Bone 81 (2015) 620–631

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Knee loading protects against osteonecrosis of the femoral head by enhancing vessel remodeling and bone healing

Daquan Liu [a,b,1], Xinle Li [a,1], Jie Li [a], Jing Yang [a], Hiroki Yokota [c], Ping Zhang [a,c,*]

[a] Department of Anatomy and Histology, School of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China
[b] Department of Pharmacology, Institute of Acute Abdominal Diseases, Tianjin Nankai Hospital, Tianjin 300100, China
[c] Department of Biomedical Engineering, Indiana University–Purdue University Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received 3 July 2015
Revised 27 August 2015
Accepted 24 September 2015
Available online 28 September 2015

Keywords:
Knee loading
Osteonecrosis
Osteoclast
Osteoblast
Vessel remodeling
Bone remodeling

ABSTRACT

Osteonecrosis of the femoral head is a serious orthopedic problem. Moderate loads with knee loading promote bone formation, but their effects on osteonecrosis have not been investigated. Using a rat model, we examined a hypothesis that knee loading enhances vessel remodeling and bone healing through the modulation of the fate of bone marrow-derived cells. In this study, osteonecrosis was induced by transecting the ligamentum teres followed by a tight ligature around the femoral neck. For knee loading, 5 N loads were laterally applied to the knee at 15 Hz for 5 min/day for 5 weeks. Changes in bone mineral density (BMD) and bone mineral content (BMC) of the femur were measured by pDEXA, and ink infusion was performed to evaluate vessel remodeling. Femoral heads were harvested for histomorphometry, and bone marrow-derived cells were isolated to examine osteoclast development and osteoblast differentiation. The results showed that osteonecrosis significantly induced bone loss, and knee loading stimulated both vessel remodeling and bone healing. The osteonecrosis group exhibited the lowest trabecular BV/TV ($p < 0.001$) in the femoral head, and lowest femoral BMD and BMC (both $p < 0.01$). However, knee loading increased trabecular BV/TV ($p < 0.05$) as well as BMD ($p < 0.05$) and BMC ($p < 0.01$). Osteonecrosis decreased the vessel volume ($p < 0.001$), vessel number ($p < 0.001$) and VEGF expression ($p < 0.01$), and knee loading increased them ($p < 0.001$, $p < 0.001$ and $p < 0.01$). Osteonecrosis activated osteoclast development, and knee loading reduced its formation, migration, adhesion and the level of "pit" formation ($p < 0.001$, $p < 0.01$, $p < 0.001$ and $p < 0.001$). Furthermore, knee loading significantly increased osteoblast differentiation and CFU-F (both $p < 0.001$). A significantly positive correlation was observed between vessel remodeling and bone healing (both $p < 0.01$). These results indicate that knee loading could be effective in repair osteonecrosis of the femoral head in a rat model. This effect might be attributed to promoting vessel remodeling, suppressing osteoclast development, and increasing osteoblast and fibroblast differentiation. In summary, the current study suggests that knee loading might potentially be employed as a non-invasive therapy for osteonecrosis of the femoral head.

1. Introduction

Osteonecrosis of the femoral head (ONFH) is a disease in which bone death occurs as a result of impairment of blood supply to the femoral head [1,2]. There are several etiologic risk factors such as trauma [3,4], excessive corticosteroid use [5–7], excessive alcohol intake [8], and Legg–Calve–Perthes disease [9,10]. Debilitated blood supply to the femoral head commonly leads to necrosis and collapse of the femoral head [11]. In spite of various research efforts and trials, ONFH is still one of the most serious orthopedic problems. More than 500,000 hip replacements were performed for ONFH patients annually in the United States, and 10% of them required surgical debridement of dead bone [12]. Many therapies have been applied to animal models and patients to prevent the progress of ONFH. Non-surgical therapies include anti-osteoporosis medicine, anti-coagulants, decreasing weight bearing, lipid-lowering medicine, electromagnetic stimulation, shockwave therapy and hyperbaric oxygen therapy, while surgical therapies consist of autologous bone marrow stem cell transplantation, core decompression and total hip replacement [13,14]. Most of these therapies, however, have limits and side-effects. It is thus important and urgent to develop safe and effective treatment for ONFH.

