411 percent] for MB plus light vs. PBS control; and (c) 2.85log10[99.859 percent] for commercial photosensitizer solution plus light vs. PBS control.

Discussion:

S. pyogenes produces a wide range of diseases, from pharyngitis to severe invasive infections such as necrotizing fasciitis [13,14]. Each year, the US Centers for Illness Control and Prevention (CDC) estimates that 11,000 to 24,000 cases of invasive group-A streptococcal disease occur, with up to 1,900 deaths [15,16]. This increased morbidity has necessitated the development of innovative antimicrobial methods that are not dependent on existing antibiotics and have broad-spectrum, rapid-cidal activity. Despite the relative relevance of this pathogen in superficial skin infections, few research have assessed the impact of aPDT against S. pyogenes in either planktonic or biofilm forms. When adequate photosensitizer concentrations and light doses are applied, results show that antimicrobial photodynamic therapy may rapidly reduce S. pyogenes titers in both planktonic and biofilm culture. Prior research [17-21] have also demonstrated the technique's broadspectrum efficacy, but the bulk of these studies used photosensitizers that haven't been proven safe in humans, or photosensitizerlight combinations that haven't been confirmed to be clinically useful.

Conclusions:

S. pyogenes is a common human pathogen that can lead to life threatening illnesses. The findings show that aPDT is capable of eradicating S. pyogenes in both planktonic and biofilm cultures quickly and effectively. Both photosensitizers create much deeper kills than controls, and lit commercial photosensitizer solution provides significantly deeper kills than lighted MB alone (PBS illuminated by light). These findings suggest the use of aPDT in the treatment of S. pyogenes infections in the future.