

Optical Monitoring and Treatment of Potentially Lethal Wound Infections In Vivo

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We report on the use of optical techniques to monitor and treat *Pseudomonas aeruginosa* wound infections in mice. Bioluminescent bacteria transduced with a plasmid containing a bacterial lux gene operon allow the infection in excisional mouse wounds to be imaged by use of a sensitive charge-coupled device camera. Photodynamic therapy (PDT) targeted bacteria, by use of a polycationic photosensitizer conjugate, which is designed to penetrate the gram-negative cell wall and was topically applied to the wound and was followed by red-light illumination. There was a rapid light dose-dependent loss of luminescence, as measured by image analysis, in the wounds treated with conjugate and light, a loss that was not seen in untreated wounds, wounds treated with light alone, or wounds treated with conjugate alone. *P. aeruginosa* was invasive in our mouse model, and all 3 groups of control mice died within 5 days; in contrast, 90% of PDT-treated mice survived. PDT-treated wounds healed significantly faster than did silver nitrate-treated wounds, and this was not due to either inhibition of healing by silver nitrate or stimulation of healing by PDT.

Photodynamic therapy (PDT) uses a combination of harmless dyes and visible light that, in the presence of oxygen, produces reactive oxygen species that damage biomolecules and kill cells [1]. Despite a century of use

of PDT to kill bacteria in vitro [2], its use to treat infections in vivo has not been developed [3]. We have discovered a method to target bacteria by use of polycationic photosensitizer conjugates, and subsequent illumination with modest levels of red light produces 6 logs of bacterial killing in vitro [4]. The method is based on the covalent attachment of photosensitizer peptides to polycationic peptides, such as poly-L-lysine, that can bind to and penetrate both gram-positive and gram-negative bacteria. We recently reported on the use of these conjugates and red-light illumination to destroy *Escherichia coli* infections in mouse wounds [5]. Because these macromolecular conjugates were administered topically into sites of infection, and because the time needed to bind to bacteria was relatively short, the treatment showed good selectivity for bacteria, compared with that of host tissue. We used genetically engineered bacteria that emit bioluminescence and that can be detected in vivo by use of an intensified charge-coupled device (CCD) camera [6]. Quantification of the luminescence images can determine, in real time, the extent of infection in living animals and thereby can provide both temporal and spatial information

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M.R.H. and T.H. are inventors on US patent 6,462,070: photosensitizer conjugates for pathogen targeting.

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