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Effects of short-term gentle treadmill walking on subchondral bone in a rat model of instability-induced osteoarthritis

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SUMMARY

Objective: Subchondral bone cyst (SBC) growth, caused by osteoclast activity during early knee osteoarthritis (OA) pathogenesis, should be treated to prevent further progressions of OA. In the present study, we evaluated the effects of gentle treadmill walking on subchondral bone and cartilage changes in an experimental rat model of destabilized medial meniscus (DMM).

Method: Twelve-week-old Wistar rats underwent DMM surgery in their right knee and sham surgery in their left knee and were assigned to either the sedentary group or walking group ($n = 42$/group). Animals in the walking group were subjected to treadmill exercise 2 days after surgery, which included walking for 12 m/min, 30 min/day, 5 days/week for 1, 2, and 4 week(s). Subchondral bone and cartilage changes were evaluated by micro-CT analysis, histological analysis, and biomechanical analysis.

Results: Treadmill walking had a tendency to suppress SBC growth, which was confirmed by micro-CT ($P = 0.06$) and positive staining for tartrate-resistant acid phosphatase (TRAP) activity for the osteoclast number per bone surface ($P = 0.09$) 4 weeks after surgery. These changes coincide with the prevention of cartilage degeneration as evaluated by the Osteoarthritis Research Society International (OARSI) score ($P < 0.05$) and biomechanically softening ($P < 0.05$). Furthermore, treadmill walking could suppressed increasing osteocyte deaths ($P < 0.01$), which was positively correlated with the OARSI score ($r = 0.77$; $P < 0.01$).

Conclusion: These results indicate biomechanical and biological links exist between cartilage and subchondral bone; preventive effects of treadmill walking on subchondral bone deterioration might be partly explained by the chondroprotective effects.

Introduction

Knee osteoarthritis (OA) is considered a multifactorial whole-joint disease. Increased subchondral bone remodeling is an important factor that contributes to OA pathology through the crosstalk bone-cartilage unit[1]. The bone-cartilage unit forms a complex functional unit, which may play complementary roles in the load-bearing joint[2], and the subchondral bone supports overlying cartilage biomechanically.

In previous animal studies, it has been shown that subchondral bone cysts (SBCs), subchondral bone plate thinning, and cartilage degeneration were confirmed in early phase instability-induced OA, such as the destabilization of medial meniscus (DMM)[3] through the dysregulation of osteoclast and osteoblast activity[4]. These animal studies recapitulated key features of human OA pathogenesis which coexist with meniscus degenerative changes[5]. Activated osteoclasts were confirmed in SBCs and further SBC growth was observed after drilling towards the articular cartilage[6]. According to an *in vitro* study, various cytokines and growth factors secreted by osteoclast and osteoblast of OA sclerotic bone promote loss of cartilage proteoglycans[7]. Therefore, preventing subchondral bone osteoporotic changes by suppressing increased subchondral

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bone remodeling in early OA may be a strategy to prevent the progression of OA[8].

Osteocytes of subchondral bone have been thought of as mechanosensing cells that influence osteoclast and osteoblast activity. Recently, osteocyte deaths were confirmed in OA subchondral bone, which may result in increased subchondral bone remodeling[9]. Cox *et al.* showed that increasing osteocyte deaths may cause SBC growth[10]. According to these previous studies, osteocyte deaths in subchondral bone lead to the dysregulation of osteoclast and osteoblast, which result in subchondral bone osteoporotic changes.

Although the exact cause of increased subchondral bone remodeling is unknown, changes involved in overlying cartilage are proposed to be a principal cause. Mechanical loading is an important factor, which regulates the maintenance of both cartilage and subchondral bone of OA. Galois *et al.* showed that moderate mechanical loading has a potential preventive effect on cartilage degeneration in instability-induced OA[11]. Recently, Boudenot *et al.* and Siebelt *et al.* investigated the effects of treadmill running on both cartilage and subchondral bone changes caused by chemically induced OA; they showed that treadmill exercise influences subchondral or trabecular bone metabolism[12,13]. However, whether treadmill exercise has a preventive effects on subchondral bone changes, particularly SBC growth and osteocyte death, remains unclear and osteoclast and osteoblast activity should be examined further to clearly determine the effects of treadmill exercise on subchondral bone remodeling in OA.

We hypothesized that in addition to its chondroprotective effects[14], gentle treadmill walking has inhibitive effects on subchondral bone porosity. In this study, we evaluated the effects of gentle treadmill exercise, consisting of milder intensity exercise compared to previous studies[12,13], on subchondral bone changes (by using histological techniques and micro-CT analysis) and cartilage degeneration in an experimental rat model of DMM.

