Inhibitory Effect of Photodynamic Therapy with a Novel Indocyanine Green-labeled Nanoparticle and Near-infrared Light on the Growth of Bone Metastasis of a Human Breast Cancer in vivo

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Introduction: Breast cancer easily metastasizes to bones. While life expectancy is prolonged by treatment, skeletal-related events such as spinal cord compression, pathological fractures, and hypercalcemia reduce the quality of life. Photodynamic therapy (PDT) uses the strong cytotoxicity of singlet oxygen and hyperthermia produced by irradiating excitation light on a photosensitizer. The phototoxic effects of indocyanine green (ICG) and near-infrared light (NIR) have been studied in different types of cancer cells [1,2]. Plasma proteins bind strongly to ICG, followed by rapid clearance by the liver, resulting in no tumor-selective accumulation after systemic administration. Kimura et al. have proposed using a novel nanoparticle labeled with ICG (ICG-lactosome) that has tumor selective accumulation owing to enhanced permeability and retention (EPR) effect [3,4,5]. In this study, we investigated the efficacy of PDT using ICG-lactosome and NIR for a bone metastatic mouse model of breast cancer.

Methods: Cells from the highly invasive human breast cancer cell line, MDA-MB-231 (MDA; ATCC, Manassas, VA) were injected into the right tibia of 26 anesthetized BALB/C nu/nu mice (4 weeks old; Oriental Bio Service, Inc., Kyoto, Japan) at a concentration of $1.0 \times 10^6$ cells/20 µL cell suspension [6]. The mice were then randomly divided into three groups: the PDT group (n = 9), the laser (laser irradiation only) group (n = 9), and the control group (n = 8). PDT was performed thrice (7, 21, 35 days after cell inoculation) following ICG-lactosome administration (5 mg•0.2 mL⁻¹•body⁻¹; Shimadzu Corporation, Japan) via the tail vein 24 hours before irradiation (Figure 1). The ICG-lactosome contained 30 nmol/mg ICG. The mice were percutaneously irradiated with an 810-nm medical diode laser (UDL-15; Olympus, Tokyo, Japan) with a power density of 125 mW/cm² for 10 min (5 min each in the supine and prone positions). In the laser group, mice were irradiated following 0.2-mL saline administration 24 hours before irradiation. Radiographic (ADX 4000V; Dexcelwone Company, South Korea) analysis, body weight (BW) measurements, and lesion observations were noted for 49 days after inoculation of MDA cells. The area of osteolytic lesion was quantified using ImageJ software. The right hind legs of 3 mice were amputated 24 hours after the third treatment. Histological analysis was performed using hematoxylin-eosin staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining (Biopathology Institute, Oita, Japan) of sagittal sections at the center of the patella. The data
was analyzed using paired t-test and Tukey-Kramer post-hoc test. P-values of <0.05 were considered significant.

**Results:** On day 49, the area of osteolytic lesion in the PDT group (7.9 ± 1.2 mm$^2$: mean ± SD) was significantly smaller than that of the control (11.4 ± 1.4 mm$^2$) and laser (11.9 ± 1.2 mm$^2$) groups (Figure 2). BW increased without any statistical difference in all 3 groups. A mouse in the PDT group was burned in the irradiation range. Histological findings are shown in Figure 3. In the PDT group, we observed many TUNEL-positive cells in the metastatic tissue 24 hours after PDT. TUNEL-positive cells clearly showed condensation of nuclei and swelling and explosion of cells (Figure 3d). In the control and laser groups, TUNEL-positive cells were occasionally observed (Figures 3e, f).

**Discussion:** We have previously reported the effect of ICG-lactosome-enhanced PDT on the cytotoxicity of human breast cancer cells *in vitro* [7] and on the delay of paralysis in a rat spinal metastasis model [8]. In this study, we demonstrated the inhibitory effect of ICG-lactosome-enhanced PDT on bone destruction caused by human breast cancer cells *in vivo*. PDT induced apoptosis and necrosis in the tumor cells. Adverse effects of repeated PDT were hardly confirmed. We examined the sequential change in accumulation of ICG-lactosome in the bone metastasis owing to the EPR effect. ICG-lactosome accumulated maximally in the metastasis 24 hours after systemic administration. ICG has recently received considerable attention as a material for intraoperative fluorescence imaging [9,10]. Intralesional resection is often performed for spinal metastases in an emergency owing to the possibility of paralysis and the location of important tissues such as the aorta, vena cava, and the spinal cord near the vertebral body. The residual tumor may regrow and cause neurological deficits. We believe that ICG-lactosome-enhanced PDT can decrease the rate of local recurrence through reduction of the residual tumor. Thus, PDT with ICG-lactosome and NIR had an inhibitory effect on the growth of bone metastasis of a human breast cancer.

**Significance:** Photodynamic therapy using ICG-lactosome, which selectively accumulates in malignant tumors, and NIR light inhibited the growth of bone metastasis of a human breast cancer. PDT induced apoptosis and necrosis in cancer cells. This treatment has the potential to decrease the rate of local recurrence of spinal metastasis through reduction of the residual tumor.
Figure 2A  Sequential change of the area of the osteolytic lesion.

Figure 2B  Radiographic image of the right tibia on day 49 in three groups. Osteolysis was observed in the proximal site of the tibia. Osteolytic lesion in the PDT group was smaller than that of the control and laser groups.
Figure 3: Histological findings of TUNEL staining (a–f) 24 hours after the third treatment. The PDT group (left row: a, d), the control group (middle row: b, e), the laser group (c, f).