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Subchondral plate porosity colocalizes with the point of mechanical load during ambulation in a rat knee model of post-traumatic osteoarthritis

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SUMMARY

Objective: This study investigated the association between spatiotemporal cartilage-subchondral bone plate alterations and mechanical load during ambulation in an experimental rat model of destabilized medial meniscus (DMM).

Design: Twelve-week-old Wistar rats ($n = 38$) underwent DMM surgery on the right knee and sham surgery on the left knee. At 2 and 4 weeks after surgery, subchondral bone changes were evaluated via micro-computed tomography with various knee flexion angles to simulate weight-bearing during rat ambulation under a 3-dimensional motion capture apparatus. Additionally, the biomechanical properties, histology, and ultrastructure of the medial tibia and femoral condyle were evaluated.

Results: Focal subchondral bone plate perforations were confirmed in the medial tibia within 2 weeks after surgery and were aggravated rapidly 2 weeks later. This subchondral plate porosity colocalized with articular cartilage lesions as confirmed by histology and scanning electron microscopy, and coincided with the likely point of contact between the posterior femoral condyle and tibial plateau during ambulation. Biomechanical properties were confirmed at the medial tibia, at which stiffness was reduced to approximately half that of the sham-operated knee at 4 weeks after surgery.

Conclusions: Cartilage-subchondral bone plate alterations localized in the region of the point of mechanical load during ambulation in DMM-operated knees, at which the mechanical integrity of cartilage was impaired. These results indicate that DMM-induced increases in mechanical load play an important role in the pathogenesis of early post-traumatic osteoarthritis (OA), and it might accelerate the development of the disease via cartilage-subchondral bone plate crosstalk through increased subchondral plate perforations.

Introduction

Osteoarthritis (OA) is a complex degenerative disease affecting articular cartilage, subchondral bone, synovium, menisci, and ligaments[1]. Although OA has been traditionally considered a cartilage disease primarily, the contribution of subchondral bone to its physiopathology is arousing interest[2].

Radin *et al.* first suggested that subchondral bone could trigger the degeneration of overlying cartilage[3]. Subsequently, several animal studies revealed some histopathological changes in the subchondral bone from the early stages of post-traumatic OA,

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including subchondral bone loss, bone microdamage, and the formation of subchondral bone cysts (SBCs)[4–6]. These structural changes of subchondral bone have also been observed after traumatic injury in humans[7]. Although the etiologies of such bone lesions remain poorly established, some researchers reported that increased subchondral bone porosity could be associated with cartilage damage in animal studies[8,9]. Furthermore, epidemiologic studies illustrated that the presence of bone marrow lesions and increased subchondral bone turnover are associated with the progression of knee OA[10,11]. However, Botter *et al.* revealed that subchondral bone plate thinning was independent of the site of cartilage damage in collagenase-injected joints[12]. Further studies investigating these alterations of subchondral bone and their association with cartilage damage in early post-traumatic OA are of particular interest to any attempt to determine the mechanism of the initiation and progression of the disease.

The mechanical load within an affected joint is a key factor regulating the development and long-term maintenance of cartilage and subchondral bone. Importantly, extreme mechanical loads are considered primarily responsible for the initiation and progression of post-traumatic OA, as indicated by a number of human and animal studies[13–16]. Ko *et al.* and Poulet *et al.* found that intense cyclic compression of the knee joint using a loading regimen could induce focal cartilage degeneration colocalized with subchondral bone damage in an intact mouse knee[14,15]. Furthermore, accumulated evidence indicates that increased mechanical load could lead to phenotypic changes of chondrocytes and osteoblasts[17,18] that might accelerate the progression of post-traumatic OA. These studies highlight the need for further studies investigating the association between mechanical load and the pathogenesis of early post-traumatic OA.

Surgically induced mechanical joint destabilization OA models are often used to represent human early post-traumatic OA[12,19]. Among surgically induced OA models, the destabilized medial meniscus (DMM) model is a frequency used chronic model with high clinical relevance that resembles some of the changes that occur in progressive human early post-traumatic OA by replicating chronic abnormal joint loading, which has different time courses or degenerative changes than chemically induced models such as collagenase- or papain-injected models[20]. Given the recent findings by Loeser *et al.* that the early phase 2 and 4 weeks after DMM surgery was the most active in terms of matrix remodeling gene expression[21], understanding the degenerative process, particularly in the early phase, might provide a basis for optimal interventions to prevent the initiation and development of human post-traumatic OA[22].

