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Bone marrow stromal cell therapy improves femoral bone mineral density and mechanical strength in ovariectomized rats

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Background

This study aimed to determine the influence of bone marrow stromal cells (BMSC) on the degree and sustainability of ovariectomy-induced bone loss.

Methods

Allogenic BMSC were injected into either the left or right femur of 15 ovariectomized rats (OVX). Saline was injected into the contralateral femur as a vehicle control. Five rats were killed at 8 weeks and 5 rats at 24 weeks. The other five OVX rats received serial injections 4 weeks after the first injection and were killed 24 weeks after the first injection. To confirm osteoporotic model, five rats received sham operation. Bone mineral density (BMD) was measured using dual-energy X-ray absorptometry. Mechanical properties were evaluated by three-point bending.

Results

The OVX rats showed significantly lower BMD compared with that of the sham operated rats. BMD at the femoral mid-shaft was significantly greater in the BMSC-injected bones compared with the control bones. At week 8, ultimate load and stiffness were also improved in the BMSC-injected bones compared with controls. At 24 weeks, the stiffness of control and BMSC-injected bones was statistically indistinguishable. The additional injection aided preservation of both BMD and mechanical properties.

Discussion

The present study suggests that bone strength may be improved by direct BMSC injection.

Keywords

bone quality, bone marrow stromal cells, bone mineral density, cell therapy, osteoporosis.

Introduction

Osteoporosis is a skeletal disease characterized by low bone mass and micro-architectural deterioration with a resulting increase in bone fragility and susceptibility to fracture. About 30% of all post-menopausal women develop osteoporosis within 10–15 years after menopause [1]. In Japan, 11 million Japanese, which constitutes almost 10% of the total population, have osteoporosis [2]. The occurrence of hip fractures from osteoporosis is associated with an increased risk of death even after pre-fracture health status is taken into account [3]. By 2050, the number of hip fractures is expected to increase to three to four times the current rate, and more than half of all hip fractures will occur in Asia [4]. The treatment of osteoporotic bone before fracture is also important because osteoporotic bone healing is 30% slower than healthy bone [5].

Bone marrow stromal cells (BMSC) are multilineage progenitor cells that can differentiate into various mesodermal tissues, including cartilage, bone, muscle, fat,

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tendon and ligament [6–9]. BMSC are readily available in clinics because they can be obtained by bone marrow (BM) aspiration under local anesthesia, which involves minimal invasiveness for patients. Thus the possibility of using BMSC for treating bone defects is being explored [10–19]. Transplanted BMSC-derived osteoblasts have been used to regenerate bone and are currently applied in orthopedic surgical cases and dental implant cases. In those clinical studies, BMSC were transplanted with carrier scaffolds such as hydroxyapetite, beta triphosphate and platelet-rich plasma-based fibrin gel, which are important for maintaining space for bone regeneration at the defect site. Osteoporosis is not always accompanied by bone defects and this type of approach may not applicable. Reduced bone mineral density (BMD) is the major cause of decreased mechanical strength in osteoporotic bone and it is of interest whether BMD can be improved by BMSC. Currently, the influence of BMSC transplanted into BM on skeletal BMD is not well understood.

In this study, we investigated the effects of BMSC injection on BMD and the mechanical properties of the femur in ovariectomized rats after 8 and 24 weeks. Furthermore, the effects of repetitive BMSC injections were compared with the effects of a single injection.

Methods

Animals

Twenty 12-week-old virgin female Sprague–Dawley rats were purchased from Chubu Kagaku Shizai (Nagoya, Japan). They were housed in individual cages and maintained in an environment at 22°C with a 12-h light/dark cycle. The animals had free access to water and food (CLEA Rodent Diet CE-2; CLEA Japan Inc., Tokyo, Japan), which contained 1.03% calcium, 0.97% phosphorous and 2.3 IU vitamin D3 per gram. All animal experiments were approved by the Animal Experiment Advisory Committee at Nagoya University School of Medicine (Nagoya, Japan) and performed in accordance with the Guidelines for Animal Experimentation of Nagoya University.

Ovariectomy

The animals received either ovariectomy ($n = 15$) or a sham operation ($n = 5$) (as described below) under systemic anesthesia by intraperitoneal (i.p.) injection of ketamine hydrochloride and xylazine (10:1 vol/vol). Ovariectomizations were confirmed by the following two procedures. First, body weight was measured to confirm weight gain [20]. Second, an autopsy was performed on all animals at the time of killing. For a successful ovariectomy, the uterus becomes atrophic and the ovaries are no longer present.

Cell culture and characteristics of BMSC

BM cells were collected by flushing the femoral lumen of 24-week-old Sprague–Dawley rats. Bilateral femora were removed by dissection and cleaned of soft tissues.

The proximal and distal ends were snipped off and the BM was flushed from the diaphysis with 10 mL MSC-GM [Cambrex MSCGM Bullet Kit (PT-3001); Cambrex, East Rutherford, NJ, USA]. A suspension of BM cells was obtained by aspiration of the marrow cell preparation through a 21-gauge needle and seeded into a 75-mm^2 plastic flask (Nunc, Roskilde, Denmark).

The BM cells were maintained in MSC-GM and kept in a humidified atmosphere at 5% CO_2 and 37°C. The medium was changed every 2 days. The cells were passaged by trypsinization (0.05% trypsin–EDTA solution; Gibco, Carlsbad, CA, USA) at 80% confluency and cells at the third passage were used for experiments.

To confirm the characteristics of BMSC, osteogenic induction was performed and alkaline phosphatase (ALP) activities were measured at days 1, 3, 7 and 14 of culture at the third passage. Cells were cultured with or without osteogenic induction medium supplements: 50 μM ascorbic acid, 100 nM dexamethazone and 10 mM β-glycerophosphate (Sigma, St Louis, MO, USA).

To measure ALP activity, an aliquot (600 μL) of media was added to 600 μL 1.0 mg/mL p-nitrophenyl phosphate containing 5 mM $MgCl_2$ (Sigma) and the mixtures were incubated for 10 min at 37°C. After incubation, 600 μL 0.2 N NaOH was added to stop the enzymatic reaction. The absorbance of each well (415 nm) was read on a plate reader (GENios spectra FLUOR plus; Tecan, Männedorf, Switzerland) and analyzed using LS-PLATE manager 2001 software (Wako Pure Chemical Industries, Osaka, Japan). All samples were run in triplicate.

Cell transplantation

Twelve weeks after ovariectomy, cell transplantation was performed. BMD was measured before the transplantation procedure to benchmark BMD. Pre-operatively, there was no statistical difference between left and right femur BMD ($n = 5$). A small skin incision was made over the contralateral side (left or right) of the femur and the