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Histopathological Changes of Joint Capsule after Joint Immobility Compared with Aging in Rats

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Abstract. [Purpose] We examined the histopathological changes in the joint capsule that occurred due to long-term immobilization of the joint, and compared them with those seen in aged rats. [Subjects] A total of 26 male Wistar rats were used in this study. [Methods] The right knee joints of the experimental group rats were immobilized for periods of 2, 4, 8, 16, or 32 weeks, and three 13-week-old "adult" rats and three 70-week-old "aged" rats were used as control groups. At the end of each immobilization period, the right knee joints of the rats were used as samples for histological examination. [Results] We observed histopathological changes in the joint capsules occurring due to joint immobilization. Increase in the thickness of the joint capsule had occurred by 4 weeks of immobilization and developed with prolongation of the immobilization period. An increase in the thickness was also observed in the joint capsule of aged rats. Further, the areas of elastic fibers in aged rats and those immobilized for 32 weeks were decreased compared to adult rats. [Conclusion] The joint capsules after immobility and aging showed similar changes, suggesting that some kind of identical mechanism causes the two conditions.
Key words: Joint capsule, Rats, Histopathology

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INTRODUCTION

Most joints in the human body move freely and have much more complex structures than immovable or slightly movable body parts. The articular adjoining ends of the bones in a freely movable joint are covered with a thick layer of articular cartilage, which is resistant to wear and produces a minimum amount of friction when it is compressed as the joint moves. Joint bones are held together by a tubular joint capsule that has two distinct layers. The outer layer consists mostly of dense fibrous connective tissue. The inner layer of the joint capsule consists of a vascular lining of loose connective tissue called the synovial membrane. This membrane covers all the surfaces within the joint capsule, except for areas that are covered by the cartilage.

The synovial membrane layer can be divided into synovial lining cells called synovial intima, which lie adjacent to the joint space and consist of zero to three layers, and the subsynovial layer called the subintima as its exterior layer[1,2]. The border of the lower synovial layer and the joint capsule is not defined and cannot be histogenetically shown by drawing a line. We describe the fibrous connective

tissue in this study, excluding the synovial lining cells, which double up as joint capsules.

A joint capsule consists of fibroblasts, fat cells, collagen fibers, and elastic fibers and contains numerous blood vessels and nerves. Since a normal joint movement is accompanied by an involuntary movement called the accessory movement that occurs in the joint capsule, the joint capsule itself needs to be moderately loose[3]. The joint capsule can be expected to be affected by a contracture because of the reason mentioned above, although the changes that occur in the joint capsule following the contracture are uncertain.

Although it has been reported that aging causes changes in the joint capsule[4], there is no evidence in support of this. We therefore conducted a study to verify the changes in the joint capsule that occur due to long-term immobilization of the joint, and the results with those for aged rats.

SUBJECTS AND METHODS

This study was carried out according to the guidelines of the Committee on Animal Experimentation of Kanazawa University (Approval no. 081174). In total, 26 male Wistar rats were used for this study. Twenty 9-week-old rats weighing 240–270 g were used to form the experimental groups. The unilateral knee joints of the experimental group rats were immobilized at the position of maximum flexion with a plaster cast for periods of 2, 4, 8, 16, or 32 weeks. Rats were randomly divided so that four were allocated to each mobilization period. In addition, three 13-week-old "adult" rats and three 70-week-old "aged" rats were used for control groups.

Cast immobilization was performed according to a previous study[5]. Animals in the experimental groups were anesthetized by intraperitoneal administration of sodium pentobarbital (40 μg/g). Following anesthetization, animals were fitted with hand-made Velfoam jackets, which were stabilized with Velcro. The right hind leg of each animal was fixed in maximal hip joint extension from the pelvis to the distal ankle joint, using a plaster cast, which was covered in gauze to prevent scratching during immobilization. The area from the distal ankle joint to the toes was left exposed for examination of edema, and the surrounding patella was left exposed for examination of bone growth. As the left hind legs and both forelimbs were not restricted, animals were allowed to move freely without restriction in their respective cages and were given access to ample food and water. If edema was discovered in the immobilized parts of the legs, the plaster casts were promptly readjusted to prevent any further development of edema. In the event that plaster casts were found to be dislocated or loosened, they were immediately readjusted to maintain immobilization. The condition of all the rats was observed daily throughout the experimental period.

