High Fibular Osteotomy Ameliorates Medial Compartment Knee Osteoarthritis in a Rabbit Model

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Research Article

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Abstract

**Purpose** Knee osteoarthritis (KOA) is a common and severe disease characterized by articular cartilage degeneration, subchondral bone remodeling and inflammation. This study aimed to investigate the therapeutic effects of high fibular osteotomy (HFO) in a KOA rabbit model and to examine the molecular mechanisms involved in medial compartment KOA protective effects.

**Methods** A rabbit model of destabilization of the medial meniscus was used to induce post-traumatic KOA. The effectiveness of HFO on protection against KOA was tested. Hematoxylin and eosin staining, Safranin O/Fast green staining and micro-CT analysis were performed to evaluate structural and morphological changes. The expression of metalloproteinase (MMP)-1, MMP-3, MMP-13, collagen type II (Col2), a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS)-5, aggrecan, interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α was assessed by real time PCR, western blotting and enzyme-linked immunosorbent assay. Additionally, western blotting was performed to test the expression of NFκB p65, phospho-IκBα and IκBα.

**Results** HFO delayed the progression of articular cartilage damage and suppressed subchondral bone remodeling. HFO also decreased MMP-1, MMP-3, MMP-13 and ADAMTS-5 expression, and increased Col2 and aggrecan expression. In parallel, HFO attenuated the expression of IL-1β, IL-6 and TNF-α. Furthermore, the molecular mechanism underlying the protective effect of HFO in medial compartment KOA was related to the NFκB signaling pathway.

**Conclusion** HFO may be a novel therapeutic approach to treating medial compartment KOA.

Introduction

Knee osteoarthritis (KOA) is one of the most common degenerative joint diseases with accompanying joint pain, joint stiffness, and deformity. The prevalence of KOA is estimated to be between 7–33% and increases with age. In addition to age, factors associated with KOA include sex, genetic predisposition, inflammation, occupation, obesity, and trauma. Pathological changes in joint components are mainly articular cartilage damage, osteophyte formation and degeneration, synovium inflammation, and injury of the subchondral bone and meniscus. However, the exact pathogenesis of KOA is not fully understood.

Medial compartment KOA is a critical part of KOA. The treatment methods of medial compartment KOA are diverse. They include medication and physiotherapy, as well as surgical procedures such as total knee arthroplasty (TKA), unicompartmental knee arthroplasty (UKA) and high tibial osteotomy (HTO), which are the main treatments known to have a definitive clinical effect. Because arthroplasty is complex, costly, and inappropriate for young patients and patients with severe comorbidities, HTO is more suitable for young and active patients and is increasing in popularity. However, HTO also has some disadvantages, such as being highly surgically and technically demanding and requiring long rehabilitation periods. Serious potential complications may arise from the surgery, including nonunion, iatrogenic fracture, surgical
wound infection, and nerve and vascular injuries. Recently, high fibular osteotomy (HFO) has been proposed for the treatment of medial compartment KOA. HFO is a simple surgical technique that relieves pain and improves joint function in medial compartment KOA patients via fibular osteotomy. HFO is easier, safer, faster, less expensive, and requires less rehabilitation compared to TKA, UKA and HTO. However, the exact molecular mechanism of HFO in medial compartment KOA remains unknown.

In the present study, we assessed whether HFO could alleviate osteoarthritis in a rabbit model of destabilization of the medial meniscus (DMM) and evaluated the molecular mechanisms that contribute to protective effects in KOA.

**Materials And Methods**

**Rabbits.**

All animal experiments were approved by the Animal Ethics Committee of The First People's Hospital of Kashi. We confirm that all experiments were performed in accordance with relevant guidelines and regulations. Rabbits were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). We confirm that all experiments were carried out in accordance with relevant guidelines and regulations. All rabbits were provided sterile rabbit diets and water, and were housed under controlled 12 h light/dark cycle conditions. Rabbits (n = 24) were randomly divided into four groups (n = 6 per group): Sham surgery only group (Sham group), destabilization of the medial meniscus (DMM) alone group (DMM group), DMM plus Sham group (DMM+Sham group), and DMM plus HFO group (DMM+HFO group).

