Therapeutic effect of Photodynamic therapy using Na-Pheophorbide a on osteomyelitis models in rats

Introduction
Osteomyelitis is a most grave infectious problem in the orthopaedic field. Although, surgical treatment, such as debridement, has been tried as a treatment for osteomyelitis, sufficient clinical results have not been reached in many of these cases. The most commonly isolated organism of osteomyelitis is Staphylococcus aureus. To obtain better clinical results, new approaches for the treatment of osteomyelitis are required. One possible approach is photodynamic therapy (PDT). Recently, we demonstrated that the combined use of semiconductor laser irradiation and Na-pheophorbide a as a sensitizer exhibited antibacterial efficacy against MRSA in vitro. Combination treatment with Na-Pheophorbide a (Na-Phde a) and laser irradiation completely eradicated MRSA.

The objective of this study was to evaluate the effectiveness of PDT using Na-Phde a on osteomyelitis models in rats. Our hypothesis was that the PDT using Na-Phde a would be an effective treatment of osteomyelitis.

Materials and methods
Ten (10) eight-week-old female SD rats were used and were kept in quarantine for one week before the study was started. A standard Methicillin sensitive Staphylococcus aureus (MSSA) strain was obtained from the American Type Culture Collection (ATCC25923). Culture of this MSSA strain was carried out for 24 hours at 37°C on a blood-agar medium. Bacterial cells were adjusted to a density of No.1 McFarland (1.0x10⁸ CFU/ml) for use in experiments.

Osteomyelitis models in rats were created as previously reported (n=10, 10 knees). 0.1 ml of MSSA bacterial solution was injected into the tibia through the bone tunnel, and the skin was sutured with surgical nylon stitches. 48 hours after this treatment, 5 of these rats were treated with PDT and the other 5 rats were used as a control group. Na-Phde a was used as a photosensitizer. Na-Phde a was dissolved in a phosphate buffered saline (PBS) to make final concentrations of 560 µmol/L. A visible light GaAlP semiconductor laser (670 nm, 125 mW, Panasonic co., Ltd., Tokyo, Japan) was used in this study. Same laser device was used in several previous reports. The laser was irradiated for 10 minutes.

In the PDT group (n=5, 5 knees), 48 hours after MSSA injection, a 0.1 ml of 560µmol/L concentration of Na-Phde a solution was injected into the bone tunnel of the tibia. The animals were kept in a darkroom for 30 minutes in order to allow the uptake of Na-Phde a to the bacteria and infected tissue. After that, laser irradiation was performed for 10 minutes directly through the bone tunnel. In the control group (n=5, 5 knees), 0.1 ml of phosphate buffered saline (PBS) was injected into the initial tibial tunnel at 48 hours after the MSSA injection. No laser irradiation was performed.

Rats were clinically monitored for leg swelling and body weight at days 0, 2, 3, 4, 6, 8, 12, 14. Tibial bone condition was evaluated using x-ray imaging at 1, 7, and 14 days after MSSA infection. Radiographic analyses were performed using the techniques of Smeltzer et al. All animals were sacrificed at 14 days after MSSA infection. Bone marrow was collected from the initial bone tunnel of the tibia and bacterial culture was performed in microaerobic conditions for 48 hours. Lower extremities were cut at the middle of their femoral bone, and then examined histopathologically. Histological evaluation was graded as reported by Smeltzer et al.

Data is presented as the mean ± standard deviation. Man-Whitney’s U test was used for statistical analysis. Values were considered significantly different at p < 0.05.

Results
From 3 days after MSSA injection to the time of sacrifice, the leg perimeters of the control group were significantly larger than those of the PDT group (p<0.05) (Figure 1). No significant difference in body weight change was observed between the control and PDT groups. The total radiological scores of the PDT group were significantly low when compared with the control group (Figure 2). In terms of bacterial growth, in the PDT group, three knee joints were rated as +, while the other 2 joints were negative, suggesting the bactericidal effect of PDT. The control group showed 3 joints were rated as +++ and two joints as ++. The total histological score of the PDT group was significantly low when compared with the control group (Figure 3).

Conclusion
In this study, PDT using Na-Phde a showed bactericidal action on osteomyelitis models in rats. PDT using Na-Phde a appears to be a useful treatment for osteomyelitis.

References