Osteoarthritis and Cartilage 25 (2017) 964–975

Osteoarthritis and Cartilage

Physiological exercise loading suppresses post-traumatic osteoarthritis progression *via* an increase in bone morphogenetic proteins expression in an experimental rat knee model

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ARTICLE INFO

Article history:
Received 27 August 2016
Accepted 6 December 2016

Keywords:
Osteoarthritis
Cartilage
Bone micro-CT
Exercise
Bone morphogenetic protein
Gremlin-1

SUMMARY

Objective: To evaluate the dose–response relationship of exercise loading in the cartilage-subchondral bone (SB) unit in surgically-induced post-traumatic osteoarthritis (PTOA) of the knee.
Design: Destabilized medial meniscus (DMM) surgery was performed on the right knee of 12-week-old male Wistar rats, and sham surgery was performed on the contralateral knee. Four weeks after the surgery, the animals were subjected to moderate (12 m/min) or intense (21 m/min) treadmill exercises for 30 min/day, 5 days/week for 4 weeks. PTOA development in articular cartilage and SB was examined using histological and immunohistochemical analyses, micro-computed tomography (micro-CT) analysis, and biomechanical testing at 8 weeks after surgery. Gremlin-1 was injected to determine the role of bone morphogenetic protein (BMP) signaling on PTOA development following moderate exercise.
Results: Moderate exercise increased BMP-2, BMP-4, BMP-6, BMP receptor 2, pSmad-5, and inhibitor of DNA binding protein-1 expression in the superficial zone chondrocytes and suppressed cartilage degeneration, osteophyte growth, SB damage, and osteoclast-mediated SB resorption. However, intense exercise had little effect on BMP expression and even caused progression of these osteoarthritis (OA) changes. Gremlin-1 injection following moderate exercise caused progression of the PTOA development down to the level of the non-exercise DMM-operated knee.
Conclusions: Exercise regulated cartilage-SB PTOA development in DMM-operated knees in a dose-dependent manner. Our findings shed light on the important role of BMP expression in superficial zone chondrocytes in attenuation of PTOA development following physiological exercise loading. Further studies to support a mechanism by which BMPs would be beneficial in preventing PTOA progression are warranted.

Introduction

Post-traumatic osteoarthritis (PTOA) is a form of arthritis that develops after joint injury, accounting for at least 12% of all osteoarthritis (OA) cases[1]. Developing effective disease-modifying therapy for PTOA is a critical medical priority, as there is no effective treatment to delay the development of the disease. OA is not limited to articular cartilage, but is considered a "whole-joint disease," with involvement of subchondral bone (SB), synovium, and the meniscus[2]. The SB plays crucial roles in the initiation and

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http://dx.doi.org/10.1016/j.joca.2016.12.008
1063-4584/

progression of cartilage degeneration in PTOA through bone remodeling involving osteoclast activity[3,4]. Cells in SB secrete local cytokines that contribute to cartilage degeneration[5,6], and that treatment to suppress abnormal bone remodeling prevents secondary cartilage damage[7,8], suggests the importance of the cartilage-bone unit, rather than cartilage or bone alone, as a therapeutic target in PTOA.

Cells within articular cartilage and SB are sensitive to mechanical loading and respond in a magnitude-dependent manner[9–11]. Moderate exercise prevents articular cartilage degeneration and inhibits osteoclast activity in SB in surgically- and chemically-induced models[12–14], whereas excessive exercise increases osteoclast activity and aggravates the disease[15]. However, much of the previous research has focused on the cartilage or SB alone, and the dose–response relationship in the cartilage-SB unit in knee PTOA has yet to be fully clarified. There is an important knowledge gap in OA concerning the use of physical exercise or increased physical activity as a potential disease-modifying therapy for knee OA.

Bone morphogenetic protein (BMP) is a candidate growth factor and has been suggested to be crucial in the maintenance of articular cartilage and SB. BMP enhances cartilage matrix synthesis as well as osteogenic activity of bone cells *in vivo*[16,17], suggesting that BMPs may contribute to the intrinsic repair capacity of damaged cartilage-bone units. Recent studies showed that messenger RNA expression of BMPs in the cartilage-bone unit was upregulated by moderate exercise *in vivo*[18], and moderate exercise increased expression of BMPs, which coincides with the prevention of the progression of PTOA in the instability-induced PTOA knee[19]. However, their function is not yet clear; further investigation of the role of BMPs in the protection against cartilage-bone damage and their interaction with the dose-dependent response of exercise intervention is warranted.

This study aimed (1) to evaluate the dose–response relationship of exercise loading in the cartilage-SB unit in surgically induced knee PTOA, and (2) to investigate the possible mechanisms of moderate exercise toward a role for BMPs in the prevention of progression of PTOA. We hypothesized that (1) exercise loading is beneficial in a dose–response manner with the further delineations that moderate exercise inhibits PTOA progression, but intense exercise deteriorate PTOA, and that (2) the beneficial effect of moderate exercise was achieved through stimulation of the BMP signaling pathway.