The animals were anesthetized with intraperitoneal sodium pentobarbital (50 μg/g) at the end of each experiment. The right knee joints of the experimental group rats were excised and used for histopathological examination. The harvested legs were fixed with 10% neutral-buffered formalin for 72 hours and were then decalcified with Plank-Rychlo's solution (7% aluminum chloride, 3.6% hydrogen chloride, and 4.6% formic acid solution) for 72 hours at 4°C. After decalcification, they were cut in the median sagittal plane through the medial condyle of the tibia for observation of the knee joint cavity and were neutralized for 72 h with 5 % sodium sulfate before being embedded in paraffin. A microtome was used to cut 2–4 μm thick serial sections from each paraffin block, which were then stained with hematoxylin and eosin (HE) stain or elastic van Gieson (EVG) stain. In addition, the right knee joints of the control group rats were excised and used as samples for histological examination, as described above.

The posterior joint capsules of outer menisci of knee joints were examined under a light microscope (BX-51; Olympus Co., Tokyo, Japan) that was interfaced with a computer system through a digital camera (DP50; Olympus). Photomicrographs of the outer and inner layers of the posterior joint capsules stained with EVG at 400-fold magnification were carefully taken to avoid overlapping. Two images were selected at random for each joint capsule with a total of four images for each animal. As shown in Figure 1, the area of elastic fibers in each image was measured with Image Tool software (Image J for Windows version 1.38). For histological examination, the sections stained with HE were used to observe the changes, various cells and collagen fiber bundles, in the joint capsule and the thickening of the posterior joint capsule that occurred due to immobilization of the joint; the results were compared with those of the control groups.

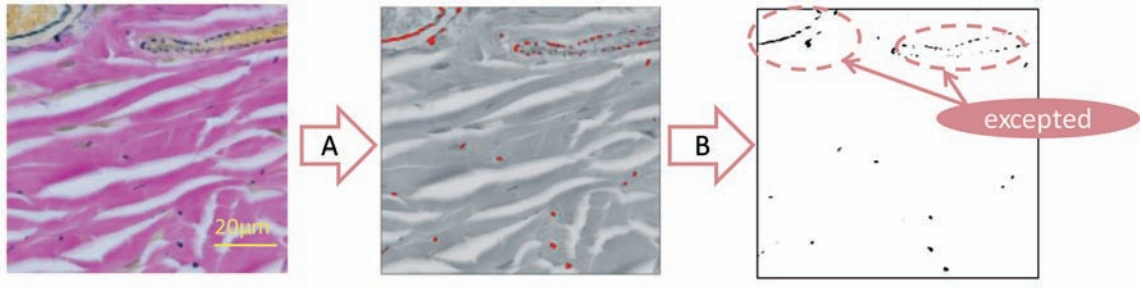

Fig. 1. Image processing with "Image J" to measure elastic fibers.
The left image shows the posterior joint capsule stained by EVG. The elastic fibers are stained black.
A: After converting to "gray scale", the thick black areas were selected. (displayed in red)
B: The areas were measured, except for elastic fibers of blood vessels

Statistical Analyses were performed with Statview 5.0 software for Windows. The differences in the areas of elastic fibers among all groups were statistically evaluated using the Tukey-Kramer test for multiple comparisons. In all statistical analyses, a level of $p<0.05$ was considered significant.

RESULTS

In images of the sections stained with HE, the joint capsule of normal adult rats was composed of coarse fibrous connective tissues, which had dispersed fibroblast cells with spindle nuclei embedded among collagen fiber bundles. There was sufficient space between each fiber bundle, and some fat cells were contained in each bundle. On the other hand, we observed the atrophy of fat cells, proliferation of fibroblasts, and narrowing of the spaces between collagen fiber bundles in the experimental groups. In addition, thickness of the posterior joint capsules in the group experiencing 4 weeks of immobilization was greater than that in the normal adult rats. The severity of this change increased as the period of immobilization was extended (Fig. 2, 3).

The changes in the joint capsules of aged rats were similar to those of the experimental groups, even though proliferation of fibroblasts was not observed, especially those immobilized for short periods of time, such as 2 or 4 weeks (Fig. 2, 3).

In the observation region of all groups, inflammatory cell infiltration into joint capsules was not observed.

The areas of elastic fibers are shown in Table 1. Aged rats and those immobilized for 32 weeks had significantly decreased areas of elastic fibers compared to the adult rats.

DISCUSSION

We histopathologically evaluated changes in the knee joint capsules that occurred due to immobilization and aging in rats. Compared to before immobilization, we observed the atrophy of fat cells in the lower synovial layer, proliferation of fibroblasts, and thickened collagen fiber bundles in joint capsules following immobilization. In addition, thickening of the joint capsule was observed after 4 weeks of immobilization, and the severity of this condition increased as the immobilization period progressed. The areas of elastic fibers decreased gradually with extension of the immobilization period, but significant decreases were only observed after 32 weeks of immobilization. Meanwhile, the changes in joint capsules associated with aging were found to be similar to those observed in the short immobilization period, but the mechanism of these histological changes is unknown. These changes may be indicative of a quantitative increase in collagen fibers and a decrease in extracellular fluid volume between the collagen fiber bundles. The increased thickness of the joint capsule and the reduced numbers of elastic fibers may contribute to joint stiffness.

