Histopathological Effects of Loading on Cartilage Repair in a Rat Full-thickness Articular Cartilage Defect Model

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Abstract. [Purpose] We investigated effects of loading on cartilage repair in rat full-thickness articular cartilage defects and histopathologically analyzed the healing process of the defect. [Subjects] A total of 40 male 9-week-old Wistar rats were used. [Methods] Full-thickness articular cartilage defects were created over the capsule at the loading portion in the medial condyle of the femur. Twenty rats were randomly allocated into each of a loading group and non-loading group. Twenty rats from these two groups were later randomly allocated to each of 2 groups for evaluation at 1 and 2 weeks after surgery. At the end of each period, knee joints were examined histopathologically and statistically. [Results] The surface of the repair tissue in the loading group was irregular and discontinuous, while that in the non-loading group was smooth and continuous. The results of the statistical analysis showed that the difference in the surface between the loading group and non-loading group was significant. The defects in the both groups spontaneously resurfaced with a mixture of hyaline cartilage and granulation tissue, and some remnants of articular cartilage with aseptic necrosis were contained in the repair tissue. [Conclusion] We concluded that loading in the early phase of an articular cartilage defect may be accompanied by a certain danger for the repair tissue.

Key words: Articular cartilage, full-thickness defect, loading

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INTRODUCTION

Traumatic articular cartilage defects of the knee are a common cause of pain and functional disability in orthopedics and sports medicine1). Unfortunately, when cartilage is damaged due to injury or disease, it has a limited capacity to heal. Human adult articular cartilage contains no blood supply, neural network, or lymphatic drainage2, 3). Furthermore, the abilities of repair and division of chondrocytes are limited, and nutrition supplied to the cartilage is exclusively provided by synovial fluid produced by the synovial membrane. As a result, full-thickness articular cartilage defects that penetrate through cartilage undergo regenerative repair of hyaline cartilage under restricted conditions4). Many researchers have studied the mechanism of articular cartilage regeneration. However, it has not been fully clarified, and there is still no consensus of opinion about the tissue repair process5–7). In addition, the gene expression, the cell differentiation and the growth factors that are needed for cartilage regeneration remain uncertain8).

It has been reported that mechanical stress like loading is essential for metabolism of cartilage. Many researchers have reported the influence of loading on cartilage metabolism. Mechanical stress that is quantitatively appropriate advances cartilage metabolism; on the other hand, stress that is quantitatively excessive or insufficient decreases the metabolism9–10). It has been reported that appropriate loading stimulates cytokines and growth factors and promotes the circulation of synovial fluid. Therefore, it is supposed that loading may play an important role for cartilage repair11, 12). However, to our knowledge, only a few in vivo studies have investigated time-sequential changes in articular cartilage regeneration under different mechanical conditions. Harada reported that dynamic compressive strain stimulates regeneration of the joint surface structure13). They also suggested that the contact condition of the defect with surface cartilage may play an important role in hyaline cartilage repair. However, the effect of loading for cartilage repair and the mechanism of it have not been fully clarified. The purposes of this study were to evaluate histopathologically the healing process of articular cartilage defects in rat knee joints that had undergone loading by walking or non-loading by tail suspension.

METHODS

Forty 9-week-old male Wistar rats were used in this study. The animals were kept under normal conditions for one week before the start of the experiments in order to acclimatize them to the environment. They were housed, 1
or 2 per a cage, in a room maintained under a 12-hour light-dark cycle, and food and water were given ad libitum.