**HFO model.**

All animal experiments were approved by the Animal Ethics Committee of The First People's Hospital of Kashi. We confirm that all experiments were performed in accordance with relevant guidelines and regulations. Rabbits were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). We confirm that all experiments were carried out in accordance with relevant guidelines and regulations. An approximately 7-8 mm longitudinal incision was created along the lateral skin of the proximal fibula. Tissue forceps were used to expose the fibia by separating the peroneus longus and gastrocnemius. An approximately 2 mm length of fibula was removed by HFO. The sham procedure was performed without osteotomy.

**DMM-induced KOA model.**

All animal experiments were approved by the Animal Ethics Committee of The First People's Hospital of Kashi. We confirm that all experiments were performed in accordance with relevant guidelines and regulations. Rabbits were purchased from Guangdong Medical Laboratory Animal Center (Guangdong,
China). We confirm that all experiments were carried out in accordance with relevant guidelines and regulations. The rabbits were subjected to surgically induced KOA by DMM as described previously. They were deeply anesthetized with 50 mg/kg phenobarbital sodium before microsurgical scissors were used to transect the medial meniscotibial ligament. The same procedure was carried out on the Sham group without transection.

**Micro-CT analysis.**

The micro-CT equipment (Skyscan 1176, Bruker micro-CT N.V., Kontich, Belgium) was used to scan the knee joint of rabbits. A three-dimensional model was reconstructed for further analysis. The region of interest was identified between the cartilage and the growth plate. The morphometric parameters of trabecular bone volume fraction, the ratio of trabecular bone volume to total volume (BV/TV) were calculated by the histomorphometric analysis.

**Hematoxylin and eosin (H&E) staining.**

Cartilage tissues were fixed in 4% paraformaldehyde for 48 h, decalcified with 13% EDTA for 8 weeks, and dehydrated in graded ethanol. After dehydration, the specimens were embedded in paraffin wax and cut into 3 μm-thick sections. The paraffin sections were hydrated in xylene and graded ethanol, and stained by H&E.

**Safranin O-Fast green staining.**

After paraffin sections were hydrated in xylene and graded ethanol, they were stained with 0.5% Fast Green for 20 min, and counterstained with 0.5% Safranin O for 5 min. The paraffin sections were then subjected to gradient alcohol dehydration, transparent xylene, and neutral gum seal. The modified osteoarthritis research society international (OARSI) scoring was used to evaluate the rabbit articular cartilage degradation as described previously.

**Real time PCR.**

Total RNA from cartilage tissues was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) as described by the manufacturer. The concentration of RNA was examined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Reverse transcription was performed using a reverse transcription kit (Takara, Dalian, China). The expression levels of mRNAs were detected using SYBR Green Real-time PCR Master Mix (Takara). β-actin was used as a loading control. The fold change in gene expression of cartilage tissues was calculated by using $2^{-\Delta\Delta Ct}$ method. All primer sequences are listed in Supplementary Table 1.
Western blotting.

Total proteins from cartilage tissues were lysed using ice-cold cell lysis buffer with protease inhibitors (Promega, Madison, WI, USA). The BCA protein assay kit (Beyotime Institute of Biotechnology, Jiangsu, China) was used to determine the protein concentration of the sample. Equal amounts of protein extraction (20 μg) were separated by SDS–polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. After blockage by 5% fat-free milk, the membranes were incubated with primary antibodies against metalloproteinase (MMP)-1 (1:500, Proteintech, Wuhan, China), MMP-3 (1:1000, Proteintech, Wuhan, China), MMP-13 (1:2000, Novus Biologicals, CO, USA), collagen type II (Col2, 1:200, Novus Biologicals, CO, USA), a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS)-5 (1:300, Absin, Shanghai, China), aggrecan (1:100, Novus Biologicals, CO, USA), IκBα (1:500, Bioss, Beijing, China), phospho-IκBα (p-IκBα, 1:500, Absin, Shanghai, China), NFκB p65 (1:500, Bioss, Beijing, China) at 4 °C overnight. Next, blots were incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for 2 h. The signals were examined using enhanced chemiluminescence. Quantification was performed using Multi Gauge software version 3.1 of the imaging system LAS-4000 (Fujifilm, Tokyo, Japan) 11.

