Histopathological Changes in Joint Components in a Rat Knee Joint Contracture Model Following Mobilization

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Abstract. [Purpose] The aim of this study was to investigate the histopathological effects of mobilization on joint components using a rat knee joint contracture model. [Method] Twenty-two male Wistar rats (aged 9 weeks) were randomly divided into an experimental group (n = 18) and a control group (n = 4). The experimental group had the right posterior limb knee joint immobilized for 8 weeks to induce contracture after which this group was subdivided into three subgroups—immobilized, treatment, and non-treatment groups. In the immobilized group (n = 6), knee joint specimens were collected immediately after immobilization. The non-treatment group (n = 6) was bred normally for 8 weeks, while the treatment group (n = 6) underwent mobilization during the same 8 weeks. After breeding, we prepared tissue specimens of the knee joint for observation in the sagittal plane and examined them using a light microscope. [Results] Compared to the non-treatment group, the treatment group showed an increase in the number of fat cells in the synovial subintima and a reduction in the density of collagen fiber bundles in the posterior joint capsule. [Conclusion] Joint mobilization appears to effectively improve joint function. [Key words: Mobilization, Contracture, Histopathology (This article was submitted Jun. 27, 2012, and was accepted Jul. 18, 2012)]

INTRODUCTION

Normal joint motion consists of bone movement and accessory movement. Accessory movement is unintentional intracapsular movement that includes two components, motion and joint play1). It requires moderate plasticity of the joint capsule1). When the normal joint motion is limited, stretching is the most popular physiotherapeutic method. During stretching, one joint is immobilized while the other extends in the direction of motion that is limited, with the aim of improving bone movement. This treatment is not usually applied in isolation, and various manual therapies are also applied prior to or in parallel with stretching in order to improve intracapsular movement. These are termed either mobilization or manipulation and correspond to the power or rate of the procedure2, 3). Because there is no general agreement on terminology, in this study, we defined a procedure for extending the joint capsule to improve intracapsular movement and overcome contracture as “mobilization.” While simple stretching is only pushing or pulling a bone on one side in the direction of motion that is limited, the mobilization techniques generally improve intracapsular movement by means of the force applied close to the joint surface to parallel shift to the plane of the joint. Therefore, this requires familiarity with the joint structure.

The first textbook to discuss mobilization was published in the 1960s4), and the technique has become well known in the physical therapy field since then. The joint capsule can be extended by mobilization5), and many recent clinical studies have reported its effectiveness when the range of joint motion is limited4, 5). These studies in which the effectiveness was verified by basic medical examination reported results derived only from the examination of fresh cadavers6–8), which led to incomplete understanding of the technique.

The aim of this study was to investigate the histopathological effects of mobilization of joint components using a rat knee joint contracture model and joint immobilization.

SUBJECTS AND METHODS

Twenty-two male Wistar rats (aged 9 weeks) were randomly divided into an experimental group (n=18) and a control group (n=4). The experimental group had the right posterior limb immobilized for 8 weeks to induce contracture. This group was then subdivided into three subgroups. In the immobilized group (n=6), knee joint specimens were collected immediately. The non-treatment group (n=6) was bred normally for 8 weeks, and the treatment group (n=6) was allowed normal mobility for the
same 8 weeks. The control group was bred normally for 16 weeks. This study was performed according to the Regulations on Animal Experiments of Nagoya Gakuin University and was approved by the Animal Experiments Committee (Approval Number: 2007-003).

Cast immobilization was performed with reference to a previous study as described below. Under inhalation anesthesia with isoflurane, the rats were fitted with custom-made jackets produced from Velfoam (Velcro USA Inc., Manchester, NH, USA) and secured at the back with Velcro. The entire posterior right limb, with the knee joint positioned centrally, was covered with gauze to prevent excoriation from the cast. An area from the pelvic girdle to the distal foot joint was immobilized by the cast with full extension of the hip joint, full flexion of the knee joint, and full plantar flexion of the foot joint. An area from the distal foot joint to the medial toe on the immobilized limb was exposed in order to monitor the possible development of edema and to confirm the absence of congestion. The patella and its surrounding area on the immobilized limb were also exposed to permit normal bone growth during the immobilization period. The contralateral posterior limb was not modified. Rats were able to move freely in their cages and had sufficient supply of water and food. The casts were replaced every 2 weeks, and if pedal edema developed or casts became loose, they were replaced immediately to maintain adequate immobilization. The immobilization period was 8 weeks, in accordance with previous studies.