In our previous study, we reported on a low-invasive method to create full-thickness articular cartilage defects in rats as our model\textsuperscript{14}. In the present study, full-thickness articular cartilage defects were created as described previously\textsuperscript{14}. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital at a dose of 40 mg/kg. After shaving the left knees, they were disinfected, and a parapatellar incision was performed to expose the knee joint. In maximum flexion of the knee, full-thickness defects (0.8 mm diameter, 2.0 mm depth) of articular cartilage were created over the capsule with a Kirschner wire (0.8 mm in diameter) in the medial condyle (Fig. 1). At the height of the center of the patella tendon, the defect was created at the medial position of the inner margin of the tendon (half the tendon width in length). The wire was marked at a position 2.0 mm from the tip to ensure invasive depth uniformity. After creation of the defect, the skin was sutured. All rats were allowed to move freely in their cages without knee immobilization after surgery. After the operation, the rats were randomly classified into two groups: a loading group (n=20) and non-loading group (n=20). Twenty rats from these two groups were later randomly allocated to each of 2 groups for evaluation at 1 and 2 weeks after surgery. The loading group was allowed to walk immediately after regaining consciousness following anesthesia. The non-loading group was subjected to hindlimb suspension for each experiment term, so their knee joints were under a non-loading condition. Hindlimb suspension in the present study was performed using the modified Andries Ferreira’s tail-suspended method\textsuperscript{15}. This modified method was low-invasive, consisting of the application of a Kirchner wire. The hindlimb was suspended so that it did not touch the floor, and both knee joints bore no weight. During suspension, rats could move their forelimbs freely for the intake of food and water.

At 1 and 2 weeks after surgery, rats were sacrificed by an intraperitoneal injection of an overdose of sodium pentobarbital. Immediately after death, their left hind limbs were disarticulated at the hip joint. All left knees were fixed in 10% neutral buffered formalin for 72 hours and decalcified with Decalcifying Solution A (Plank-Rychlo Method, Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 72 hours. The knees were excised, decadified in 5% sodium sulfate solution for 72 hours, dehydrated in ethanol after washing with water, and embedded in paraffin wax. Sagittal sections (3 μm) were stained with hematoxylin and eosin and examined and imaged using a light microscope and digital camera (BX-51 and DP-50; Olympus Corporation, Tokyo, Japan). This investigation was approved by the Animal Research Committee of Kanazawa University Graduate School of Medicine, Kanazawa, Japan (approval No. 112206). All procedures for animal care and treatment were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals at Kanazawa University.

The histological character of the repair tissue was evaluated qualitatively in regard to surface and matrix based on the International Cartilage Repair Society Visual Histological Assessment Scale\textsuperscript{16}. The surface of the repair tissue was observed to determine whether it contained any subsidence, discontinuance, and irregularity or not. The matrix was observed to determine whether it contained any granulation tissue, fibrous-like tissue, fibrocartilage, and hyaline cartilage or not.

Data for histological characteristics were analyzed statistically using Fisher’s exact test, and a value of p < 0.05 was accepted as statistically significant.

RESULTS

All animals were conscious and started to walk within several hours after surgery. No rat showed signs of knee infection or swelling or died during the experimental period. Thus, inflammation was macroscopically and microscopically well controlled.

In the histological examination, the surface of the repair tissue in most of the specimens in the loading group was irregular and discontinuous at 1 and 2 weeks after surgery (Table 1, Fig. 2). The surface in the non-loading group was smooth and continuous, and the results of the statistical analysis showed that the difference in the surface between the loading group and non-loading group was significant (p<0.05). Regarding the matrix of the repair tissue in both groups at 1 and 2 weeks after surgery, most of the defects spontaneously resurfaced with a mixture of hyaline cartilage and granulation tissue, and some remnants of articular cartilage with aseptic necrosis were contained in the repair tissue. The results of the statistical analysis showed that there was no difference in the matrix between the loading group and non-loading group.
The purposes of this study were to evaluate histopathologically the influence of loading on the healing process of articular cartilage defects in the rat knee joint. The results showed that the difference in the surface of the repair tissue between the loading group and non-loading group was significant and that the surface in the loading group was irregular and discontinuous, while that in the non-loading group was smooth and continuous. The defects in both groups were spontaneously resurfaced with hyaline cartilage and granulation tissue. (A-D) Scale bar = 200 μm.

**DISCUSSION**

The purposes of this study were to evaluate histopathologically the influence of loading on the healing process of articular cartilage defects in the rat knee joint. The results showed that the difference in the surface of the repair tissue between the loading group and non-loading group was significant and that the surface in the loading group was irregular and discontinuous. Many researchers have reported the risk of weight bearing in the early phase after surgery. Gill suggested that weight bearing or joint loading delays healing and that up to 2 months of bearing no weight may be required to promote early fibrous tissue maturation.

