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Mini Review

Keywords

Porphyromonas gingivalis,
Blue light,
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Protoporphyrin IX

Potential use of antimicrobial photodynamic therapy in dentistry

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Abstract

After antibiotics were discovered as a method of sterilization microorganisms, the interest of people has been diminished from the research and application of photodynamic sterilization method. However, the necessity for the innovative antimicrobial strategies instead of the conventional antibiotics have required, because the speed of microbial antibiotic resistance acquisition is recently to be on the increase. Therefore, the photodynamic therapy has been paid attention again. Also, antibacterial photodynamic therapy (aPDT) is starting to be applied to the periodontal treatment in the dental field. However, the main evidences of aPDT in dentistry are the clinical reports by practitioner, therefore, little consideration is given to the sterilization mechanism and safety for the body. In this review, we introduce that background of aPDT and our report of the examination of bactericidal mechanism of aPDT applying periodontal bacterial pigment and its sterilizing capacity.

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Photodynamic therapy (PDT) has been listed by Japan's national health insurance as a treatment for lung, stomach, esophagus, cervix, and bladder cancer since 1996, and for brain cancer since 2014. In the United States, the Food and Drug Administration (FDA) approved the use of some photosensitizing drugs for PDT in 2003. In the same year, the Japanese Ministry of Health, Labor, and Welfare approved the domestic use of photosensitizers and semiconductor lasers, which were already in use for ophthalmic PDT in Western countries. In addition, the Japan Ophthalmological Society has established guidelines for the appropriate implementation of, and prevention of side effects induced by, light exposure[1)].

Cytotoxicity caused by the photosynthesis of compounds and visible light by microorganisms was established early in the last century. Von Tappeiner and Jadblauer reported incidents of phototoxicity that were not due to heat. In 1904, von Tappeiner coined the term "photodynamic response" to describe the reaction of non-toxic photosensitizers to visible light[2,3)]. This reaction was believed to be an effect of microorganisms.

The first antimicrobial substance discovered was penicillin, which marked the beginning of the "golden age" of antibiotics. However, there has been a severe lack of control in the use of antibiotics, and they have been greatly abused for applications such as addition to livestock feed and unnecessary prescriptions for viral infections, leading to increasing rates of antibiotic resistance in microorganisms globally. The discovery of new antibiotics cannot keep up with the speed at which microorganisms develop antibiotic resistance.

This phenomenon has prompted research into the development of new antibacterial strategies, such as antimicrobial PDT (aPDT)[4].

In particular, aPDT may become a substitute for local antibiotic oral and skin infection treatments[5]. It was suggested the possibility of PDT application in medicine as early as the 1970s, when the idea of using its photodynamic effect to selectively destroy malignant tumors was considered[6]. It is thought that tumor cells and microorganisms share a high proliferation rate and an active metabolism. Since microorganisms are able to accumulate different photosensitizers, it is believed that photodynamic inactivation may be an effective antibacterial approach[7].

Even though bactericidal photodynamic effects have been known for a long time, interest in the potential applications of these effects has increased recently[8-11]. The development of resistance to aPDT is unlikely to occur, since in microbial cells, reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) and some free radicals, interact with several cell structures and different metabolic pathways. aPDT is equally effective against antibiotic-resistant and antibiotic-susceptible bacteria, and repeated photosensitization has not been found to induce the selection of resistant bacterial strains[12]. Against this background, there has also been interest in the use of aPDT in dentistry, as a treatment for *Porphyromonas gingivalis* (*P. gingivalis*), a periodontal pathogen.

Periodontal disease is a chronic inflammatory infection that affects the gingiva and is associated with a loss of gingiva, periodontal ligament connective tissue, and alveolar bone[13]. Recently it has been found that periodontal disease is associated with an increased risk of systemic symptoms, such as coronary heart disease and diabetes, so the disease burden of *P. gingivalis* in the body may be higher than previously thought[14-16]. In addition, *P. gingivalis* is widely recognized to cause the production of the black pigment used by anaerobic gram-negative bacteria involved in the initiation and progression of periodontal disease[15, 17-19]. Dental aPDT is a sterilization treatment method involving staining the target bacteria with a dye (methylene blue), and then using the ROS generated by photoexcitation with a red light (670 nm) to kill the affected cells[20, 21]. However, conventional aPDT does not have a bactericidal effect on periodontal debridement sites, which cannot be stained. Furthermore, methylene blue also stains the nuclei of vital living cells, causing cytotoxicity by the photoexcitation-induced ROS[22]. In addition, conventional aPDT is mainly a clinical method conducted by clinical dentists, and there has been little basic research on the bactericidal mechanisms and safety of this procedure[23]. Since treatment guidelines for aPDT for periodontal disease have not yet been developed, the Japanese Ministry of Health, Labor, and Welfare has not yet approved dental aPDT, but this treatment is nonetheless offered to patients.

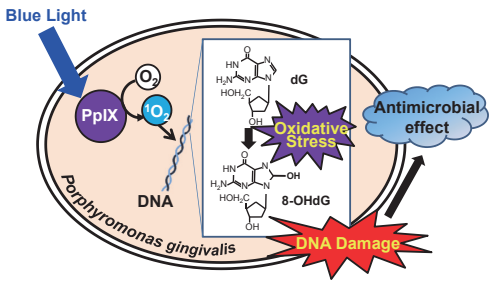

Fig 1. Blue-light-irradiation-induced antimicrobial mechanisms via protoporphyrin IX (PpIX) in *Porphyromonas gingivalis*. Modified from *Sci Rep*, 7(1): 5225, 2017. dG indicates deoxyguanosine and 8-OHdG indicates 8-hydroxy-2'-deoxyguanosine.

P. gingivalis is a bacterium that produces a black pigment derived from porphyrin[15,17-19]. Both gram-negative and gram-positive bacteria are reported to incorporate porphyrin in order to metabolize energy from their host. Furthermore, it has been reported that 5-aminolevulinic acid, a porphyrin precursor and amino acid, is metabolized to porphyrin when it is systemically administered to mice. PDT has also been used to improve skin ulcers caused by methicillin-resistant *Streptococcus aureus* infections[24]. Based on the above, we have focused on the porphyrin of *P. gingivalis*, which contains an essential pigment for bacterial energy metabolism, and have investigated the effects of aPDT on it. We found that the porphyrin concentration of the dye present in the bacterium depends on the number of bacteria, and the fluorescence in response to the blue light depends on the porphyrin concentration. We confirmed the blue-light-irradiation-intensity-dependent generation of $^1O_2$ by protoporphyrin IX (PpIX), and have discovered that blue-light irradiation of *P. gingivalis* is bactericidal at 100 Joule. The sterilization mechanism was oxidative damage of the DNA by the $^1O_2$ generated in the bacterial cells[25] (Fig. 1). As discussed above, aPDT have not been regarded to induce the development of resistant bacteria. We

therefore used the conventional method for additive photosensitizer dye. However, it is possible that dye-resistant bacteria that have a mechanism for resisting uptake of the photosensitizer might develop.

PpIX in *P. gingivalis* is used for energy production. It is likely that this aPDT treatment method, applied to the PpIX of *P. gingivalis*, carries a low risk of emergence of resistant bacterial strains. This approach might therefore suggest an effective treatment not only for new periodontal diseases, but also for antibacterial treatment against the multiple-drug-resistant bacteria that continue to proliferate.

Conflict of interest

The authors declare that they have no competing interests.

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