The mobilization technique was performed with rats in the lateral position under inhalation anesthesia. With the knee joint in the loose-packed position, the proximal tibia was compressed in a posterior–anterior direction in order to align it parallel to the plane of the knee joint (Fig. 1). Loads approximately equivalent to the rats’ weights were applied and were measured once weekly. We applied a strain gauge sensor (LMA-A; Kyowa Electronic Instruments Co., Ltd., Tokyo, Japan) to the first digit and increased the tension in a controlled manner. Each cycle consisted of continuous extension for 25 s followed by rest for 5 s. The treatment was performed 10 times per day (i.e., a total of 5 min) and 5 times per week. Loads were calculated according to the preliminary examination in which the stress–strain curve of the joint capsule (including the articular ligament) was described using a composite of the femur, joint capsule, and tibia obtained from a fresh dead rat. We observed that the “linear phase” corresponded to tissue extension and thereby decided that the period of mobilization would need to conform to that utilized in human studies.

After the breeding period, all groups underwent perfusion fixation using 4% paraformaldehyde under intra-abdominal Nembutal anesthesia. The right posterior limb was disarticulated at the hip joint, and specimens were permeated and fixed for 72 h, followed by decalcification with Plank–Rychlo solution for 72 h at 4°C. The knee joints were then excised, neutralized with 5% sodium sulfate solution, delipidated with 100% ethanol, and paraffin-embedded in order to study the sagittal plane. Using a sliding microtome, approximately 3–5-μm slices were obtained from the prepared paraffin block and stained with hematoxylin-eosin (HE).

Examination of the entire joint cavity was conducted using a light microscope (BX-51; Olympus Corporation, Tokyo, Japan) linked to a digital microscope camera (DP50; Olympus Corporation, Tokyo, Japan). We measured the thickness of the posterior joint capsule using whole joint images under ×10 magnification. A reference line was created following the course of the patellar ligament. We drew a line orthogonally from the reference line passing through the center of the gap between the femur and tibia as far as possible and also measured the length of the posterior joint capsule along that line (Fig. 2). Using images captured at ×400 magnification, we also measured the cross-sectional area of the collagen fiber bundles (including fibroblasts) as an index of the density of the posterior joint capsule. The values measured were then divided by the total area,
omitting any blood vessels apparent, and these values are shown as percentages. We captured images near the center of the mediolateral area below the meniscus and used 3 images recorded once each from 4–6 randomly selected areas.

We used the ImageJ image processing software (ver. 1.45s for Windows; NIH, USA), and the IBM SPSS ver. 19 statistical software was used for comparison of the thickness and density of the posterior joint capsule among groups. We used the Kolmogorov–Smirnov test to evaluate normality and performed one-way analysis of variance. Using Scheffe’s multiple comparison test, we evaluated significant differences. The significance level was set at 5%.

RESULTS

Soon after the removal of casts, the average weight of the treatment group was 254 ± 7.4 g; this increased to 340 ± 11.4 g 8 weeks later. The power level used during the study ranged from 2.5 N to 3.3 N.

In the control group, the anterior joint cavity evidenced many fat cells beneath the first 3 layers of the synovial lining cells, and the joint cavity was of normal size (Fig. 3). On the other hand, the immobilized group showed atrophy/decrease in fat cells, and fibrosis and adhesion of hyperplastic synovia-like tissue to the cartilage surface layer were observed (in 5 of 6 limbs). Accordingly, the joint cavity size was reduced. In the treatment group, fibrosis and a slight degree of atrophy/decrease in fat cells were recognized, but these changes were more marked in the non-treatment group. Hyperplastic tissue and adhesions to the cartilage surface layer were not observed in the treatment group or non-treatment group, but part of the cartilage surface layer had been replaced by fibrotic tissue, and misalignment of this layer was observed in those groups (Fig. 4; in 5 of 6 limbs in the treatment group and in 5 of 6 limbs in the non-treatment group). These findings did not lead to any marked difference between the treatment and non-treatment groups.

In the normal group, the posterior joint capsule consisted of fibrous connective tissue with fibroblasts with spindle-shaped nuclei were scattered between the collagen fiber bundles. The spaces between collagen fiber bundles were normal. The immobilized group, however, evidenced dense hyperplastic tissue between the collagen fiber bundles. In both the treatment and non-treatment groups, the spaces between collagen fiber bundles were enlarged, more so in the treatment group but less than in the control group (Fig. 5).

In the observation region of all groups, no inflammatory cell infiltration into joint capsules was observed.

There was no significant difference in the thickness of the posterior joint capsule among groups, but the density was significantly different (Table 1). Following immobilization, the density of collagen fibers in the joint capsule was increased. It had been improved by mobilization, but not to normal levels.

DISCUSSION

Joint contracture is a commonly occurring dysfunction in the physical therapy field that can benefit from physical therapy and more specifically, manual therapy. The aim of this study was to investigate the histopathological effects of joint mobilization on joint components using a rat knee joint contracture model.