Enzyme-linked immunosorbent assay (ELISA).

The levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in the synovial uid were examined using ELISA kits (Biorbyt, Cambridge, UK) according to the manufacturer’s instructions.

Statistical analysis.

Data are expressed as mean ± standard deviation (SD). Comparison between two groups was conducted by using a Student’s t-test. Analysis was performed using GraphPad Prism 5.0 software (San Diego, CA, USA). P-values less than 0.05 were considered significant.

Results

HFO reduces articular cartilage degradation in DMM-induced KOA model.

To explore the effect of HFO treatment on cartilage degradation in the DMM-induced KOA model, histological analysis with H&E and Safranin O-Fast Green staining was carried out. As shown in Fig. 1A, B, the Sham group showed normal cartilaginous structures. Comparing to the Sham group, superficial cartilage destruction and chondrocyte death were observed in the DMM group and DMM + Sham group. Compared to the DMM + Sham group, the DMM + HFO group had increased articular cartilage thickness and reduced cartilage damage. Furthermore, the cartilage score of each group was assessed. The
modified OARSI score was significantly higher in the DMM group and DMM + Sham group than in the Sham group, and the DMM + HFO group exhibited lower OARSI scores than the DMM + Sham group (Fig. 1C). These data indicate that HFO could protect against articular cartilage damage.

**HFO attenuates subchondral bone remodeling.**

To investigate whether HFO could reduce the progression of KOA after DMM in rabbits, the bone phenotype of rabbit knee joints was assessed using the three-dimensional image. As shown in Fig. 2A, HFO prominently decrease DMM-induced osteophyte formation. Moreover, we found that the DMM group and DMM + Sham group developed serious osteosclerosis, this effect was significantly reversed by HFO treatment (Fig. 2A, B). To analyze the extent of osteosclerosis among the groups, the value of BV/TV was calculated. The value of the DMM group and DMM + Sham group was significantly higher than that of the Sham group, whereas the value of the DMM + HFO group was lower compared to the DMM + Sham group (Fig. 2C). These results indicate that HFO restrains the subchondral remodeling in the progression of KOA.

**HFO suppresses the metabolism of cartilage matrix.**

MMPs and ADAMTS are well-known matrix-degrading enzymes. MMPs play a key role in the degradation of Col2 to destruct the cartilage. ADAMTSs demonstrate proteolytic activity toward the hyalectan group of chondroitin sulfate proteoglycans, such as aggrecan 12. Therefore, MMPs and ADAMTSs play important roles in detecting cartilage degeneration. As shown in Fig. 3, 4, the mRNA and protein expression levels of MMP1, MMP3, MMP13 and ADAMTS-5 in the DMM group and DMM + Sham group were much higher than those in the Sham group. In contrast, the DMM + HFO group had an opposing expression pattern of these genes. Compared to the Sham group, the mRNA and protein expression levels of Col2 and aggrecan in the DMM group and DMM + Sham group were remarkably diminished. These inhibitory effects were reversed in the DMM + HFO group. These findings demonstrate that HFO plays a protective role in articular cartilage degeneration by restraining the degradation of Col2 and aggrecan.

**HFO protects against inflammatory response in rabbit models of KOA.**

Pro-inflammatory factors are key mediators in inflammatory reactions. Abnormal expression of inflammatory factors in osterarthritis (OA) can lead to abnormal cartilage matrix metabolism and an increase of apoptotic cells, which eventually causes cartilage degeneration 13,14. To investigate the anti-inflammatory effects of HFO treatment in a KOA model, the expression levels of pro-inflammatory factors TNF-α, IL-6 and IL-1β in cartilage tissue and joint synovial fluid were measured. TNF-α, IL-6 and IL-1β mRNA expression levels in cartilage tissue were markedly enhanced in the cartilage of the DMM group and DMM + Sham group. On the contrary, the mRNA expression levels of IL-1β, IL-6, and TNF-α were
decreased in the DMM + HFO group (Fig. 5A). Meanwhile, the DMM group and DMM + Sham group led to a significant upregulation in the protein expression levels of TNF-α, IL-6 and IL-1β in joint synovial fluid compared to the Sham group, while the DMM + HFO group showed a downregulation compared to the DMM + Sham group (Fig. 5B). These results show that the inflammation in KOA was significantly improved after HFO surgery.

