Osteoarthritis and Cartilage 22 (2014) 1036–1043

Osteoarthritis and Cartilage

Destabilization of the medial meniscus leads to subchondral bone defects and site-specific cartilage degeneration in an experimental rat model

H. Iijima †, T. Aoyama ‡, A. Ito †, J. Tajino †, M. Nagai †, X. Zhang †, S. Yamaguchi †, H. Akiyama §, H. Kuroki † *

† Department of Motor Function Analysis, Human Health Sciences, Graduate School of Medicine, Kyoto University, Japan
‡ Department of Development and Rehabilitation of Motor Function, Human Health Sciences, Graduate School of Medicine, Kyoto University, Japan
§ Department of Orthopaedic Surgery, Graduate School of Medicine, Gifu University, Japan

ARTICLE INFO

Article history:
Received 20 February 2014
Accepted 7 May 2014

Keywords:
Destabilization of medial meniscus
Subchondral bone defects
Cartilage degeneration
Cartilage covered by menisci

SUMMARY

Objective: This study aimed to investigate subchondral bone changes using micro-computed tomography (micro-CT) and regional differences in articular cartilage degeneration, focusing on changes of cartilage covered by menisci, in the early phase using a destabilization of the medial meniscus (DMM) model.
Method: The DMM model was created as an experimental rat osteoarthritis (OA) model (12 weeks old; $n = 24$). At 1, 2, and 4 weeks after surgery, the rats were sacrificed, and knee joints were scanned using a Micro-CT system. Histological sections of the medial tibial plateau, which was divided into inner, middle, and outer regions, were prepared and scored using the modified OARSI scoring system. The cartilage thickness was also calculated, and matrix metalloproteinase 13 (MMP13), Col2-3/4c, and vascular endothelial growth factor (VEGF) expression was assessed immunohistochemically.
Results: Subchondral bone defects were observed in the middle region, in which the cartilage thickness decreased over time after surgery, and these defects were filled with MMP13- and VEGF-expressing fibrous tissue. The OARSI score increased over time in the middle region, and the score was significantly higher in the middle region than in the inner and outer regions at 1, 2, and 4 weeks after surgery. Col2-3/4c and MMP13 expression was observed primarily in the meniscus-covered outer region, in which the cartilage thickness increased over time.
Conclusion: Loss of meniscal function caused cartilage degeneration and subchondral bone defects in the early phase site-specifically in the middle region. Furthermore, our results might indicate cartilage covered by menisci is easily degraded resulting in osmotic swelling of the cartilage in early OA.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and a major cause of pain[1] and disability[2] in older adults. The common risk factors for knee OA include age, sex, obesity, prior joint injury, and mechanical factors, including malalignment and abnormal joint kinematics during ambulation[3]. Despite the multifactorial nature of knee OA, the mechanical environment of the knee during ambulation has a profound influence on the initiation and progression of knee OA[4]. The increased incidence of medial compartment knee OA is therefore believed to result from higher mechanical loading of the medial compartment.

The menisci play an important role in load-bearing distribution at the knee[5]. Loss of meniscal function due to meniscal extrusion and meniscectomy result in increased mechanical loading of the articular cartilage and subchondral bone of the affected knee[6,7]. Total meniscectomy increases the risk of initiation and progression of knee OA radiographically by 14-fold after 21 years[8]. However, the initiating events in knee OA after the loss of meniscal function remain unknown.

* Address correspondence and reprint requests to: H. Kuroki, Department of Motor Function Analysis, Human Health Sciences, Graduate School of Medicine, Kyoto University, 53 Shogoin, Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. Tel: 81-75-751-3963; Fax: 81-75-751-3909.
E-mail addresses: iijima.hirotaka.75s@st.kyoto-u.ac.jp (H. Iijima), aoyama.tomoki.4e@kyoto-u.ac.jp (T. Aoyama), ito.akira.27n@st.kyoto-u.ac.jp (A. Ito), tajino.junichi.57z@st.kyoto-u.ac.jp (J. Tajino), nagai.momoko.36v@st.kyoto-u.ac.jp (M. Nagai), zhang.xiangkai.48v@st.kyoto-u.ac.jp (X. Zhang), yamaguchi.shouki.26n@st.kyoto-u.ac.jp (S. Yamaguchi), hakiyama@kuhp.kyoto-u.ac.jp (H. Akiyama), kuroki.hiroshi.6s@kyoto-u.ac.jp (H. Kuroki).

http://dx.doi.org/10.1016/j.joca.2014.05.009
1063-4584/

Recently, subchondral bone changes are determined to play an important role in the pathogenesis of knee OA[9,10]. In human studies, subchondral bone fracture frequently occurs after meniscectomy, worsening the patient's clinical condition[11–13]. Additionally, meniscal extrusion is associated to increase subchondral bone cyst[7]. However, whether these subchondral bone changes occur in early phase after loss of meniscal function are still unknown. Therefore, understanding of the subchondral bone changes in the early phase after loss of meniscal function is important for preventing the initiation and progression of knee OA.

An experimental OA model of surgical destabilization of the medial meniscus (DMM) has been used for common knee OA basic research[14]. Previous studies showed subchondral bone changes by histological methods after DMM[15–17]. Recently, extensive micro-computed tomography (micro-CT) studies which observed morphological changes of subchondral bone in a rat OA model have been conducted[9,18]. Also in DMM rat model, trabecular porosity was increased in 4 weeks after surgery by micro-CT study[19], however the time-course of subchondral bone changes in early phase after DMM is still unknown.