Following an 8-week period of immobilization, atrophy/disappearance of fat cells in the synovial subintima and fibroblastic hyperplasia occurred in the anterior joint synovia, and hyperplastic tissues were found adherent to the cartilage surface layer. The number of fat cells increased in the groups who underwent normal breeding or therapeutic intervention with remobilization of joints following immobilization. The number of fat cells was particularly increased following therapeutic intervention. Though we found no adhesions to the cartilage surface layer, we observed misalignment of this layer and partial replacement by fibroid tissue. The changes
in joint components following the period of immobility in this study matched those of a previous study[9] during which we presumed we could create a similar contracture model leading to arthrogenous limitation. Regarding changes in fat cells, many studies reported that atrophy or fibrosis was observed from the initial stage of joint immobility[9, 12, 13]. Few histopathological reports have, however, documented such changes following joint remobilization. Takemura et al.[13] recorded increased changes in rat knee synovia when joints were immobilized for 2 weeks compared with those in stretching and normal breeding groups over the same period. Regardless of therapeutic interventions, the status of the fat cells in the synovial subintima was improved, with the stretching group more closely approximating the normal state. Our study indicated that mobilization, which is different from stretching, facilitated intracapsular movement and thus effectively improved the status of fat cells. Fat cells within joints are classified as structure fat based on their functions[14, 15]. With reference to previous reports, it has been suggested that changes in fat cell flexibility depend on a certain degree of joint movement. Further investigation will be required to reveal this mechanism, as well as its relationship to the degree of range of motion or resistance to movement. With regard to changes in the cartilage surface layer following 8 weeks of immobilization, the immobilized group should have shown adhesion of hyperplastic tissues to the cartilage surface layer occurring with high probability, as was found in the non-treatment and treatment groups. The findings in both groups might be the result of denudation of the adhered hyperplastic tissues and cartilage surface layer because of joint remobilization. Regeneration of joint cartilage is very poor, and it is not clear whether hyaline cartilage or fibrocartilage can repair the cartilage surface layer[16, 17]. Our results suggested that tissue repair was in process. No reports to date have discussed changes in joint cartilage following the formation of adhesions. Further investigation will be required on this subject, but our findings indicate that the adhesions observed in this study were improved even in rats who were allowed unrestricted movement (normal breeding).

There were no significant differences noted in the thickness of the posterior joint capsule in this study. In a previous study[18] using immobilization of between 2 and 32 weeks with a similar immobilized model[18], we demonstrated a hyperplastic joint capsule. The findings of the present study are different from those of the previous study, however, leading us to consider the reason for these different outcomes. In the present study, the density of collagen fiber bundles showed significant differences between the groups, and the posterior joint capsule showed quantitative and qualitative changes in density because of joint immobilization. Akenson et al.[19] used a biochemical approach and reported that the amount of extracellular matrix (i.e., water and glycosaminoglycan) decreased in connective tissues around the immobilized knee joint of rabbits, leading to less spacing between collagen fibers, and our histological findings corroborated this. The posterior joint capsule became more dense after 2 weeks of immobilization[20], and the whole joint capsule became thicker[19], indicating a quantitative change in addition to a qualitative change. After joint excision, collagen fiber bundles could move against each other more easily, and the spaces between them expanded, resulting in a decreased density of the bundles in both the treatment and non-treatment groups. We consider that mobilization effec-

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**Fig. 4.** Cartilage surface layer findings (HE Staining). Control group: Cartilage cells in the cartilage cavity align like columns toward the surface layer and are surrounded by a homogenous matrix. Immobilized group: Adhesions to the cartilage surface layer, creating an unclear border (dotted line shows the assumed original border). Hyaline cartilage, which is different from conventional cartilage, can be seen near the border (arrow). Non-treatment group/treatment groups: Fibrotic tissues can be seen near the border (arrow).

**Fig. 5.** Posterior joint capsule (HE Staining). The deeply colored area indicates collagen fiber bundles, and the white area indicates the spaces between them. In the immobilized group, there are only a few spaces, while in the treatment and non-treatment groups, the extent of the spaces is similar to that in the control group.
tively led to such density decreases, as the treatment group showed significant improvement. Studies using shoulder joint specimens from fresh cadavers\(^6\)-\(^8\) reported that mobilization (repetitive stretch stimulation) increased the range of joint motion and altered the rigidity of the joint capsule. It is possible that only the cross-linking between collagen fibers was damaged, though we did not investigate cross-linking. The expansion observed in the spaces between the bundles tends to support the above possibility. Further investigation to include the relationship with range of motion will be required.

The altered spaces between collagen fiber bundles found in this study may possibly have been induced by certain artifacts introduced in the process of tissue specimen preparation. However, we processed all specimens in the same way, so the differences were probably a reflection of the ease of separation of bundles, and this may be an index of the qualitative properties of the joint capsule.

No reports on mobilization effects using animal experiments have been reported to date. Continuous investigation will be required to determine whether our interventions are of practical use. It is difficult to demonstrate quantified findings using only histopathological examinations, and multiple investigations involving a biochemical approach are a future aim.

ACKNOWLEDGEMENTS

We would like to offer our deep thanks to the staff of the Department of Morpho-Functional Pathology, Division of Cancer Medicine, Graduate School of Medical Sciences, Kanazawa University. This work was supported by a Grant-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (23700641).

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