Research Article
Volume 6:2, 2020

Journal of Antimicrobial Agents

ISSN: 2472-1212 Open Access

Antimicrobial Photodynamic Therapy for Rapid Eradication of *S. Pyogenes*

J Cross, C Romo*, R Andersen and N Loebel

Ondine Research Laboratories, Bothell, WA, USA

Abstract

Antimicrobial photodynamic therapy (aPDT) employs the combination of a photosensitive agent and activating light source to destroy microbes across the prokaryotic spectrum. Disinfection is mediated by high levels of oxidative stress exerted on microbial membranes, resulting in rapid kill without genomic exposure and consequent upregulation of resistance. Specificity results from the electrostatic interaction between cationic photosensitizers and generally anionic microbial membranes, an effect largely absent in zwitterionic human cells. *S. pyogenes*, a member of Group A streptococci, is a member of skin microbiota strongly associated with invasive skin and soft tissue infections, including necrotizing fasciitis. This study aimed to determine susceptibility of the microorganism in both planktonic and biofilm culture to aPDT, as a preparatory step to deployment in human clinical studies.

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

Introduction

Streptococcus pyogenes, a Gram-positive aerotolerant coccus, is a member of skin microbiota and an opportunistic pathogen in cases of trauma, altered pH, or immunosuppression.This pathogen is one of the most important causes of skin and soft tissue infections[1]and can lead to relatively serious infectionsranging from pharyngitis to severe invasive infections such as necrotizing fasciitis, a soft-tissue infection that can lead to sepsis, shock, organ failure and death[2,3].The reported lethality of severe *S. pyogenes* infections is high, ranging from 10%–30% with 650,000 deaths occurring each year[4]. Skin invasion is mediated by erythrogenic exotoxins including streptolysins and streptococcal superantigens among others[5].

Antimicrobial photodynamic therapy (aPDT) employs the combination of a photosensitive agent and activating light source to destroy microbes as well as their endogenous virulence factors[6].The technique has been shownin a number of *in vitro* and in vivo studies to eradicate multidrug-resistant organisms across the prokaryotic spectrum[7,8,9]. Disinfection results from oxidative stress exerted on pathogen membranes which immediately disrupts function without genomic exposure[10].This kill mechanism avoids upregulation of microbial resistance [11].No known harmful effects occur in eukaryotic cells, making the technique uniquely suited to topical disinfection of patients[12]. The objective of this study was to demonstrate the efficacy of aPDT using two different photosensitizer formulations against the Group A Streptococcusin both planktonic and biofilm forms.

Materials and Methods

Bacterial culture

Streptococcus pyogenes (ATCC® 700951™) was received from ATCC and placed in tryptic soy broth (TSB)(Becton, Dickinson and Company, Franklin, NJ) for 18-24 hr incubation at 35 ± 2°C. After incubation, streak cultures were plated on tryptic soy agar (TSA) plates (Becton, Dickinson and Company, Franklin, NJ) and grown aerobically at 35 ± 2°C. After incubation, an inoculumwas prepared in phosphate buffered saline (PBS) and density adjusted to approximately 5×10^7 cells/mL using a spectrophotometer set to 420 nm (Genesys 10s, Thermo Scientific, Pittsburgh, PA).For biofilm growth, inoculum was adjusted to a cell density of 5×10^8-1×10^9 cells/mL and then diluted 1:40 in tryptic soy broth (TSB).

Test solutions and illumination system

The photosensitizer formulations (PS) used included a commercial formulation (Steriwave™ ND, Ondine Biomedical Inc., Vancouver BC) and a solution of 0.01% methylene blue USP in sterile water for injection. The control solution consisted of phosphate buffered saline (PBS) at pH 7.4.The illumination for the photodynamic disinfection procedure was provided by a 670nm non-thermal diode laser (PW1100 system, Ondine Biomedical Inc.) coupled to a light delivery handpiece via a 400 µm diameter glass fiberoptic cable. Output from the handpiece was calibrated to 200 ± 10 mW, and was verified prior to each experiment using an optical multimeter (ILX Lightwave, Bozeman, MT).Optical intensity was measured at 150 mW/cm^{2}at a distance of 7 mm from the emitting aperture of the handpiece using the same multimeter. All experiments were carried out with the surface of the test solutions located at this distance to ensure that light emission filled the entire surface of the solution.