**HFO antagonizes activation of NF-κB signaling pathway.**

Because the NF-κB signaling pathway is related to cartilage degeneration, cartilage matrix metabolism, and inflammatory response \(^{15-17}\), we hypothesized that HFO regulates cartilage degeneration, cartilage matrix metabolism, and inflammation through the NF-κB signaling pathway. To confirm this idea, we analyzed p-IkBα, IkBα and p65 expression levels with western blotting. The expression levels of p-IkBα were statistically upregulated in the DMM and DMM + Sham groups while this effect was reversed in the DMM + HFO group (Fig. 6A). Similarly, the DMM and DMM + Sham groups displayed a significant increase in the expression of p65. HFO surgery decreased p65 expression levels in the DMM + HFO group (Fig. 6B). These results suggest that HFO may inhibit cartilage degeneration, cartilage matrix metabolism, and inflammatory response by downregulating the NF-κB signaling pathway.

**Discussion**

Biomechanically, KOA results from an imbalance in biological resistance and mechanical stress. Reducing stress on affected articular cartilage is one important treatment for KOA\(^ {18}\). Osteotomy around the knee attenuates the stress on arthritic articular cartilage. High tibial osteotomy has been regarded as a satisfactory surgical method to treat medial compartment KOA with varus deformity\(^ {19}\). The procedure aims to ameliorate the stress acting on the mechanical axis of the lower limb and to relieve the forces acting on the medial compartment of the knee joint. HTO can stop or delay the progression of medial compartment KOA while improving knee joint pain and patient mobility \(^ {20}\). However, the surgery is complex and technically demanding for young doctors \(^ {21}\). High fibular osteotomy is an innovative approach to medial compartment KOA treatment, which is based on the nonuniform settlement theory \(^ {7}\). According to the theory, lateral support provided to the osteoporotic tibia by the fibula–soft tissue complex may lead to the bilaterally nonuniform settlement and degeneration of the plateau \(^ {7}\). This may lead to a medial shift of the load distribution to the medial plateau, causing knee varus and thus accelerating medial compartment knee OA progression \(^ {22}\). HFO aims to weaken lateral fibular support, relieve nonuniform settlement, correct varus deformity, and alleviate the knee joint. In this study, we used rabbit models of KOA to verify the effect of HFO on the development of medial compartment KOA.

Several animal studies have been reported that the change of subchondral bone takes place in the early stages of OA, and is associated with degeneration of articular cartilage \(^ {23,24}\). Subchondral bone
undergoes remodeling and modelling in response to an abnormal and excessive mechanical loading environment, causing abnormal bone remodeling and osteophyte formation. Our results revealed that DMM induced the changes of subchondral bone was restored via HFO surgery, indicating that HFO restrained the subchondral bone remodeling with less osteophyte formation in the KOA. Additionally, articular cartilage degeneration and inflammation have been reported to play a crucial role in the initiation and progression of OA. IL-1β and TNF-α are recognized as the major players in synovial inflammation and cartilage damage. TNF-α not only cooperates with IL-1β, but also induces the secretion of IL-6, which plays an important role in the genesis and development of OA. In OA patients, pro-inflammatory cytokines including TNF-α, IL-6 and IL-1β were expressed in the synovial, cartilage and subchondral bone. These pro-inflammatory cytokines can increase the expression levels of MMP-1, MMP-3, MMP-13, ADAMTS4, ADAMTS5 and other catabolic enzymes, which degrade the extracellular matrix and destroy the articular cartilage. In this study, we observed increased articular cartilage degeneration and pro-inflammatory factors TNF-α, IL-6, and IL-1β in our DMM group, whereas in our DMM+HFO group, these effects were reversed.

