Is low-intensity pulsed ultrasound effective for revitalizing a severely necrotic small-bone?

**An experimental rabbit model**

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Introduction:
Kienböck disease is a progressive wrist disorder characterized by osteonecrosis of the lunate. Bone marrow (BM) transplantation for the treatment of osteonecrosis of the femoral head was reported to have good clinical results and bone regeneration (1-2). As such, we applied a new method that composed of drilling, BM transplantation, external fixation and radiating low-intensity pulsed ultrasound (LIPUS) for Kienböck disease and reported good clinical results obtained by this new method (3-4). To better understand the four factors in our new method, we histopathologically examined the efficacy of drilling and of BM transplantation for regenerating necrotic bone in a small-bone rabbit model. BM transplantation, with drilling, was effective for revitalizing a severely necrotic small bone in an experimental rabbit model (5-6). The purpose of this study was to investigate whether bone regeneration can be accelerated by LIPUS and/or multiple drilling and/or BM transplantation in an experimental rabbit model.

Materials and Methods:

**Bone necrosis model and grouping**

Eighteen adult female Japanese white rabbits, weighing 2.5 to 3.0 kg, were used in this study. Bilateral fourth tarsal bones were exposed, subsequently soaked in liquid nitrogen for 5 minutes. The surface of the bone, which is approximately 1 cm², consists of cartilage and cortical bone. The bones were inserted bilaterally into subcutaneous pouches formed by a blunt dissection of the back of the rabbit. The rabbits were divided into three groups: the BM transplantation group (group BM, 6 rabbits), the drilling group (group D, 6 rabbits) and the control group (group C, 6 rabbits). In group BM, a drill hole (2 mm in diameter) using a Kirschner wire was formed in 3 cortical surface facets of the fourth tarsal bone, with the remaining cartilage surface left intact. BM (3 mL), obtained from the iliac crest of the same rabbit, was injected into 1 of the 3 holes slowly. After filling the hole, the rest of the BM was used to surround the bone in the pouch. In group D, 3 drill holes were also formed in the same fashion but no BM transplantation was performed. In group C, the bones were simply inserted into the subcutaneous pouch without any manipulation after soaking in liquid nitrogen.

**LIPUS Treatment protocol**

LIPUS energy was provided by a sonic Accelerated Fracture Healing System (SAFHS; Smith & Nephew, Memphis, TN, USA; Teijin Pharma, Tokyo, Japan). The treatment head module delivered an ultrasound signal comprising a burst width of 200 µs containing 1.5 MHz sine waves, with a repetition rate of 1 kHz and a spatial average and temporal average intensity of 30mW/cm². For all rabbits, only the right transplanted necrotic bone was treated with LIPUS. LIPUS treatment, coupled with protection of the skin with standard ultrasound gel, was introduced daily for 20 min, 6 days per week and continuing from 2 days after the operation until the experiments were terminated. The left-side necrotic bones were left untreated as controls. We sacrificed all rabbits at 8 weeks post-transplantation. A calcein fluorochrome label was injected subcutaneously 2 weeks before and 2 days before sacrifice.

**Histopathological and histomorphometric analyses**

Just after extraction, the necrotic fourth tarsal bone was cut across the center. Half of the specimens were decalcified sections and the other half of the specimens were undecalcified sections. In undecalcified sections, serial sections (5 µm thick) of the central part of the specimens were prepared for toluidine blue staining. ALP and TRAP stainings were also performed. Calcein were visualized by fluorescent microscopy. In the histomorphometric analysis, five fields were randomly selected from each section, and the mineralizing surface area (labeled surface/bone surface) and the osteoblast surface area (osteoblast surface/bone surface) were measured at ×100 magnification. The TRAP positive osteoclasts were counted in all the selected fields of the histological sections.

**Statistical analyses**

For the data on mineralizing surface area, osteoblast surface area and osteoclast number, the mean and standard deviations were calculated for the LIPUS treated group (LIPUS(+)) and for the untreated group (LIPUS(-)) by Student’s t-test in groups C, D and BM. Statistical significance was set at p < 0.05.

Results:

**Histomorphometric findings**

A fluorochrome-labeled surface was rarely observed in groups C or D, regardless of having LIPUS (+) or (-) (p=0.57, p=0.07) (Fig 1). In group BM with LIPUS (+), the mineralizing surface area showed a significant increase as compared with LIPUS (-) (Fig 2). And the osteoblast surface area and the number of osteoclasts also increased. However, there were no significant differences for LIPUS (+) or (-).

**Discussion**

LIPUS treatments did not accelerate revitalization of a severely necrotic small bone in this experimental rabbit model; however, LIPUS combined with BM transplantation tended to promote new bone formation. Takayama et al. reported that LIPUS stimulation did not affect the rate of cell proliferation; rather, it increased osteogenic differentiation (7). Generally, the mineralizing surface area reflects osteogenic differentiation, whereas the osteoblast surface area reflects cell proliferation. In light of this, LIPUS treatment, combined with BM transplantation, may be an effective approach for the revitalization of small necrotic bones. These results demonstrate feasibility for our new clinical approach in the treatment of Kienböck disease.

References:
3. Ogawa T. Is transplantation of the bone marrow effective for clinical approach in the treatment of Kienböck disease. Takayama et al. reported that LIPUS stimulation did not affect the rate of cell proliferation; rather, it increased osteogenic differentiation. Generally, the mineralizing surface area reflects osteogenic differentiation, whereas the osteoblast surface area reflects cell proliferation. In light of this, LIPUS treatment, combined with BM transplantation, may be an effective approach for the revitalization of small necrotic bones. These results demonstrate feasibility for our new clinical approach in the treatment of Kienböck disease.

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