Planktonic culture aPDT

Under dark room conditions, test and control solutions were prepared by adding 180 µL of (a) PBS, (b) 0.01% methylene blue in sterile water for injection, and (c) commercial PS, to triply spaced wells of black 96-well plates (VWR, Tualatin, OR) along with a 0.25-2mm magnetic stir bars. The wells were covered with sterile aluminum foil. Plates were placed on a magnetic stir plate (Corning PC420; Fisher Scientific, Pittsburgh, PA) set at 800 rpm and the light delivery handpiece positioned as described above over the first well. To conduct the test, one test well at a time was opened by using a pipette tip to break through and remove the foil covering the well, followed by addition of 20 µL of bacterial inoculum to the solution. Illumination was initiated for 60 sec while magnetically stirring. After illumination, samples were plated onto TSAplates and incubated at 37°C for 18 hrs prior to enumeration.

Biofilm culture aPDT

Under dark room conditions, 200 µL of working inoculum was added to each well of a 96-well plate and incubated while shaking at 125 rpm, 37°C for 48 hr. Media was changed after the first 24 hrs of incubation and visual confirmation of the presence of biofilm carried out at 48 hrs. The resulting biofilm was washed 3X with PBS. Plates were further prepared by adding 250 µL of PBS to each well to maintain biofilm hydration and then covered with sterile aluminum foil. Test solutions (a) PBS; (b) 0.01% methylene blue in

***Address for Correspondence:** Dr. C Romo, Ondine Research Laboratories, 19017 120th Ave N.E., Bothell, WA, USA, Tel: +14254891123; E-mail: cromo@ondinebio.com*

***Copyright:** © 2020 Cross J, et al. This is an open-access article distributed under the terms of the creative commons attribution license, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.*

Received 28 December, 2019; **Accepted** 23 April, 2020; **Published** 30 April, 2020

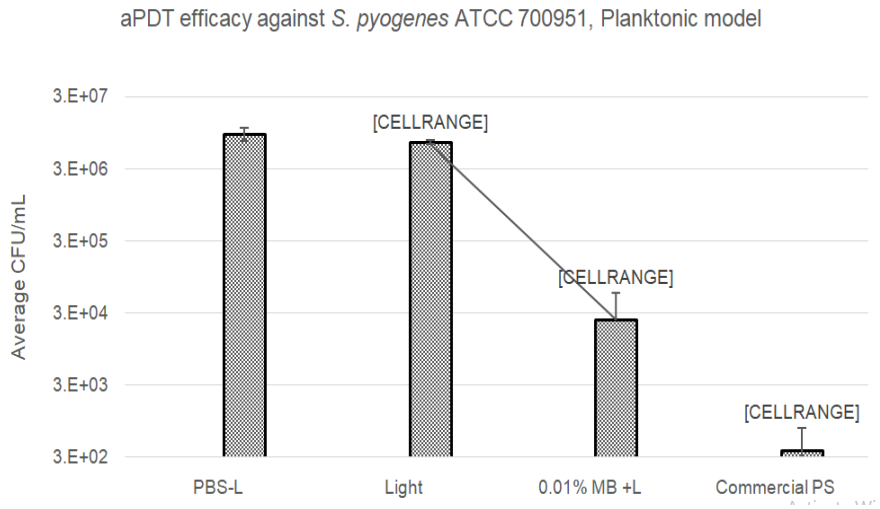

Figure 1. aPDT kill of *S. pyogenes* in a Planktonic Culture model. Values are mean (n=3) ± SD CFU/mL. Data on bars represent log10 reduction from control (PBS).