Methods

Animals and surgical procedure

This study was approved by the animal research committee of Kyoto University. Ninety male Wistar rats (12 weeks old; mean body weight = 272.1 g) were purchased and placed in plastic cages with sawdust bedding, with three to four animals per cage. The room had a 12 h dark/light cycle and was at a constant temperature. Animals were allowed to move freely in the cages and had free access to food and water.

As described previously, our preclinical model of DMM[3] was performed under anesthesia using 0.85 mL/kg somnopentyl. All these surgery were conducted in the light phase. The surgery involved an incision of the medial capsule with a transection of the anterior medial meniscotibial ligament (MMTL) on the right knee. For internal controls, a sham operation was performed on the left knee joint using the same approach without MMTL transection.

Exercise protocol

Animals were randomly divided into either the sedentary group ($n = 42$) or walking group ($n = 48$). Animals in the walking group were subjected to treadmill exercise 2 days after surgery on a motor driven treadmill. A treadmill performance scale on a 1–5 Likert scale was used to assess trainability before the walking exercise in each animal[15]. Animals with a rating of ≥ 3 were included in the present study and those with a mean rating of 1 or 2 were excluded to avoid using differentially stressed animals[16]. Six out of the 48 rats in the walking group were excluded due to an insufficient score. In total, 42 rats were included into each group (i.e., sedentary and walking groups) for the present study. All these exercise training were conducted in the light phase.

Animals in the walking group were then subjected to treadmill exercise for 1, 2, and 4 week(s) ($n = 14$ per time point) for 5 days/week, 30 min/day. The initial velocity of 6 m/min at 2 days was increased to 12 m/min at or after 3 days. Animals in the sedentary group were allowed to move freely in standardized cages without any treadmill exercise for 1, 2, and 4 week(s) ($n = 14$ per time point). Seven rats in the sedentary and walking groups at each time point were used for biomechanical analysis and the remaining seven rats at each time point were used for micro-CT and histological analysis.

Biomechanical analysis

After the rats at each time point were sacrificed, a micro-indentation test at the center of the medial tibia plateau was performed, to determine the biomechanical properties of cartilage in each group, according to a previously validated method[17,18]. A preload of 0.01 N was applied and allowed to equilibrate for 100 s, followed by loading at a strain rate of 0.005 mm/s up to 0.1 N, which was maintained for 300 s[17]. A stress–strain curve and creep (mm) were obtained from the micro-indentation test[17,18].

Micro-CT analysis of subchondral bone changes

Prior to histological sectioning, all rat knee joints were scanned using a micro-CT system (SMX-100CT, Shimadzu, Kyoto, Japan) with the following parameters: 600 views over 360° increment, 20 exposures averaged per view, voltage of 43 kV, current of 40 μA, voxel size of 21 μm, and a scan time of approximately 7 min per knee. The reconstructed data sets were examined using three-dimensional data analysis software (Amira5.4, Visage, Berlin, Germany).

To measure subchondral bone plate thickness (Sb thickness), we selected subchondral bone of the weight-bearing region in the medial tibia, which was defined as a mediolateral width of 0.5 mm and a ventrodorsal length of 1 mm in the frontal plane[19]. In addition, the maximum SBC diameter[4] and the average diameter of 3 SBCs were compared between the DMM knee of the sedentary and walking groups at each time point.

To further analyze the proximal tibia, 2 separate cylindrical regions of interest (ROI), with a diameter of 1 mm were placed at the trabecular bone of the epiphysis in the medial tibia. The first cylinder, which had a height of 0.6 mm, was placed on epiphysis distal to the growth plate in the trabecular bone. These ROIs were determined based on anatomical landmarks with reference to previous report such as that by McErlain *et al.*[20] Details about the ROIs are presented in Supplementary Fig. 4. Then, the following parameters were calculated for the trabecular bone of the epiphysis: trabecular bone volume fraction (Trab BV/TV), trabecular bone thickness (Tb.Th), and trabecular spacing (Tb.Sp)[21]. To examine whether Trab BV/TV could be changed locally as indicated by Botter *et al.*[21], the other cylinder, which had a height of 0.2 mm, was placed in the trabecular bone which was located epiphysis proximal to the medial subchondral bone plate of the weight-bearing region. By using the same algorithm as that used for the first cylindrical ROI, the subchondral trabecular bone volume fraction (Sb BV/TV) was also calculated[21].

Histological and immunohistochemical analysis

As described previously[3], decalcified paraffin sections were prepared from the medial tibial plateau in the frontal plane. A