Williams reported that weight bearing, especially in the first 6 weeks after surgery, can cause potential propagation or collapse of the subchondral bone and that shear stress or excessive pressure in this early phase can flatten the repair cartilage or displace the mesenchymal cells and clot from the defect. Kuroki also reported that the acoustic stiffness of implanted cartilage after autologous osteochondral transplantation decreased up to 12 weeks after surgery. In the present study, we presumed that the surface of the repair tissue became irregular because weight bearing against the defect arose before the tissue got mature sufficiently and acquired the strength against mechanical stress. Accordingly, these results indicated that weight bearing in the early phase of an articular cartilage defect may be accompanied by a certain danger for the repair tissue.

Most of the defects in both groups were spontaneously resurfaced with a mixture of hyaline cartilage and granulation tissue, and some remnants of articular cartilage with aseptic necrosis were contained in the repair tissue. Generally, full-thickness articular cartilage defects are believed to induce fibrin clot formation in the area of the chondral defect. This clot contains pluripotent marrow-derived mesenchymal stem cells. These cells are able to differentiate into fibrocytes and chondrocytes, resulting in hyaline cartilage or fibrocartilage repair with varying amounts of type I, II, and X collagen content. However, there is no consensus regarding which cartilage is generated, hyaline cartilage or fibrocartilage. It is known that cartilage repair is influenced by species and age and by position, depth and size of the defect.

Many studies have reported that full-thickness articular cartilage defects regenerated with granulation tissue at 1 or 2 weeks after surgery, and the results of the present study supported these reports. Some of fragments of articular cartilage were found in the repair tissue in both groups. In previous studies, the fragments were removed by surgical equipment. Accordingly, we considered them as the remnants of original articular cartilage that were produced secondarily by low-invasive method used in the present study. The remnants were regenerated concomitantly with aseptic necrosis because the chondrocyte nucleus had disappeared in the repair tissue and an inflammation response was not observed in the repair tissue. Accordingly, we assumed that the remnants may affect the process of regeneration and repair tissue and may promote regeneration. However, we presumed the difference in the method of creating the defect and the existence of the remnants hardly affected the regeneration of cartilage because the results concerning the repair tissue supported the previous studies.

It has been reported that mechanical stress like loading is essential for metabolism of cartilage. Many researchers have reported the influence of loading on cartilage metabolism. Mechanical stress that is quantitatively appropriate advances cartilage metabolism; on the other hand, stress that is quantitatively excessive or insufficient decreases the metabolism. Application of appropriate mechanical stress to articular cartilage advances the expression of transforming growth factor-β1, which promotes matrix metabolism, and sox 9, which promotes differentiation of mesenchymal stem cells into chondral cells, and increases the production of type II collagen and aggrecan. However, insufficient stresses decrease the metabolism and bring about full-thickness articular cartilage defect, thinning of articular cartilage, and expansion of subchondral ossification. Excessive stresses bring about rhexis of collagen fiber and edema of cartilage tissue. In addition, the disappearance of glycosaminoglycan

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and proteoglycan and apoptosis and necrosis arise by the alteration of cell activity and the production of proteolytic enzymes and matrix metalloproteinase9, 22).

In the present study, the amount of loading that is quantitatively appropriate was not clarified; however, it was clarified that weight bearing in the early phase of an articular cartilage defect may make the surface of the repair tissue irregular and discontinuous. Mechanical stress that is quantitatively appropriate for the cartilage defect can produce better cartilage regeneration by regulation of the cartilage differentiation and the increase in cartilage matrix because the mechanical stress can influence catabolically and anabolically cartilage metabolism. Therefore, it is important to clarify the appropriate mechanical stress and the necessity of joint exercise for rehabilitation to promote cartilage regeneration. Further study including study of the long-term course of the non-loading period should be conducted because the immunohistochemical characteristics of the repair tissue are fully unknown.

REFERENCES


