Antimicrobial Blue Light Inactivation of *Pseudomonas aeruginosa* by Photo-Excitation of Endogenous Porphyrins: In Vitro and In Vivo Studies

Rehab M. Amin, PhD,[1,2,3]* Brijesh Bhayana, PhD,[1,2] Michael R. Hamblin, PhD,[1,2] and Tianhong Dai, PhD[1,2]**
[1]*Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts 02114*
[2]*Department of Dermatology, Harvard Medical School, Boston, Massachusetts 02114*
[3]*National Institute of Laser Enhanced Science, Cairo University, Giza 12613, Egypt*

Pseudomonas aeruginosa is among the most common pathogens that cause nosocomial infections and is responsible for about 10% of all hospital-acquired infections. In the present study, we investigated the potential development of tolerance of *P. aeruginosa* to antimicrobial blue light by carrying 10 successive cycles of sublethal blue light inactivation. The high-performance liquid chromatographic (HPLC) analysis was performed to identify endogenous porphyrins in *P. aeruginosa* cells. In addition, we tested the effectiveness of antimicrobial blue light in a mouse model of nonlethal skin abrasion infection by using a bioluminescent strain of *P. aeruginosa*. The results demonstrated that no tolerance was developed to antimicrobial blue light in *P. aeruginosa* after 10 cycles of sub-lethal inactivation. HPLC analysis showed that *P. aeruginosa* is capable of producing endogenous porphyrins in particularly, coproporphyrin III, which are assumed to be responsible for the photodynamic effects of blue light alone. *P. aeruginosa* infection was eradicated by antimicrobial blue light alone (48 J/cm^2) without any added photosensitizer molecules in the mouse model. In conclusion, endogenous photosensitization using blue light should gain considerable attention as an effective and safe alternative antimicrobial therapy for skin infections. Lasers Surg. Med. 48:562–568, 2016. © 2016 Wiley Periodicals, Inc.

Key words: *Pseudomonas aeruginosa*; blue light; endogenous porphyrins; drug resistance; mouse

INTRODUCTION

Pseudomonas aeruginosa is one of the most common causes of infection, especially in patients with compromised host defenses [1]. The increased number of patients diagnosed as immuno-suppressed, coupled with those subjected to invasive medical techniques, and those at high risk of surgical site infections, has contributed to the rise in acquired *Pseudomonas* infections.

Although microbiologists have been ringing the alarm bell for years, the threat of resistance to antimicrobial drugs in healthcare settings has reached such new prominence in the popular press that the issue should be added to the list of global emergencies [2–6]. *P. aeruginosa* infections are among the most difficult to treat because effective therapeutic options are either very limited or nonexistent. There is consequently a critical need for the development of new therapeutics to tackle drug resistance [6,7]. Antimicrobial photodynamic therapy (aPDT) [8–12] has been extensively investigated as an alternative for localized infections. However, the major disadvantages of PDT are (i) the sub-optimal uptake of photosensitizers by bacteria; and (ii) the lack of selectivity of many photosensitizers for bacterial cells over host cells [13]. Recently, antimicrobial blue light therapy (aBLT) has attracted considerable attention due to its intrinsic antimicrobial effect without the involvement of exogenous photosensitizers [14,15–17]. A common hypothesis regarding the mechanism underlying the antimicrobial effect of blue light is that the natural endogenous photosensitizers, mainly porphyrins, are converted to their triplet state when exposed to light. These excited photosensitizers may generate free radicals or superoxide ions resulting from hydrogen or electron transfer (Type I), and/or they can produce singlet oxygen (Type II), all of which can react with cellular components and cause microbial cell death [8,18].

In a previous study, we investigated the effectiveness of antimicrobial blue light for treatment of lethal third degree *P. aeruginosa* burn infections in mice [14]. In the present study, we investigated the potential development of tolerance of *P. aeruginosa* to antimicrobial blue light by

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*Correspondence to: Rehab M. Amin, PhD, National Institute of Laser Enhanced Sciences, Cairo University, Giza 12316, Egypt. E-mail: rehabamin@niles.edu.eg
**Correspondence to: Tianhong Dai, PhD, Harvard Medical School, Boston, MA 02114. E-mail: tdai@mgh.harvard.edu
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