Joint loading is one form of non-invasive physical treatment. Joint loading has been applied to synovial joints such as the elbow, knee and ankle [15]. In our previous studies, joint loading was applied 3 to

Abbreviations: ONFH, osteonecrosis of the femoral head; RANKL, receptor activator of nuclear factor kappa-B ligand; M-CSF, macrophage-colony stimulating factor; BMD, bone mineral density; BMC, bone mineral content; TRAP, tartrate resistant acid phosphatase; ALP, alkaline phosphatase.

* Corresponding author at: Department of Anatomy and Histology, School of Basic Medical Sciences, Tianjin Medical University, 22 Qixiangtai Road, Tianjin 300070, China.
E-mail address: pizhang2008@163.com (P. Zhang).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bone.2015.09.012
8756-3282/

5 min per day for 2 to 3 weeks. Our bone histomorphometric studies demonstrated that knee loading stimulates bone formation [16]. We have also demonstrated that knee loading can accelerate the healing of surgical wounds in the femoral neck and tibia [15,17].

The mechanism of knee loading is considered to change intramedullary pressure of femoral and tibial bone cavities. The load-driven pressure may generate fluid flow in a lacuna canalicular network in bone cortex. The pressure also activates bone metabolism-related genes in femur and tibia [18,19]. In our previous works, knee loading stimulated bone formation by conducting bone histomorphometry using the cross-sections at 25% (distal femur), 50% (midshaft), and 75% (proximal femur) of the length of the femur from the loading site. Knee loading also induced bone formation and enhanced bone healing in the femoral neck [20]. In this study, we addressed a question whether knee loading improves experimentally induced ONFH by modulating both vessel remodeling and bone remodeling. Our hypothesis was that knee loading would enhance vessel remodeling and bone healing through the modulation of the fate of bone marrow-derived cells.

To test the hypothesis, a rat model of ONFH was induced by transecting the ligamentum teres followed by a tight ligature around the femoral neck. The knee joint received loading for 5 weeks. We evaluated the effects of knee loading focusing on BMD and BMC using pDEXA. Ink infusion and histology assay were performed to evaluate vessel remodeling and bone healing. We also evaluated the effects of knee loading on the functions of osteoblast and osteoclast of bone marrow-derived cells.

2. Materials and methods

2.1. Animals and material preparation

Male Sprague–Dawley rats (~12 weeks of age, Animal Center of Academy of Military Medical Sciences, China) were used. The rats were housed on a 12:12 h light–dark cycle under pathogen-free conditions and were feed with food and water ad libitum. All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Ethics Committee of Tianjin Medical University. Murine receptor activator of nuclear factor kappa-B ligand (RANKL) and murine macrophage-colony stimulating factor (M-CSF) were purchased from PeproTech (Rocky Hills, NC, USA). VEGF polyclonal antibody was purchased from Proteintech (Chicago, IL, USA). Immunohistochemical staining kit and 3, 3′-diaminobenzidine (DAB) substrate kit were purchased from ZSGB-BIO (Beijing, China). Dulbecco's Modified Eagle's Medium (DMEM), Minimum Essential Medium Alpha (MEM-α), fetal bovine serum, penicillin, streptomycin and trypsin were purchased from Invitrogen (Carlsbad, CA, USA). Other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Experimental design

Eighteen rats were randomly divided into 3 groups: sham operated control group (Sham), osteonecrosis group (ON), and knee loading treated osteonecrosis group (ON + loading) ($n = 6$). Ischemic osteonecrosis of the bilateral femoral heads were induced using the previously described method with minor modifications [3].