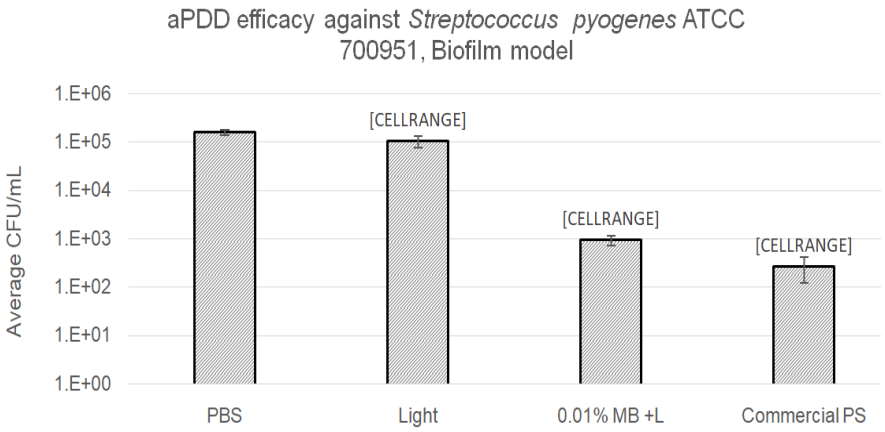

Figure 2. aPDT kill of *S. pyogenes* in a Biofilm Model. Values are mean (n=3) ± SD CFU/mL.Data on bars represent log_{10} reduction from control (PBS).

sterile water for injection; and (c) commercial PS were prepared. To conduct the test, one test well at a time was opened by using a pipette tip to break through the foil covering the well, followed by removal of PBS solution and addition of 250 µL of test solution which was allowed to incubate for 3.5 mins. Residual test solution was removed and illumination conducted for 4 min. Immediately following illumination, the biofilm surfaces within each treated well were swabbed using a sterile calcium alginate swab (Puritan Medical Products Co LLC, Guilford, ME) following a reproducible "X" pattern. Swab samples were added to 500 µL of recovery solution (10% Tween™-80, 3% lecithin, in 0.3% sodium thiosulphate), and disruption of biofilm was carried by vortexing for 10-30 sec, sonication for 15 min (Ultrasonicator250HT, VWRTualatin, OR), and a second vortexing for 10-30 sec before samples were serially diluted and plated on TSA plates. Plates were incubated at 37°C for 18 hrs prior to enumeration by colony counting. All test and control experiments were run in triplicate.

Statistical analysis

Raw counts for replicates of each experimental condition were averaged and data presented as CFU/mL of surviving organisms after treatment. Kill rate was calculated as surviving organisms expressed in CFU/mL divided by control (no light, no photosensitizer) values expressed in CFU/mL, and presented as log_{10}reduction value.

Results

Results for the eradication of *S. pyogenes* in both planktonic and biofilm models are shown in Figs. 1 and 2 respectively. In planktonic culture, aPDT was demonstrated to reduce *S. pyogenes* populations by an average of (a) 0.1 log_{10}[20.567%] for light alone vs. PBS control; (b) 4.19 log_{10}[99.993%] for MB 0.01% plus light vs. PBS control; and (c) 5.5 log_{10}[99.999%] for commercial photosensitizer solution plus light vs. PBS control. In biofilm culture, aPDT produced average reductions of (a) 0.2 log_{10}[36.904%] for light alone vs. PBS control; (b) 2.23 log_{10}[99.411%] for MB plus light vs. PBS control; and (c) 2.85log_{10}[99.859%] for commercial photosensitizer solution plus light vs. PBS control.

Discussion

S. pyogenes expresses a wide variety of virulence factors resulting in disease ranging from pharyngitis to severe invasive infections like necrotizing fasciitis [13,14].The US Centers for Disease Control and Prevention (CDC) estimate 11,000 to 24,000 cases of invasive group-A streptococcal disease occur each year, resulting in up to 1,900 deaths [15,16]. This morbidity has created an urgent need for novel antimicrobial approaches that do not rely upon existing antibiotics and are capable of broad-spectrum, rapidly-cidal activity.

Few studies have evaluated the effect of aPDT against *S. pyogenes* in either planktonic or biofilm forms, despite the relative importance of this microorganism to superficial skin infections.