DMM results in an elevation of peak local contact stress in the medial compartment[20,21] and even changes the distribution of mechanical loading in menisci-covered and uncovered cartilage. In recent *ex vivo* studies, cartilage covered by menisci differed from uncovered cartilage in terms of histology[22,23], mechanical properties[22–24], and metabolic activities[25]. These studies indicated cartilage covered by menisci is potentially susceptive to mechanical loading than cartilage uncovered by menisci. Some animal studies indicated that the kinetics of cartilage degeneration differ between these two regions[24,26,27], and cartilage covered by menisci displays greater degeneration after meniscectomy than uncovered cartilage[28]. However the time-course change and regional change of cartilage especially cartilage covered by menisci after loss of meniscal function are still unclear.

The first purpose of this study was to investigate subchondral bone changes over time after loss of meniscal function using micro-CT and the second was to investigate regional differences in articular cartilage degeneration, focusing on changes of cartilage covered by menisci, in the early phase using a DMM model.

Method

Experimental animals and surgical procedures

This study was approved by the animal research committee of Kyoto University (approval number: 13602). An experimental OA model was created *via* DMM[14] in male Wistar rats (12 weeks old; $n = 24$; mean body weight, 272.4 g). After the rats were anesthetized by 8.5 ml/kg somnopentyl, the right knee joint was exposed following a medial capsular incision and gentle lateral displacement of the knee extensor muscles without transection of the patellar ligament. Then, the medial meniscotibial ligament (MMTL) was transected, and the medial meniscus could be displaced medially. After replacement of the extensor muscles, the medial capsular incision was sutured, and the skin was closed. A sham operation was performed on the left knee joint using the same approach without MMTL transection. The animals were then permitted unrestricted activity and provided free access to food and water.

Micro-CT analysis of the subchondral bone

At 1, 2, and 4 weeks after surgery ($n = 8$ for each time point), the rats were sacrificed, and knee joints were scanned using a Micro-CT system (SMX-100CT, Shimadzu, Kyoto, Japan) at voxel size 21 μm resolution. After scanning, the knee joint was three-dimensionally reconstructed using a software package (Amira5.4, Visage, Berlin, Germany). Changes of the subchondral bone were observed in the sagittal and frontal sections qualitatively.

Histological analysis

The knee joints were fixed in 4% paraformaldehyde overnight and decalcified in 10% EDTA. The samples were dehydrated and embedded in paraffin. Paraffin sections were prepared from the medial tibial plateau in the frontal plane according to previously described methods[29] and alternately stained with hematoxylin-eosin (H-E) and toluidine blue. The toluidine blue-stained sections were evaluated using the OARSI score established by Pritzker *et al.*[30], which scores the product of six grades (depth of lesion) and four stages (extent of involvement) on a scale of 0 (normal) to 24 (severe OA). To detect regional differences in cartilage, the method was slightly modified such that the cartilage of the medial tibia was divided into outer, middle, and inner regions, each comprising approximately one-third of the total arc length[31,32]. The cartilage in the outer regions is covered by the meniscus, whereas the cartilage in middle and inner region is not covered by the meniscus. To assess cartilage thickness, the frontal sections were used for thickness histomorphometric analysis, and digital images of each region (×40) were captured. The cartilage thickness of each region was defined as the mean value of three thickness measurements performed at regular intervals perpendicular to the cartilage surface[33] in the photo image of histology using the Image-J software.

Immunohistochemistry

Immunohistochemical staining of vascular endothelial growth factor (VEGF), matrix metalloproteinase 13 (MMP13), and Col2-3/4c was performed. Col2-3/4C antibody was kindly donated by Dr A.R. Pool (Shriners Hospitals for Children, Department of Surgery, McGill University, Montreal). The Col2-3/4C antibody is a rabbit polyclonal antibody directed against the COOH terminus of the three-quarter fragment. It is generated specifically *via* cleavage of native type II collagen by mammalian collagenases. Deparaffinized sections were treated with 0.3% hydrogen peroxide to reduce endogenous peroxidase activity. Then, the sections were treated with 1.25 (anti-VEGF) or 0.6% (anti-MMP13) hyaluronidase (Sigma–Aldrich Co., St Louis, MO, USA) in PBS for 60 min at room temperature and 1% hyaluronidase in PBS for 30 min at 37°C (Col2-3/4c). Non-specific staining was blocked by incubation of the sections with 5 (anti-VEGF) or 10% (anti-MMP13, Col2-3/4c) normal goat serum for 60 min.

Subsequently, the sections were treated with anti-VEGF (diluted 1:50), anti-MMP13 (diluted 1:1000), and Col2-3/4c (diluted 1:800) and further incubated overnight at 4°C. Detection was performed using the streptavidin-biotin-peroxidase complex technique with an Elite ABC kit (diluted 1:100; Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was visualized by incubation with diaminobenzidine solution (Vector Laboratories) followed by counterstaining with hematoxylin. The primary antibody was not added to negative controls.

Statistical analysis

The software program JMP 11 (SAS Institute, Cary, NC USA) was used for the statistical analysis. Descriptive statistics were calculated as median and interquartile range for OARSI score and as means and 95% confidence intervals (CIs) for cartilage thickness. The Mann–Whitney *U* test was used for pair-wise differences of OARSI score and the paired *t*-test was used for pair-wise differences