Matsumoto et al.[6] reported histological findings and immunohistochemically semiquantified them in the joint capsules of rat knee joints, which were fixed in the flexion position for 2–32 weeks. They showed new characteristic collagen fibers with high cellularity, a fibrotic reaction in the posterior lower

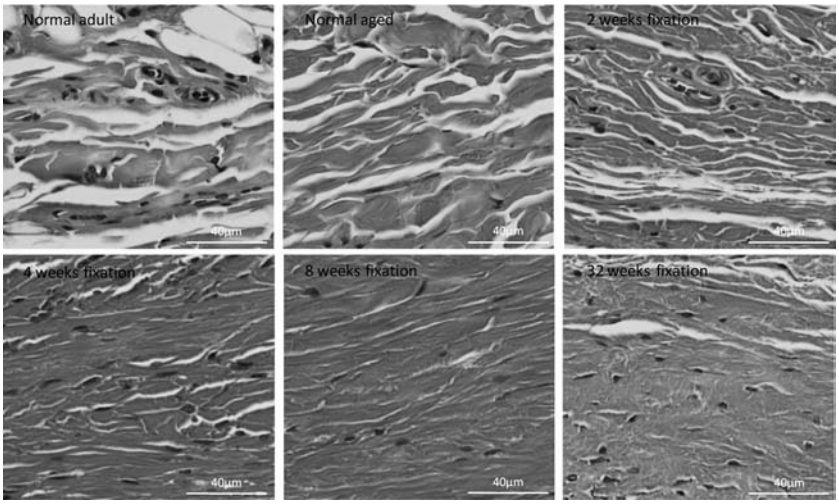

Fig. 2. Histological findings of the posterior joint capsule. (HE stain × 400)
The narrowing of the space among collagen fiber bundles in immobilized rats can be seen.

Table 1. Area of elastic fibers in the joint capsule

control group		experimental group				
13W	70W	after 2W	after 4W	after 8W	after 16W	after 32W
171.1 ± 38.48	62.9 ± 22.83	102.7 ± 25.22	148.6 ± 46.24	130.1 ± 54.10	107.2 ± 17.12	54.8 ± 30.18
*				*		
*						

mean ± SD (μm^2), *; $p<0.05$, significant difference according to the Tukey-Kramer multiple comparison test.

synovial layers after 2 weeks of immobilization, and increased fibrillization in the posterior lower synovial layers after 4 weeks of immobilization. They also showed that the tissue was converted into a hypocellular and organized connective tissue after 16 and 32 weeks of immobilization, and reported that the production of type I collagen was significantly increased after 2, 4, and 6 weeks of immobilization. Similarly, Hildebrand et al.[7], Hibino et al.[8] also reported similar changes of collagen production in similar situations. Further, Peacock et al.[9] reported that shortening and thickening of the joint capsule were seen in a study in which a dog's hind leg was fixed using a fixing pin for 4 weeks to produce contracture, following which, tissues such as the skin surrounding the knee joint and muscles were removed for macroscopic observation. Based on a subsequent biochemical evaluation, it was reported that the tissue contained twice the normal amount of hydroxyproline which is the principal component of collagen. Akenson et al.[10] adopted a biochemical approach and reported that the amount of extracellular matrix components such as moisture and glycosaminoglycans decreased in the connective tissue surrounding the knee joint in an immobilized rat. The spaces between collagen fibers were reduced, resulting in decreased smoothness and ultimately cross-linking of the fibers. The results of our study are consistent with the findings of the previous studies, and we consider they have clarified the histological changes. Moreover, the fact that thickening of the joint capsule was observed after 4 weeks of immobilization may support the findings of Trudel et al.[11] who showed the dominance of an articular limit, and not a muscular limit, after 2 weeks of immobilization.

