Is transplantation of the bone marrow effective for revitalizing a severely necrotic small bone in an experimental rabbit model?

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Introduction:
Kienböck disease is a progressive wrist disorder characterized by osteonecrosis of the lunate. We decided to investigate whether transplantation of bone marrow (BM), a less invasive procedure, is an effective treatment for cases of severe small bone necrosis such as Kienböck disease. BM transplantation for the treatment of osteonecrosis of the femoral head was reported to have good clinical results and bone regeneration (1,2). In cases of small bone necrosis, which are different from those of epiphyseal necrosis such as osteonecrosis of the femoral head, necrotic area has not continuity to normal cancellous bones area such as diaphysis that has rich bone marrow cells. We investigated the effectiveness of BM transplantation in an animal model of severe small bone necrosis with poor vascular surroundings of the bone.

Materials and Methods:

Severe small bone necrosis model The study was approved by the University Committee for Animal Experimentation. We used the bilateral fourth tarsal bones of rabbit as small bones (Fig 1-a), whose surface consisted of cartilage and cortical bone. The tarsal bones were removed carefully, soaked in liquid nitrogen, and inserted into subcutaneous pouches of the rabbit’s back.

Study design The rabbits were divided into four groups, the BM transplantation group (group M), peripheral blood (PB) transplantation group (group P), drilling group (group D), and control group (group C).
In group M, three drill holes were formed on the cortical surface of the fourth tarsal bone using a Kirschner wire (2 mm in diameter), with the remaining cartilage surface left intact. After inserting subcutaneously, the tarsal bones were filled with BM (3 ml) obtained from the iliac crest. BM was aspirated using an 18G needle and was injected into one of the three holes, filling the inside and surrounding areas of the bone in the pouch. In group P, drill holes were also formed and PB was transplanted. In group D, three drill holes were also formed but no BM or PB was transplanted. In group C, the bones were simply inserted into the subcutaneous pouch without any manipulation after soaking in liquid nitrogen and thawing. We sacrificed three rabbits, obtaining six specimens from each group at 2, 4, 8, 12, and 20 weeks posttransplantation.

Histomorphometrical analysis A fluorochrome label, calcine (Wako®, Osaka, Japan; 20 mg/kg body weight) was injected subcutaneously 1 week (2- and 4-week posttransplantation groups) or 2 weeks (8-, 12- and 20-week posttransplantation groups) before and 2 days before sacrifice. Bone morphological study (toluidine-blue), Alkaline phosphatase (ALP) staining, and tartrate-resistant acid phosphatase (TRAP) staining were carried out, using undecalcified sections. In the analysis, five fields were randomly selected from each section, and the areas of the mineralizing surface (labeled surface / bone surface) and osteoblast surface (osteoblast surface / bone surface) were measured at × 100 magnification. The number of osteoclasts that were positive for TRAP staining was determined in all the selected fields of all the histologic sections.

Statistical analyses For the data on mineralizing surface, osteoblast surface and osteoclast number, the mean and standard deviation were calculated for each group at five time points using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post hoc test. Statistical significance was set at p<0.05.

Results:

Macroscopic findings None of the rabbits showed infection during the experiments. The area of surrounding tissues increased to a greater extent with the vascularization of necrotic bones in group M than in any other group. In groups C, D, and P, the necrotic severe small bones retained their original shape, and their surfaces were smooth and white (Fig 1- b, c, d). In group M, at 12 and 20 weeks, necrotic bones no longer retained their original shape. They were red and soft with a collapsed shape (Fig 1-e).

Histomorphometrical findings A fluorochrome-labeled surface was rarely observed at 2 weeks in any of the groups. At 4, 8, 12, and 20 weeks, group M showed a significant increase (p<0.01) in mineralizing surface area (Fig 2, 3). The surface area of osteoblasts also increased significantly in group M at 4, 8, and 12 weeks (p<0.05). A significant increase in the number of osteoclasts in group M was observed at 4, 8, and 12 weeks (p<0.01). However, in the osteoblasts surface and the number of osteoclasts, at 20 weeks the differences were not significant.

Discussion:
Our severe small bone necrosis model is useful for evaluating the effect of only transplanted BM, transplanted PB, and drilling. Drilling enables the invasion of vessels and cells into the bones in this subcutaneous condition. PB transplantation introduces some types of cytokines such as growth factors. However, new bone formation in PB transplantation is significantly poorer than that in BM transplantation. BM transplantation introduces osteoprogenitor cells and cytokines. We considered that good bone regeneration was due to the interactions of implanted mesenchymal stem cells with many types of cytokine and platelets in BM(3). Our study suggests that BM cells are necessary for bone regeneration, and BM transplantation accelerates bone formation and resorption in severe small bone necrosis.

References: