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Ultrastructural change of the subchondral bone increases the severity of cartilage damage in osteoporotic osteoarthritis of the knee in rabbits

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ABSTRACT

Osteoporotic osteoarthritis is a phenotype of osteoarthritis (OA) manifested as fragile and osteoporotic subchondral bone. However, the ultrastructural features of subchondral bone in osteoporosis OA have not been determined. The study was aimed to investigate the ultrastructural dynamic changes of subchondral bone in osteoporotic OA model and how the ultrastructural damage in the subchondral bone caused by osteoporosis deteriorated the cartilage damage in OA. Eighteen rabbits were equally randomized to three groups, including the control, the OA and the osteoporotic OA groups. The structural changes of cartilage were evaluated by HE and safranin-O fast green staining, the Mankin's grading system was used to assess the stage of OA progression. And microstructural or ultrastructural changes in subchondral bone were assessed by micro-computed tomography or by scanning electron microscopy. According to the changes of cartilage histopathology, the OA group was in the early pathological stage of OA while the osteoporotic OA group was in the middle stage of OA based on Mankin's grading system. In addition, the damage of cartilage surface, reduction in the number of chondrocytes and the matrix staining were more increased in the osteoporotic OA group compared to the OA group. Compared to the OA group, the subchondral bone in the microstructure and ultrastructure in the osteoporotic OA group showed more microfracture changes in trabecular bone with more destructions of the tree-like mesh. Moreover, the collagen fibers were random rough with a fewer amount of bone lacunae in subchondral cortical plate in the osteoporotic OA group compared to the OA group. These findings indicated that the subchondral bone ultrastructure in the osteoporotic OA model was characterized by the destruction of the network structure and collagen fibers. The subchondral bone ultrastructural damage caused by osteoporosis may change mechanical properties of the upper cartilage and aggravate OA cartilage. Therefore, early diagnosis and treatment of osteoporosis is of great significance to prevent early OA from further developing osteoporotic OA.

1. Introduction

Osteoarthritis (OA) is a disease in joints involving mainly the articular cartilage, subchondral bone and osteophytosis. The risk factors of OA are sex, age, inflammation and so on [1]. Interestingly, osteoporosis (OP) is related to the pathogenesis of OA [2,3]. Furthermore, osteoporotic OA, a specific phenotype of OA, is considered as a disease of fragile subchondral bone that causes microfractures to accelerate OA progression [4,5]. There is an intimate biomechanical relationship between the articular cartilage and the subchondral bone, and the mechanical property of the subchondral bone can affect the integrity of the articular cartilage and trigger cartilage degeneration [6–8]. Besides, both OA and OP have the changes of structure in cartilage and subchondral bone in their pathological process [9,10]. Therefore, the study

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on the structural changes of cartilage and subchondral bone is of great significance in the evaluation development of osteoporotic OA.

Current studies have been less focus on the osteoporotic OA, and most research only focus on either OA or OP [11,12]. In fact, clinical experience suggests OA and OP can coexist in the same individual [13–15]. However, the clinical researchers have not completely individuals of osteoporotic OA and normal control because it is difficult to receive age-and gender-matched samples without any diseases [11]. In this case, use of osteoporotic OA animal models without interference from other complex factors is valuable [16]. OP may increase the severity of cartilage damage, which was correlated with the microstructure impairment at subchondral bone [16,17], however, it is not known how the ultrastructural changes of the subchondral bone and cartilage affect the pathological process of osteoporotic OA.

Scanning electron microscopy (SEM) can determine collagen fibril orientation and bone lacuna in different bone tissues [18], micro-computed tomography (micro-CT) can quantify structural changes in the subchondral bone [19]. According to clinical imaging for early OA diagnosis, the simultaneous use of SEM and micro-CT could reveal some unpredictable features of the microstructure of the subchondral bone [20–22]. Thus, for a deeper investigation of the subchondral bone on ultrastructural level, exploring the changes of in mechanical properties combined with morphological observations appears to be a promising approach [23].

We hypothesized that the ultrastructural damage of subchondral bone caused by osteoporosis could contribute to the cartilage damage. To test this hypothesis, we compared the microstructure of cartilage and subchondral bone in the osteoporotic OA and OA rabbit model, trying to demonstrate the characteristic bone structure alterations in OP and OA.

2. Material and methods

2.1. Animals

Eighteen female New Zealand rabbits (Shanghai, China) were housed in the Animal Center of Fujian University of Traditional Chinese Medicine. The rabbits were free access to food and water with a constant temperature conditions. The study was approved by Animal Care and Use Committee of the Fujian University of Traditional Chinese Medicine (protocol number: 2017-010).

2.2. Experimental design

After one week of acclimation, six out of 18 rabbits were randomly selected to induce to OA model in the right knees as before [24,25]. Another six rabbits were firstly induced to OP model by receiving bilateral ovariectomy for four weeks as before [26] but without corticosteroid, followed by again inducing to the osteoporotic OA model as before [24,25]. Another six intacted rabbits acted as the control group.

All animals were sacrificed at 5 weeks after the right knee surgery. Right knee specimens were harvested for histopathologic examination, micro-CT scan and SEM observation.

2.3. Histopathological examination

The femoral sections were fixed in 4% paraformaldehyde for 3 days and then decalcified in 10% EDTA at room temperature for 16 weeks. Subsequently, the medial femoral condyle were cleaved in a sagittal plane along the central portion of the articular surface, and then embedded in paraffin wax. The cartilage sections (4 μm) were stained with haematoxylin and eosin for observation of morphological changes in the articular tissues and safranin-O fast green for evaluation of the proteoglycan contents using a light microscope (Leica, Germany). Cartilage histological changes were assessed according to Mankin's grading system for the evaluations of cartilage structure, cartilage cells, and tidemark integrity, with a score from 0 to 14 [27].

2.4. Micro-computed tomography scan

The microarchitecture of the tibial in the region at $4.2 \times 1.2 \times 1.8$ mm from the subchondral bone was studied using micro-computed tomography (Zhongke kaisheng, China). The X-ray source was set at 60 kV and 666 μA, with a pixel size of 50 μm. 2°. A 3D analysis was used to calculate morphometric parameters of the trabecular bone. The following indices were measured: bone mineral density (BMD), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), bone volumetric fraction (BV/TV).

2.5. Scanning electron microscopy observation

After micro-CT scan, the medial two-thirds of the medial tibial condyle was sampled, rinsed with 0.1 M phosphate-buffered saline in deionized water. After dehydrating with tertiary butanol, the specimen was dried in vacuum drier (Jinghong, China), then observed under tabletop scanning microscope (Hitachi, Japan).

2.6. Statistical analysis

Results are represented as the mean ± standard deviation and analyzed by using the SPSS 20.0 for Windows. The intragroup comparison was performed with one-way ANOVA or Kruskal-Wallis. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Cartilage histopathology

The changes of cartilage structure, cartilage cells and tidemark integrity were assessed using H&E staining. There was no evidence of cartilage degeneration in the control group, the cartilage surface was smooth, chondrocyte arranged in rows and tidemark was observed (Fig. 1A). However, the OA group exhibited the partial damaged cartilage surface, with disordered chondrocyte, tidemark replication, and a thickening of cartilage layer (Fig. 1B). By contrast, the osteoporotic OA group showed the cartilage surface damaged, and the number of chondrocytes was reduced and arranged disorderly with tidemark replication (Fig. 1C). These results suggested that the structural impairment at cartilage in the osteoporotic OA group was more serious than that in the OA group, indicating OP may contribute to cartilage damage in the OA animals.

To evaluate the content of proteoglycan in cartilage, we performed safranin-O fast green staining. The control group exhibited the normal cartilage proteoglycan staining (Fig. 1D), by contrast, the moderate or the severe proteoglycan loss was observed in the OA group (Fig. 1E) or in the osteoporotic OA group (Fig. 1F), respectively. These results indicated that OP may increase the loss of proteoglycan in cartilage of OA animals.

Cartilage histological was assessed according to the Mankin score. Compared with the control group, Mankin score was generally increased in both the OA and the osteoporotic OA groups (Fig. 1G, $P < 0.05$ or $P < 0.01$). However, the osteoporotic OA group showed slightly higher in Mankin score than that in the OA group, although there were no statistical differences between the OA and the osteoporotic OA group. According to Mankin score, the score 1–5, 6–9 or 10–14 is regarded as in the early, middle or the late stages of OA. The results suggest that the cartilage damage in the osteoporotic OA group was in the middle stages of OA, while the OA group was in the early stages of OA.