Our recently published evidence illustrated that DMM induces site-specific changes of cartilage and cortical subchondral bone plate porosity from the early phase, particularly in the medial tibia[6], which could increase crosstalk between cartilage and subchondral bone plate, contributing to disease progression[23,24]. However, the mechanisms underpinning these phenomena and the mechanism by which the loading environment and spatiotemporal cartilage-subchondral bone plate alterations are interrelated in the knee joint in DMM-induced post-traumatic OA remain unclear. The present study assessed the association between the spatiotemporal distribution of cartilage-subchondral bone plate alterations in the early phase after DMM induction and loading environment evaluated using a 3-dimensional motion capture apparatus for assessing rat ambulation.

Methods

Surgical procedures and experimental design

The experimental design for this study was approved by the College Animal Research Committee of Kyoto University (approval number: Med Kyo 14032). Thirty-eight male Wistar rats (12 weeks old; mean body weight = 274.3 g) were purchased and placed in plastic cages with sawdust bedding (3–4 animals per cage). The cages were kept in a room with a 12-h/12-h dark/light cycle and constant temperature. Animals were allowed to move freely in the cages and provided free access to food and water. As described previously[6], we performed DMM surgery on each animal's right knee and sham surgery on the left knee under anesthesia using Somnopentyl (Kyoritsu Seiyaku Corp., Tokyo, Japan). A minor incision (1 cm) was made on the medial parapatellar side of the right knee joint, followed by incision of the medial capsule. Then, the medial meniscotibial ligament (MMTL) was transected, allowing the medial meniscus to be displaced medially in knee flexion and extension positions. Finally, the joint capsule and skin were sutured separately. As an internal control, sham surgery was performed on the left knee joint using the same approach without MMTL transection. At 2 and 4 weeks after surgery ($n = 19$ for each time point), the rats were sacrificed and divided into three groups for micro-computed tomography (μ-CT) analysis with histological analysis ($n = 8$ for each time point), scanning electron microscopy (SEM) ($n = 3$ for each time point), and biomechanical testing ($n = 8$ for each time point).

Three-dimensional motion analysis of rat ambulation

To investigate whether site-specific subchondral bone changes corresponded to the region that received a repetitive mechanical load during ambulation, we performed a 3-dimensional gait analysis, prior to sacrifice, at 2 and 4 weeks after surgery ($n = 4$ for each time point). The kinematic properties of both knees during ambulation on the treadmill were recorded using a 3-dimensional motion capture apparatus (Kinema Tracer, Kissei Comtec, Nagano, Japan). The treadmill speed was set at 12 m/min for each experiment. Details regarding the apparatus and measurement setup for recording knee kinematics during ambulation were described in our previous reports[25,26]. In brief, from 3-dimensional motion capture analysis of rat ambulation, we automatically obtained the trajectory of the knee angle, defined as the angle formed by a line connecting the trochanter major (hip) with the knee joint cavity (knee) and a line connecting the lateral malleolus (ankle) with the knee joint cavity (knee). The maximum knee extension and flexion angles and total knee range of motion in the stance and swing phases were calculated.

μ-CT analysis

After sacrifice, we performed μ-CT using a modification of our previously described methods[6]. Rat knee joints were examined in various angles using a μ-CT system (SMX-100CT, Shimadzu, Kyoto, Japan) to determine whether the region of cartilage-subchondral bone alterations coincided with the likely points of contact in the region between the tibia and femur during rat ambulation. We restricted knee joints to a fixed angle using clay after removing the soft tissue to expose the bodies of femur and tibia. Knee angles were determined as the angles formed by the intersection of two lines originating from points bisecting the femur and tibia and measured by a protractor. Scans were performed with the following parameters: 600 incremental views over 360°, 20 exposures averaged per view, voltage of 43 kV, current of 40 μA, and voxel size of 21 μm. Scan time was approximately 5 min per scan. The reconstructed data sets were examined using 3-dimensional data analysis software (Amira 5.4, Visage, Berlin, Germany), and changes in subchondral bone were observed qualitatively.