OSTEOGENESIS IN NECROTIC BONE WITH A SINGLE INJECTION OF FIBROBLAST GROWTH FACTOR-2 (FGF-2) AND VASCULAR BUNDLE IMPLANTATION

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[Introduction]
Avascular necrosis of bone is a devastating problem most often seen in the femoral and humeral head, as well as the carpal and tarsal bones. We reported at the 49th annual meeting of the Orthopaedic Research Society that a local single injection of fibroblast growth factor-2 (FGF-2) into necrotic bone accelerated surgical angiogenesis in necrotic bone at one and two weeks after surgery. The aim of this study is to clarify the acceleration of osteogenesis in necrotic bone by the same methods.

[Methods]
Ten Japanese white rabbits, weighing 3.0 to 3.5 kg, were used in this study. A portion of rabbit iliac crest bone was removed as a free bone graft and frozen for 5 minutes in liquid nitrogen to ensure complete cellular necrosis. Then, the sphenous artery and its venae comitantes were exposed and mobilized as a vascular bundle in the medial aspect of the right thigh. A narrow hole was created in the necrotic bone and the graft was placed in the proximal thigh. In group 1, FGF-2 was injected into the hole at a single dose of 100 microgram, and the saphenous artery and its venae comitantes were passed through the hole of the bone. In group 2, injection of saline into the hole and vascular bundle implantation was used as a control.

Fluorochrome label was administered to allow later analysis of new bone formation. Calcine was injected intramuscularly at a dose of 5 mg/kg at 2 days before death. At 4 weeks after surgery, the animals were sacrificed by euthanasia using an overdose of sodium pentobarbital, and the bone and vascular bundle were collected. The specimens were fixed in 70% ethanol and stained with Villanueva bone stain for 14 days. They were then dehydrated in a graded series of ethanol and acetone and embedded in methylmethacrylate. 7-mm-thick sections at the middle portion of the specimens were prepared for Villanueva-Goldner staining as well as fluorescence microscopic analysis. Bone measurements were accomplished using a computerize image analysis system. Perimeters of trabecular bone surface, fluorochrome-labeled surface, and osteoid surface were measured with this system in all areas of each section. Each animal was treated according to the guidelines of the Institutional Animal Care and Use Committee and the protocol was approved by the Ethics Committee for Experimental Animals of Hiroshima University. The Mann-Whitney U test was used for statistical analysis. Significance was set at p < 0.05.

[Results]
Osteoid surface visualized with Villanueva-Goldner staining (Fig 1) was seen on 38.7 % of the trabecular bone surface in group 1 and 23.0 % in group 2. There was significant difference between two groups (Fig 3A). Fluorochrome-labeled surface (Fig 2) occupied 24.9% of the trabecular bone surface in group 1 and 13.1 % in group 2. This difference was also statistically significant (Fig 3B).

[Discussion]
Recent clinical and basic research studies have revealed that FGF-2 plays an important role as regulators of bone development, remodeling, and repair. In vitro, FGF-2 acts via autocrine and paracrine mechanisms to variably promote pre-osteoblast proliferation, stimulate or inhibit osteoblast differentiation, and regulate bone nodule formation and calcification in isolated cell or organ cultures in a complex manner. In vivo, single local application or systemic infusion of FGF-2 into animals exerts anabolic actions on bone tissue, and increases endosteal bone formation or accelerates fracture healing. Our results show that combining vascular bundle implantation and a single local application of recombinant human FGF-2 into necrotic bone can accelerate osteogenesis in the bone. This method may contribute to the treatment of avascular necrosis of bone.