Abstract

Possibility of application of 310 nm ultraviolet light-emitting diode
as a new treatment and prevention tool for periodontitis
- A study of bactericidal effects on oral bacteria -

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【Introduction】

Ultraviolet (UV) light is used for phototherapy in dermatology, and Narrowband (NB)-UVB light is effective for treatment of psoriasis and atopic dermatitis. NB-UVB light, the wavelength of which is around 310 nm with a narrow peak, has less side effects for humans than broadband (BB)-UVB light and has a greater therapeutic effect than these of other UV therapies. NB-UVB may also be useful for treatment of oral mucosal disorders such as periodontitis, but there has been no report on the use of NB-UVB in dentistry. It has been shown that UVC light has bactericidal effect. The peak of DNA absorption of UV light is 260 nm and its wavelength impairs bacterial DNA by forming cyclobutane pyrimidine dimers (CPD). The DNA damage also leads to the repression of its transcription, replication and finally induces to cell death. UVB light has the same effects to DNA, however, there have been few studies on the bactericidal effect of UVB, especially on the effect on oral bacteria. We hypothesized that UVB would show bactericidal action like UVC, and we produced a small NB-UVB (310 nm) LED device for intraoral use to examine the bactericidal effects on oral bacteria. In this study, we examined the bactericidal effects and mechanism of UVB-LED irradiation on oral bacteria to explore the possibility of using a 310 nm UVB-LED device for treatment of oral infectious diseases. This is the first study on the possibility of a narrowband UVB-LED device for intraoral use.

【Materials and Methods】

Experiment using a prototype device #1 Experiment using a prototype device #2

1. Bactericidal effects : We investigated bactericidal effects on *S. mutans, S. sauguinis, P. gingivalis, and F. nucleatum* at the states of Planktonic by the 310 nm UVB-LED device for intraoral use (Prototype device #1was provided by NIKKISO Co. Ltd.). And we investigated bactericidal effects on these bacteris at the states of Planktonic and Biofilm using the prototype device #2 (NIKKISO, Co. Ltd.).

2. Detection of CPD in bacteria : We measured CPD formation in bacteria irradiated with 310 nm UVB-LED by using High Sensitivity CPD ELISA kit Ver.2 (COSMOBIO, Tokyo).
3. Cytotoxicity to oral epithelial cells : We investigated cytotoxicity of 310 nm UVB-LED on human oral squamous epithelial carcinoma cell line Ca9-22 by measuring the formation of formazan in the cells.

4. NO and H_2O_2 induction in oral epithelial cells : The production of NO by 310 nm UVB-LED irradiation in Ca9-22 cells was detected using DAF2-DA. We also

examined the production of iNOS in Ca9-22 cells by immunostaining and Real-time-PCR. The production of H_2O_2 by 310 nm UVB-LED irradiation in Ca9-22 cells was also measured by a kit.

5. Bactericidal effects of NO and H_2O_2 : The bactericidal effects of NO and H_2O_2 on oral bacteria were examined.

6. Expression of Claudin-1 by 310 nm UVB-LED irradiation: We examined production of Claudin-1in immortalized human gingival epithelial cell line (OBA-9) irradiated with 310 nm UVB-LED by Western blotting.

【Results】

At Experiment, irradiation by the 310 nm UVB-LED 105 mJ/cm^2 (for 60 sec) of, viabilities of *S. mutans* and *P. gingivalis* were decreased to 40-50%. On the other hand, irradiation of 265 nm UVC at 17 mJ/cm^2 (for 10 sec) killed more than 97% of these oral bacteria. In floating bacterial cultures, all tested bacteria were suppressed the viability by irradiation with 310 nm UVB-LED at 1050 mJ/㎠ (60 sec). Especially, the %avility of *P. gingivalis* was strongly reduced after the irradiation. In addition, 310 nm UVB-LED irradiation (1050 mJ/cm^2, 60 sec) significantly induced the formation of CPD in *P. gingivalis*. Irradiation with the 310 nm UVB-LED (420 mJ/㎠ and 1050 mJ/㎠) also had bactericidal effects on biofilms of all tested bacteria. Irradiation with 310 nm UVB-LED at 105 mJ/cm^2 (for 60 sec) did not have any cytotoxicity to Ca9-22 cells, though irradiation with the 265 nm UVC-LED at 17 mJ/cm^2 (for 10 sec) showed strong cytotoxicity to the cells. Production of NO and H_2O_2 was observed by 310 nm UVB-LED irradiation at 105 mJ/cm^2 (60 sec) in Ca9-22 cells. Addition of the NO and H_20_2 for 1 h in the cultures strongly killed *P. gingivalis*, but other bacteria were not killed by the treatment. Furthermore, the expression of claudin-1 protein was induced in OBA-9 cell cultures irradiated with 10.5 mJ/㎠ of 310 nm UVB-LED.

【Discussion】

Low dose (105 mJ/㎠) of 310 nm UVB-LED irradiation using the prototype device #1 showed bactericidal effect on oral bacteria weaker than the same dose of 265 nm UVC-LED irradiaiton. On the other hands, high dose (1050 mJ/㎠) of 310 nm UVB-LED irradiation using the prototype device #2 elicited bactericidal effect on biofilm of oral bacteria as well as on planktonic state of the bacteria. General sterilization agents like mouth wash do not exhibit sufficient bactericidal effects, then the effects on oral biofilm is an advantage of UVB-LED

irradiation against those reagents. Cytotoxicity of UVB-LED irradiation was observed at over 210 mJ/cm^2 of UVB-LED. However, the outermost layer of the oral epithelium has a stratum corneum stratum structure. Therefore, it is considered that it is possible to use further irradiation in the oral cavity, and enhancement of bactericidal effect can be expected.
Since 310 nm UVB-LED irradiation induces the production of reactive oxygen species from oral epithelial cells and it may contribute to the sterilization and growth suppression of anaerobic bacteria, such as periodontal pathogenic bacteria. Especially, *P. gingivalis* is sensitive to ROS and generates many CPD in responses to the irradiation, then 310 nm UVB-LED irradiation is effective for *P. gingivalis* antibacterial effects. Therefore, 310 nm UVB-LED irradiation is useful as a device for selective sterilization for periodontal pathogens including *P. gingivalis*. Recently, the relationship between decrease of intercellular adhesion molecule expressions in junctional epithelium of gingiva and pathogenesis of periodontal disease. The decrease of barrier function in gingival epithelium may contribute to the pathogenesis of periodontal disease. In this study, low dose of 310 nm UVB-LED irradiation induced the elevated expression of Claudin-1 in oral epithelial cells. These results suggest that 310 nm UVB-LED suppresses the onset and progression of periodontitis by inhibiting the invasion of bacteria and the sensitization with various antigens in gingival epithelium.

【Conclusion】

Irradiation with 310 nm UVB-LED exhibits direct bactericidal effect by inducing profound amount of CPD formation, and indirect bactericidal effect by inducing the production of ROS from oral epithelial cells, which in turn, enhanced bactericidal activity of UVB-LED to *P. gingivalis*. In addition, 310 nm UVB-LED irradiation exhibited low cytotoxicity to gingival epithelial cells, induced the production of Claudin-1 and enhanced the barrier function in gingival epithelium. Therefore, 310 nm UVB-LED device may be useful as a new therapeutic device for the prevention and treatment in periodontitis.