Photodynamic therapy in-vivo for multi-drug resistant wound infections

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Despite advances in wound management, infections remain a leading cause of mortality and morbidity in wound patients. Many wound infections, and in particular those caused by bacteria, are treated with antibiotics. Unfortunately, due to the excessive use of antibiotics, there are a growing number of microorganisms resistant to multiple classes of antibiotics. The initial and seemingly guaranteed success of antibiotics, the fruit of human ingenuity, has been countered by an escalation of resistance mechanisms in bacteria. This has led to the present time being described as the "end of the antibiotic era". Recently, the emergence of *S*. *aureus* strains resistant to vancomycin, which was considered as a last line of defense, has been documented. As a result, there is an urgent need for developing new treatment regimens to combat wound infections.

Photodynamic inactivation of microorganisms is based on the concept that a non-toxic dye, known as a photosensitizer (PS), should be localized preferentially in the bacteria and not in the surrounding host tissue or cells, and subsequently activated by low doses of visible light of an appropriate wavelength to generate free radicals or singlet oxygen that are toxic to target bacteria. The use of photodynamic therapy (PDT) is attractive for wound infections in that it is a non-pharmacological treatment that is likely to be non-injurious to wounds. Despite a century of using PDT to inactivate bacteria *in-vitro*, its use *in-vivo* to treat actual infections has hardly been developed.

The overall objective of this proposal is to explore this hypothesis: **Anti-microbial photo-dynamic therapy is an efficacious alternative option for treating multi-drug resistant wound infections**. The specific aims are to:

1) Use PDT *in vivo* to treat our established models of surgical (excisional) and burn wounds on the mice infected with *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* or *Staphylococcus aureus*, which are the species frequently encountered in wound infections and are clinically most problematic.

We have discovered a method of targeting polycationic PS conjugates to bacteria and subsequent illumination with red light produces up to 99.9999% of bacterial inactivation *in vitro*. Multi-drug resistant strains are as sensitive as naïve strains. In addition, we have been fortunate to be able to use a unique optical detecting technique, *in-vivo* bioluminescence imaging, to continuously monitor in real time the extent of infection. The bioluminescent intensity is linearly proportional to bacterial colony forming units (CFU), therefore, the extent of infection can be monitored in real time by using a photon counting ICCD camera. In an earlier study, we developed a mouse model of excisional wound infected with *P. aeruginosa*, which was found to be invasive. Since then, we have developed several new mouse models of different bacterial infections, including *P. aeruginosa* burn-wound infection (fatal infection), *A. baumannii* burn-wound infection (chronic infection), *S. aureus* burn-wound infection (chronic infection), and *A. baumannii* excisional wound infection in neutropenic mice (fatal infection).

PDT will be carried out at different times after infection to investigate efficacies of treatment for both early and established infections. For fatal infections, survival analysis will be conducted for different groups of mice. For chronic infections, the time courses of bio-burden (indicated by bioluminescence intensity), morbidity (indicated by body weight), and wound healing will be compared between different groups.

We have carried out preliminary studies on PDT of *A. baumannii* burn infection. Fig. 1A shows the dose response of bioluminescent intensity of a mouse burn infected with *A. baumannii* and treated with PDT. PDT was carried out at 24 hours after infection, allowing the bacteria to penetrate the burn and establish infection. After 240 J/cm^2 light of 660-nm wavelength had been delivered, approximately 99% inactivation of bacterial population was achieved as indicated by luminescence intensity. Fig. 1B compares the time course of bioluminescence intensity of the PDT treated mouse burn with that of a mouse burn without treatment. It can be seen that only modest re-growth occurred in the treated mouse burn after PDT, while in the untreated mouse burn the infection remained strong throughout the follow-up period (from day 2 to day 7).

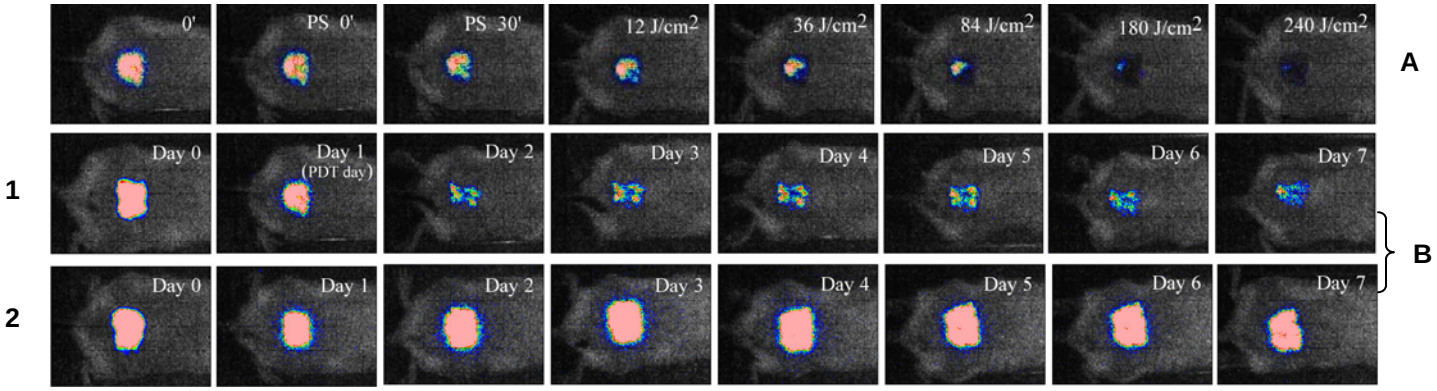

Fig. 1. A) Dose response of bioluminescent intensity of a mouse burn infected with *A. baumannii* and treated with PDT. PDT was carried out at 24 hours after infection. B) Time courses of bioluminescence intensity of the PDT treated mouse burn shown in Fig 1A and a mouse burn without treatment. 1: PDT treated burn shown in Fig. 1A; 2: un-treated burn.

2) Determine whether the wound pathogenic bacteria *P. aeruginosa, A. baumannii,* or *S. aureus* can develop resistance to PDT by repeated cycles of inactivation and re-growth of bacteria *in vitro*. Elucidate the mechanism of mutant development if bacterial resistance to PDT develops.

We will perform up to 20 successive cycles of *in vitro* inactivation and re-growth for each bacterial species. In the 1st growth-inactivation cycle, the PDT doses will be adjusted to leave about 1% surviving bacterial cells after irradiation and the same PDT doses will then be used throughout the PDT resistance study. If no increase in the survival rate of bacterial population is observed after 20 cycles of inactivation and re-growth, we will assume bacterial resistance to PDT dose not occur. If resistance develops, the hypothesis has been proposed that repeated cycles of PDT could select for bacterial strains/mutants with decreased outer membrane permeability. We will attempt to answer the question of whether the outer membrane has changed when and if PDT resistance is observed by using the method of measuring the uptake of the fluorescent probe 1-N-phenylnaphthylamine.

Accomplishing the specific aims outlined in this proposal will provide the foundation required to assess the efficacy (Aim 1) as well as possible side effects (Aim 2) of PDT for wound infections. Given the significant drawbacks in current treatments, this new technology has great potential. If this treatment option can be shown to be both effective and safe, public health would benefit tremendously including possible life saving intervention for those unfortunate people who die of a multi-drug resistant wound infection.