Method

Animals and surgical induction of PTOA

This study was approved by the animal research committee of Kyoto University (approval number: Med Kyo 16017), and was conducted in accordance with the ARRIVE guidelines[20]. A total of 106 Wistar rats (12 weeks old; mean body weight = 261.9 g) were purchased and placed in plastic cages with sawdust bedding (2–3 animals per cage) in a 12-h light/dark cycle at constant temperature. Animals were allowed to move freely in cages and provided free access to food and water. A destabilized medial meniscus (DMM) was created in the right knee and sham surgery (i.e., incision of the skin and medial capsule) was performed on the left (i.e., contralateral) knee under anesthesia induced with 0.85 ml/kg pentobarbital sodium (Somnopentyl; Kyoritsu Seiyaku Corp., Tokyo, Japan) as described previously[21,22].

Grouping for the rats used in this study is described in Fig. 1(A-(c)). After the surgery, the 66 animals were randomly assigned to either of the following post-intervention assessment groups: *sedentary* ($n = 20$) or *exercise* ($n = 46$) groups. To prevent the animals from becoming differentially stressed, treadmill performance on a 1–5 Likert scale[23] was evaluated for each animal in the *exercise* group, and animals with a rating of ≥3 were included[12]. Six of the 46 rats in the *exercise* group were excluded because they displayed insufficient Likert scores (i.e., 1 or 2 points), leaving 40 rats in the *exercise* group. The remained 40 animals were randomly assigned to baseline assessment ($n = 16$), activity assessment ($n = 6$), and gremlin-1 inhibitory assessment ($n = 18$).

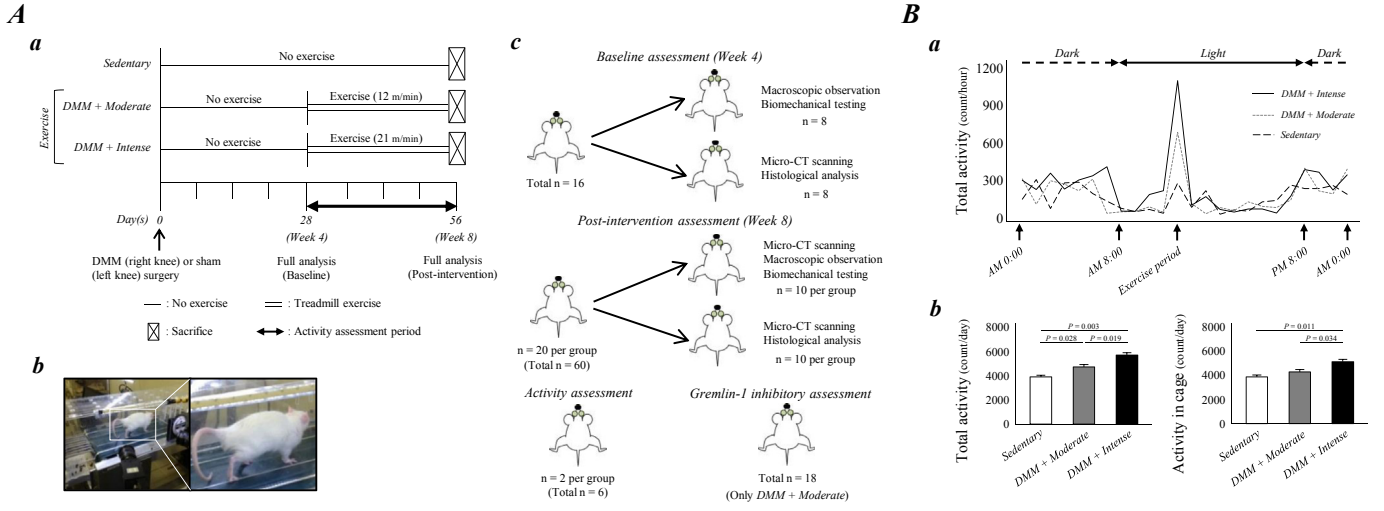

Fig. 1. Experimental design and physical activity measurements. **A**, Experimental design describing exercise protocols and analytic methods. The animals in each *exercise* group were subjected to exercise for 30 min/day, 5 days/week, from weeks 4 through 8, at a constant speed of (1) 12 m/min for the *DMM + moderate* group, and (2) 21 m/min for the *DMM + intense* group (a). A treadmill was used to encourage exercise (b). The animals were sacrificed 4 ($n = 16$) and 8 ($n = 84$) weeks after surgery (c). The rats sacrificed at 8 weeks were used for (1) histological, biomechanical, and micro-CT analyses ($n = 60$), (2) activity assessment ($n = 6$), and (3) gremlin-1 inhibitory assessment ($n = 18$). **B**, Physical activity evaluated by KSN-200. Representative activity patterns during a day when treadmill exercises (30 min) were performed in the daytime (a). Total activity of rats in each group, including activities during the exercise period, and activity in the cage other than that during exercise period (b). In the calculation of activity in the cage in the *sedentary* group, 30 min of daytime activity was also excluded as for the other two groups. Values are the means ± 95% CI of the independent experiment. In this figure, *P*-values were calculated using the Tukey–Kramer test for *post hoc* analysis of variance ($n = 2$ per group).