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Original Article

5-Aminolevulinic acid induced photodynamic inactivation on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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ABSTRACT

The aim of the present study was to develop a simple and fast screening technique to directly evaluate the bactericidal effects of 5-aminolevulinic acid (ALA)-mediated photodynamic inactivation (PDI) and to determine the optimal antibacterial conditions of ALA concentrations and the total dosage of light *in vitro*. The effects of PDI on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the presence of various concentrations of ALA (1.0 mM, 2.5 mM, 5.0 mM, 10.0 mM) were examined. All bacterial strains were exponentially grown in the culture medium at room temperature in the dark for 60 minutes and subsequently irradiated with 630 ± 5 nm using a light-emitting diode (LED) red light device for accumulating the light doses up to 216 J/cm^2. Both bacterial species were susceptible to the ALA-induced PDI. Photosensitization using 1.0 mM ALA with 162 J/cm^2 light dose was able to completely reduce the viable counts of *S. aureus*. A significant decrease in the bacterial viabilities was observed for *P. aeruginosa*, where 5.0 mM ALA was photosensitized by accumulating the light dose of 162 J/cm^2. We demonstrated that the use of microplate-based assays—by measuring the apparent optical density of bacterial colonies at 595 nm—was able to provide a simple and reliable approach for quickly choosing the parameters of ALA-mediated PDI in the cell suspensions.

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1. Introduction

The widespread inappropriate use of antibiotics has resulted in multiresistant bacterial strains and increased rates of infection [1,2]. This health care problem is therefore particularly urgent because there is a clear need for a more effective anti-infective strategy against these organisms. Many of the new antibiotics are more potent, but they also increase the risk of systemic toxicity. The versatility and potency of photodynamic therapy (PDT) may be an interesting alternative choice against many types of microorganisms and the lack of resistance with repeated use [3]. Basically, PDT is the result of the use of three autonomously nonactive elements in combination: (1) a nontoxic photoactive molecule called a photosensitizer (PS); (2) light of the appropriate wavelength to excite the PS; and finally, (3) oxygen, which is transformed into the highly reactive singlet oxygen species upon energy transfer from the light-activated PS.

The photodynamic effect uses nontoxic dyes or PS in combination with exposure to harmless visible light in the presence of oxygen to induce the generation of highly reactive, cytotoxic reactive oxygen species, causing selective photodamage to tumor tissues or leading to localized cell death [4]. It provides the following advantages for treatment of microbial infections: (1) broad light spectrum of action; (2) efficient inactivation of antibiotic-resistant strains; (3) low mutagenic potential; and (4) less likelihood of inducing photoresistant cells [5,6]. Bacterial organisms such as Gram-positive bacteria can be killed by photodynamic inactivation (PDI) *in vitro* with exogenous PS such as porphyrins. The complex outer membrane of Gram-negative bacteria acts as a barrier that hinders the PS to transport through the cell membranes; hence, Gram-negative bacteria appear to be less sensitive to the lethal action of PDI with exogenously supplied porphyrins [7–9].

5-Aminolevulinic acid (ALA) is a naturally occurring intermediate in the hemesynthesis pathway [10]. It is a precursor of porphyrins that can be biosynthesized in nearly all aerobic cells in mammals. The first topical application of ALA in the treatment of basal cell carcinoma was reported in 1990 [11]; since then, the clinical use of ALA-PDT continues to grow. ALA-PDT has been widely studied and marketed around the world [12], and the methyl-ester derivative of ALA has been approved for the treatment of basal cell carcinoma and actinic keratosis [13]. In addition, the European Medicines Agency has approved the hexyl-ester derivative of ALA for diagnostic application in endoscopic photodynamic detection of bladder cancer and ALA for intraoperative photodiagnosis of residual malignant glioma [14].

The reasons why ALA was extensively used in the field of PDT can be summarized as follows: (1) ALA is the only PDT agent that is a biochemical precursor of a PS, which is naturally produced by the body, and alone shows low dark toxicity to cells; (2) the topical delivery of ALA does not induce any prolonged photosensitivity reactions, because the drug can be selectively applied in areas to be treated; (3) endogenously produced protoporphyrin IX is rapidly cleared from the body (24–48 hours), because it has a natural clearance mechanism; and (4) the short time interval (1–8 hours, depending on the mode of administration) needed between the administration of ALA and the maximal accumulation of protoporphyrin IX in target tissues makes ALA attractive for patients.

Furthermore, ALA has been shown to have considerable photobactericidal activity. Compared to exogenously administered hydrophobic porphyrin derivatives, ALA is highly water soluble and may enter the intracellular compartment of Gram-negative bacteria through the hydrophilic pores of its outer membrane [15]. Treatment with exogenous ALA could effectively accumulate considerable amounts of photoactive porphyrins (PAPs) within the targeted cells [16]. Under the irradiation of the appropriate wavelength of light, the accumulated porphyrins will induce PDI to destroy the cells [17]. Recently, a few reports showed that ALA could induce PDI effectively against various kinds of bacterial strains such as Gram-positive *Staphylococcus aureus* and Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* [18–24].

Although PDI of bacteria has been known for more than 100 years [25], its use for treatment of infections has not been extensively developed [26]. This may be partly attributable to the lack of a standardized and reliable *in vitro* screening method to evaluate the antibacterial efficacy of PDT. Our study aimed to assess the effectiveness of ALA-mediated PDI on *S. aureus* and *P. aeruginosa* by directly judging the apparent optical density (OD) caused by light scattering of colonies and further to determine the optimal antibacterial conditions of ALA doses and light exposure *in vitro*. We have developed a more economic and rapid *in vitro* screening technique to evaluate the antimicrobial activity of ALA-PDT in contrast to the traditional antibacterial susceptibility testing.

2. Materials and methods

2.1. Materials

ALA and phosphate buffer solution (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nutrient broth medium (BD 234000) and nutrient agar medium (BD 4311472) were purchased from Difco (Detroit, MI, USA).

2.2. Preparation of ALA solution

A stock solution of 100 mM ALA was prepared by dissolving ALA in 0.1 M PBS (pH 7.4) prior to the experiment. The ALA stock and diluted solutions were used within 2 hours after the preparation to ensure its stability.

2.3. Bacterial strains and growth conditions

This study was conducted with a Gram-negative strain (*P. aeruginosa*; American Type Culture Collection Strain 27853) and a Gram-positive strain (*S. aureus*; American Type Culture Collection Strain 29213) purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan). The strains were grown in nutrient broth separately for 24 hours at 37°C in a humidified atmosphere of 95% air and 5% CO_2 controlled by a low-temperature incubator (LE-509; YIH DER Instruments, Taipei, Taiwan). The broth cultures were then spread on nutrient agar medium and then incubated at 37°C