Results of the present study demonstrate that antimicrobial photodynamic therapy can rapidly reduce *S. pyogenes* titers in both planktonic and biofilm culture when appropriate photosensitizer concentrations and light doses are used. Prior investigations [17-21] have also demonstrated the broad-spectrum efficacy of the technique, but the majority of these studies utilized photosensitizers not necessarily proven safe in humans, or with photosensitizer-light combinations resulting in poor clinical utility. For example, Kashef, et. alevaluated the effect of photodynamic inactivation in 14 clinical strains of

Methicillin Resistant *Staphylococcus aureus* (MRSA) and 26 clinical strains of *Staphylococcus aureus* susceptible to methicillin (MSSA) [22].Bacteria were exposed to 50 µg/mL methylene blue, pre-incubation time of 10 min and light intensity of 26 mW/cm^2 for 15 min. Despite these lengthy exposure times, kill levels still remained <1 log$_{10}$. In this work, our group demonstrated a >99.999% (>4 log$_{10}$ reduction) in 60 sec at 150 mW/cm^2 using either 0.01% MB or the commercial PS (a methylene blue-based photosensitizer formulation) against *S. pyogenes*. Previous studies by our group have demonstrated similar kills levels and exposure times in MSSA, MRSAand *P. aeruginosa* [11,23].

Antibiotic treatment failure of *S. pyogenes* infections has been demonstrated to be associated with biofilm formation [24]. Biofilm is characterized by the presence of extracellular polymeric substances including polysaccharides and proteins which are relatively heavily crosslinked, and therefore protect against environmental stress, dehydration and antibiotic effectiveness [25,26]. Methylene blue is a cationic small molecule which easily penetrates crosslinked, multi-species biofilms and can generate oxidative stress across the entire biofilm structurewhen appropriately illuminated. At the same time, aPDT has been shown to destroy extracellular virulence factors of bacteria such as protease and leukocidin, representing an additional advantage to antibiotics [27-29].

Conclusion

S. pyogenes is a ubiquitous human pathogen capable of causing life-threating infections. Results of this study indicate that aPDT is capable of rapidly and potently eradicating *S. pyogenes* in both planktonic and biofilm culture. Illuminated commercial photosensitizer solution produces significantly deeper kills than illuminated MB alone, and both photosensitizers produce significantly deeper kills than controls (PBS illuminated by light). These results provide support for future clinical testing of aPDT in the treatment of *S. pyogenes* infections.

Conflict of Interest Statement

All authors are employees of Ondine Biomedical Inc.