NF-κB is a critical signaling pathway involved in the pathogenesis of OA. NF-κB activation can regulate the expression of catabolic cytokines and chemokines, including IL-6, TNF-α, IL-1β, IL-8 and receptor activator of NF-κB ligand (RANKL). These catabolic cytokines and chemokines can increase the expression of many degradative enzymes, such as MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP13, ADAMTS4 and ADAMTS5, and inhibit collagen and proteoglycan synthesis, resulting in ECM damage and articular cartilage destruction. Once the NF-κB p65 signaling pathway is activated, IκB-α is phosphorylated and degraded. NF-κB p65 is then phosphorylated and translocated from the cytoplasm into the nucleus, where it triggers OA-related gene expression. In this study, we demonstrated that our DMM group facilitated the phosphorylation of IκB-α and p65, which was reversed in our DMM+HFO group.

In conclusion, our study is the first to demonstrate that HFO successfully ameliorated cartilage degeneration, subchondral bone remodeling and inflammation in KOA rabbits by regulating the NF-κB signaling pathways. Thus, our study provides new insights into understanding HFO treatment for medial compartment KOA.

Declarations

Acknowledgment

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Authors Contributions
Feihua Yan made plans for the experiment. Xujun Zhao, Shisheng Duan and Aini Maimaiti performed experiments. Yong Qi and Maozhao Li supported their method. Yong Qi, Maozhao Li, Muteli Maimaiti and Wenqiang Li joined the discussion, and then Feihua Yan and Xujun Zhao wrote the manuscript.

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**Conflict of interest**

The authors declare no conflicts of interest.

**Ethics approval**

All animal experiments were approved by the Animal Ethics Committee of The First People's Hospital of Kashi. We confirm that all experiments were performed in accordance with relevant guidelines and regulations. We confirm that the study was carried out in compliance with the ARRIVE guidelines.

**Data Availability Statement**

The data used to support the findings of this study are available from the corresponding author upon request.

**References**


HFO reduces articular cartilage degradation in the rabbit models of KOA. (A) H&E and Safranin O-Fast Green staining (B) were performed to analyze the morphological structure of rabbit articular cartilage samples. (C) The modified OARSI scores were used to evaluate the degree of articular cartilage destruction. Values are means ± SD, n= 6. *p<0.05 vs Sham group; #p<0.05 vs DMM+Sham group.
Figure 2

HFO attenuates subchondral bone remodeling. (A) Three-dimensional reconstruction images. (B) Two-dimensional reconstructions. (C) Quantification of the bone morphological parameters (BV/TV). Values are means ± SD, n= 6. *p<0.05 vs Sham group; #p<0.05 vs DMM+Sham group.
Figure 3

The mRNA expression levels of MMP1, MMP3, MMP13, ADAMTS-5, Col2 and aggrecan were examined by real time PCR. Values are means ± SD, n= 6. *p<0.05 vs Sham group; #p<0.05 vs DMM+Sham group.
Figure 4

The protein expression levels of MMP1, MMP3, MMP13, ADAMTS-5, Col2 and aggrecan were examined by real time PCR. Values are means ± SD, n= 6. *p<0.05 vs Sham group; #p<0.05 vs DMM+Sham group.
Figure 5

HFO protects against inflammatory response in rabbit models of KOA. (A) TNF-α, IL-6 and IL-1β mRNA expression levels in cartilage tissue were tested by real-time PCR. (B) ELISA analysis of TNF-α, IL-6 and IL-1β protein expression levels in joint synovial fluid. Values are means ± SD, n= 6. *p<0.05 vs Sham group; #p<0.05 vs DMM+Sham group.
Figure 6

HFO antagonizes activation of NF-κB signaling pathway. (A) The expression of p65, p-κBα and κBα (B) in each group was assayed by western blotting. Values are means ± SD, n= 6. *p<0.05 vs Sham group; #p<0.05 vs DMM+Sham group.

Supplementary Files

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- SUPPLEMENTARYMATERIAL.docx
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