Antimicrobial Blue Light Inactivation of *Neisseria gonorrhoeae*: Roles of Wavelength, Endogenous Photosensitizer, Oxygen, and Reactive Oxygen Species

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Background and Objectives: The aim of this study was to investigate the efficacy, safety, and mechanism of action of antimicrobial blue light (aBL) for the inactivation of *Neisseria gonorrhoeae*, the etiological agent of gonorrhea.

Study Design/Materials and Methods: The susceptibilities of *N. gonorrhoeae* (ATCC 700825) in planktonic suspensions to aBL at 405- and 470-nm wavelengths were compared. The roles of oxygen in the anti-gonococcal activity of aBL were studied by examining the effects of hypoxic condition (blowing N_2) on the anti-gonococcal efficiency of 405-nm aBL. The presence, identification, and quantification of endogenous photosensitizers in *N. gonorrhoeae* cells and human vaginal epithelial cells (VK2/E6E7 cells) were determined using fluorescence spectroscopy and ultra-performance liquid chromatography (UPLC). Finally, the selectivity of aBL inactivation of *N. gonorrhoeae* over the host cells were investigated by irradiating the co-cultures of *N. gonorrhoeae* and human vaginal epithelial cells using 405-nm aBL.

Results: About 3.12-$\log_{10}$ reduction of bacterial colony forming units (CFU) was achieved by 27 J/cm^2 exposure at 405 nm, while about 3.70-$\log_{10}$ reduction of bacterial CFU was achieved by 234 J/cm^2 exposure at 470 nm. The anti-gonococcal efficacy of 405-nm aBL was significantly suppressed under hypoxic condition. Spectroscopic and UPLC analyses revealed the presence of endogenous porphyrins and flavins in *N. gonorrhoeae*. The concentrations of endogenous photosensitizers in *N. gonorrhoeae* (ATCC 700825) cells were more than 10 times higher than those in the VK2/E6E7 cells. In the co-cultures of *N. gonorrhoeae* and VK2/E6E7 cells, 405-nm aBL at 108 J/cm^2 preferentially inactivated *N. gonorrhoeae* cells while sparing the vaginal epithelial cells.

Conclusions: aBL at 405-nm wavelength is more effective than 470-nm wavelength in inactivating *N. gonorrhoeae* while sparing the vaginal epithelial cells. Reactive oxygen species generated from the photochemical reactions between aBL and endogenous photosensitizers play a vital role in the anti-gonococcal activity of 405-nm aBL. Lasers Surg. Med.

Key words: antibiotic resistance; antimicrobial blue light; *Neisseria gonorrhoeae*; endogenous photosensitizers; porphyrins; flavins; reactive oxygen species; singlet oxygen

INTRODUCTION

Gonorrhea is the second most prevalent sexually transmitted infection globally [1]. Despite the public health efforts to control gonorrhea for 70 years, these infections remain a significant public health concern. In 2015, a total of 395,216 cases of gonorrhea were reported in the United States. Worldwide, 106.1 million people are affected by gonococcal infections annually [2]. If gonococcal infections are not appropriately treated, they can result in severe complications and sequelae such as salpingitis and pelvic inflammatory disease, which may lead to sterility and/or ectopic pregnancy. In addition, epidemiologic and biologic studies have provided evidence that the failure to curb the transmission of gonorrhea facilitates the transmission of HIV infection [3]. Repeated infections are common and no state of protective immunity appears to develop as a consequence of infection. Since there is no gonococcal vaccine, treatment of gonorrhea relies especially on antibiotics. However, *Neisseria gonorrhoeae*, the etiological agent of gonorrhea, is evolving into a superbug and may become untreatable due to its resistance to almost all the antibiotics previously and currently widely used (e.g., sulfonamides, penicillins, earlier cephalosporins, tetracyclines, macrolides, and fluoroquinolones) [4]. As such, the

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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