References

1. Ferretti, Joseph, Dennis Stevens, and Vincent Fischetti "The Streptococcal Proteome-*Streptococcus pyogenes*: Basic Biology to Clinical Manifestations" (2016).
2. Cunningham, Madeleine W "Pathogenesis of group A streptococcal infections" *Clin Microbiol Rev* 13(2000): 470-511.
3. Morgan, William, Michael Caldwell, Jennifer Brady, Mary Stemper et al. "Necrotizing Fasciitis due to a Methicillin-Sensitive *Staphylococcus aureus* Isolate Harboring an Enterotoxin Gene Cluster" *Journal of Clin Microb* 45(2007): 668-671.
4. www.cdc.gov/groupastrep/diseases-public/necrotizing-fasciitis.html
5. Terao, Yutaka "The virulence factors and pathogenic mechanisms of *Streptococcus pyogenes*" *J Oral Bios* 54(2012): 96-100.
6. Dai, Tianhong, Ying-Ying Huang, and Michael R. Hamblin "Photodynamic therapy for localized infections-state of the art" *Photodiagnosis Photodyn Ther* 6(2009): 170-188.
7. Skwor, Troy, Stephanie Klemm, Hanyu Zhang and Brianna Schardt, et al. "Photodynamic inactivation of methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*: a metalloporphyrin comparison" *J Photochem and Photobiol B* 165(2016): 51-57.
8. Belibasakis, Georgios and Thomas Thurnheer "Validation of antibiotic efficacy on *in vitro* subgingival biofilms" *J Periodontol* 85(2014): 343-348.
9. Cieplik, Fabian, Andreas Pummer, Christoph Leibl and Johannes Regensburger, et al. "Photodynamic inactivation of root canal bacteria by light activation through human dental hard and simulated surrounding tissue" *Front Microbiol* 7(2016): 929.
10. Castano, Ana, Tatiana Demidova, and Michael Hamblin "Mechanisms in photodynamic therapy: part two-cellular signaling, cell metabolism and modes of cell death" *Photodiagnosis Photodyn Ther* 2(2005): 1-23.
11. Pedigo, Lisa, Aaron Gibbs, Robert Scott and Cale Street "Absence of bacterial resistance following repeat exposure to photodynamic therapy. Proc. SPIE 7380, Photodynamic Therapy: back to the future" (2009).
12. Demidova, Tatiana, Michael Hamblin "Photodynamic therapy targeted to pathogens" *J Immunopathol Parmacol* 17(2004): 245-254.
13. Goldstein, Ellie, Daniel Anaya, and E. Patchen Dellinger "Necrotizing soft-tissue infection: diagnosis and management" *Clin Infect Dis* 44(2007): 705-710.
14. Ustin, Jeffrey S., and Mark A. Malangoni "Necrotizing soft-tissue infections" *Crit Care Med* 39(2011): 2156-2162.
15. www.cdc.gov/groupastrep/surveillance.html
16. www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf
17. Banfi, Stefano, Enrico Caruso, Loredana Buccafurni and Valeria Battini, et al. "Antibacterial activity of tetraaryl-porphyrin photosensitizers: an *in vitro* study on Gram negative and Gram positive bacteria" *J Photochem Photo Biol* 85(2006): 28-38.
18. Alves, Eliana, Liliana Costa, Carla MB Carvalho and João PC Tomé et al. "Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins" *BMC Microbiol* 9(2009): 70.
19. Bhatti, Manpreet, Alexander MacRobert, Sajeda Meghji and Brian Henderson, et al. "A study of the uptake of toluidine blue O by *Porphyromonas gingivalis* and the mechanism of lethal photosensitization" *Photochem Photobiol* 68(1998): 370-376.
20. Brooun, Alexei, Songhua Liu, and Kim Lewis "A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms" *Antimicrob Agents Chemother* 44(2000): 640-646.
21. Maisch, Tim, Claudia Bosl, Rolf Markus Szeimies and Norbert Lehn et al. "Photodynamic effects of novel XF porphyrin derivatives on prokaryotic and eukaryotic cells" *Antimicrob Agents Chemother* 49(2005): 1542-1552.
22. Kashef, Nasim, Mahboobeh Akbarizare, and Seyed Kamran Kamrava "Effect of sub-lethal photodynamic inactivation on the antibiotic susceptibility and biofilm formation of clinical *Staphylococcus aureus* isolates" *Photodiagnosis Photodyn Ther* 10(2013): 368-373.
23. Street, Cale, Aaron Gibbs, Lisa A Pedigo and Dane Andersen et al. "*In vitro* photodynamic eradication of *Pseudomonas aeruginosa* in planktonic and biofilm culture" *Photochem Photobiol* 85(2009): 137-143.
24. Conley, Joslyn, Merle E. Olson, Linda S. Cook and Howard Ceri, et al. "Davies Biofilm formation by group a streptococci: is there a relationship with treatment failure?" *J Clin Microbiol* 41(2003): 4043-4048.
25. Abdi-ali, Ahya, Mojgan Mohammadimehr and Y Agha Alaei "Bactericidal activity of various antibiotics against biofilm-producing *Pseudomonas aeruginosa*" *Int J Antimicrob Agents* 27(2006): 196-200.
26. You, Jian Lan, Xiao Li Xue, Li Xiang Cao and Xin Lu, et al. "Inhibition of Vibrio biofilm formation by a marine actinomycete strain A66" *Appl Microbiol Biotechnol* 76(2007): 1137-1144.
27. Shafreen, Raja Mohmed Beema, Shanmugam Srinivasan, Paramasivam Manisankar and Shunmugiah Karutha Pandian "Biofilm formation by *Streptococcus pyogenes*: modulation of exopolysaccharide by fluoroquinolone derivatives" *J Biosc Bioeng* 112(2011): 345-350.
28. Bartolomeu, Maria, Sónia Rocha, Ângela Cunha and Maria da Graça P M S Neves, et al. "Effect of photodynamic therapy on the virulence factors of *Staphylococcus aureus*" *Front Microbiol* 7(2016): 267.
29. Tubby, Sarah, Michael Wilson, and Sean P. Nair "Inactivation of staphylococcal virulence factors using a light-activated antimicrobial agent" *BMC Microbiol* 9(2009): 211.

How to cite this article: Cross, Julia, Cristina P Romo Bernal, Roger Andersen, Nicolas G. Loebel. "Antimicrobial Photodynamic Therapy for Rapid Eradication of *S. Pyogenes*." J Antimicrob Agents 6 (2020):187.