2.3. Surgical procedure to induce osteonecrosis

The rat was placed in an anesthetic induction chamber to cause sedation and then mask-anesthetized using 2% isoflurane (IsoFlo, Abbott Laboratories, North Chicago, IL, USA) at a flow rate of 1.0 to 1.5 l/min. A longitudinal incision was made on the skin over the large trochanter. The gluteus maximus muscle and the gluteus medius muscle were separated from the bone. The joint capsule of hip was transected and the femoral head was dislocated (Fig. 1A). Osteonecrosis of the femoral head was induced by transecting the ligamentum teres and tightly placing a ligature (#3–0 Vicryl, Ethicon) around the femoral neck (Fig. 1B). The femoral head was relocated, and gluteal muscles and skin were sutured with #3–0 and #4–0 stitches, respectively. Osteonecrosis was induced on both left and right sides. For sham operation, a joint capsule was not transected and a femoral neck received no ligation. In order to alleviate the pain associated with surgery, analgesia was conducted. The rat was given a dose of buprinorphine hydrochloride (0.05 mg/kg) at a rate of 0.2 ml subcutaneously before incision. 1% pramoxine hydrochloride ointment was applied on the incision sites after surgery. Buprinorphine hydrochloride (0.05 mg/kg) was also administered per 8 h for the first three postoperative days, and applied until a week if necessary. In addition, antibiotic prophylaxis (enrofloxacin, 5 mg/kg) was administered per day for the first three postoperative days.

2.4. Knee loading

The joint loading equipment in the form of knee, ankle, and elbow loading is a recently devised treatment modality (Fig. 1C) [21]. In this current study, knee loading was achieved through dynamic loads applied to the left and right knee joints of rats in the lateral–medial direction, respectively. To position the knee properly, the lower end of the loading rod and the upper end of the stator were designed to form a pair of semispherical cups. The lateral and medial epicondyles of the femur together with the lateral and medial condyles of the tibia were confined in the cups (Fig. 1D) [22]. The tip of the loader had a contact area of 15 mm in diameter. Knee loading was conducted one week after operation. Anesthesia was induced with 2% isoflurane at a flow rate of 1.0 to 1.5 l/min. With the custom-made loader, knee loading (5 N force) was laterally applied to the left and right knees successively at 15 Hz for 5 min/day for 5 weeks. Animals were sacrificed 5 weeks after loading. Femurs and tibias were collected, and bone marrow cells were isolated.

2.5. Measurements of bone mineral density (BMD) and bone mineral content (BMC)

The animals were anesthetized by 2% isoflurane at a flow rate of 1.0 to 1.5 l/min, placed on the platform in the prone position, and images were acquired in about 5 min. Bone mineral density (BMD, g/cm^2) and bone mineral content (BMC, g) of the femur were measured by peripheral dual-energy X-ray absorptiometry (pDEXA) before surgery and sacrifice. Changes in BMD and BMC were determined and statistical analysis was conducted.

2.6. Vessel remodeling assay

To evaluate vessel remodeling of the femoral head, a blood circulation assay was performed. Chinese ink was infused 6 weeks after the operation using the procedure with minor modifications [23]. The animals were anesthetized by an intraperitoneal injection of 10% chloral hydrate (3 ml/kg). The chest was opened and the heart was exposed. A needle was inserted into the left ventricle for ink infusion, and the right atrium was cut. Animals were flushed with a heparin-saline solution (25,000 units in 250 ml of 0.9% sodium chloride) until clear liquid flowed from the circulation. The 5% gelatin/ink solution (the ratio between Chinese ink and water was 1:1) was injected into the circulation until the skin of animals became uniformly black. After euthanizing the animals, a pair of femoral heads was harvested.

2.7. Histology and immunohistochemistry assay

After sacrifice, bilateral femoral heads were harvested and fixed in 10% neutral buffered formalin for 2 days. Samples were decalcified in 10% ethylenediaminetetraacetic acid (EDTA, pH 7.4) for 40 days and embedded in paraffin. The samples were cut with a Leica RM2255 microtome (Leica Microsystems Inc., Bannockburn, IL) into 5-μm thick slices along