Previously, no study had histologically evaluated the changes in joint capsules that occur with aging in rats. In general, metabolism in rats is known to decline with aging. The changes associated with aging that were observed in this study were similar

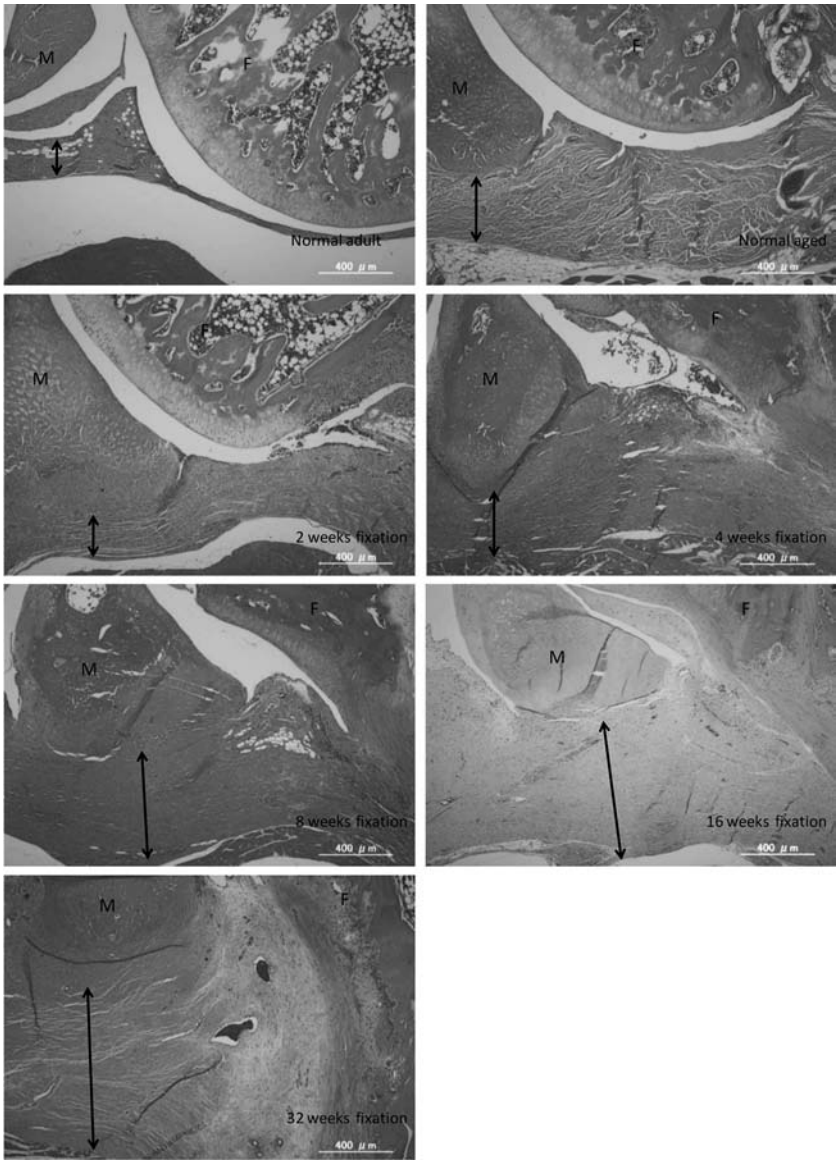

Fig. 3. Histological findings of the posterior joint capsule. (HE stain × 40)
F: femur M: meniscus. ↔ : thickness of posterior joint capsule.
Increase of thickness of the joint capsule with prolongation of immobilization. Aged rats also showed similar tendency.

to those observed in the short immobilization period and may be attributable to the fact that the decline in metabolism observed with aging was similar to the changes in the metabolic environment following immobilization. Regarding the change in elastic fibers, Takemura et al.[12] reported decreased elastic fibers following immobilization based on the findings of a study, in which the knee joint of a rat was fixed for 2 weeks and then histologically examined. Our study is supportive of theirs since a similar tendency was observed, though no significant difference was found. Moreover, Gigante et al.[13] histologically examined the distribution of elastic fibers in the knee joint of an aging rabbit and reported that observable fibers were scarce in aged rabbits. This finding is similar to that of our study despite the use of a different animal species. Although the reason for these changes is unknown as mentioned above, metabolism within the knee joint capsule may have

been affected.

Since the changes associated with immobilization or aging were not quantitatively assessed in our study, evaluation from multiple angles including biochemical assessment will be needed in future. Moreover, in order to treat contractures, it may be necessary to clarify whether the changes observed in this study are reversible with the addition of various treatment agents.

In conclusion, changes of the joint capsule after immobilization occurred in the first 2 weeks of immobilization, and thickening of the joint capsule had occurred by 4 weeks of immobilization, with this change developing along with prolongation of the immobilization period. The areas of elastic fibers of the joint capsule decreased after long term immobilization. The increase of the thickness and decrease of the area of elastic fibers of joint capsule occurred not only after immobility but also with aging.

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