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Progression of an arthrogenic motion restriction after immobilization in a rat experimental knee model

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Abstract

Background. Contracture is defined as a decrease in both active and passive ranges of motion after immobilization. A fibrotic change of a capsule is suggested to be one of the main causes of the joint contracture. The goal of this study is to determine the effect of capsule on limiting the range of motion after immobilization.

Materials and Methods. We immobilized the knee joint of 35 rats with an internal fixator with the knee joint flexed at 150 degrees. The rats were sacrificed at 1, 2, 4, 6, 8, 12, and 16 weeks after surgery and the lower extremities were disarticulated at the hip joint. After extra-articular myotomies around the tibia and femur, x-rays were taken to measure the angles of extension of the knee joint under 3 different torques. The measurements were repeated after releasing the posterior capsule in order to observe their effects on knee motion.

Results. Joint contracture was rapidly progressed until 8 weeks and advanced slowly after 8 weeks. After releasing the posterior capsule, both the immobilized and the control groups gained the angle of knee extension. The acquired angle in the immobilized group was significantly greater than in the control group after 4 weeks and became plateau after 8 weeks.

Conclusion. Joint contracture develops at the early stage of immobilization and progresses over time. The posterior capsule significantly contributes to the limitation in extension.

Introduction

The definition of joint contracture is a limitation of range of motion. The joint contracture is often seen in daily examinations, but its pathogenesis has been unsolved. Disadvantages of limited motion of the joints include various degrees of limitation in the activities of daily living. Once the joint contracture is established, it is extremely difficult to regain a full range of motion with vigorous and extensive rehabilitation or even with surgical treatment [1, 2]. Therefore, prevention of joint contracture is of prime importance for clinicians.

Joint contracture is classified in two types according to its etiology: arthrogenic and myogenic. The arthrogenic contracture is defined as the one caused by the bone, cartilage, synovial membrane, capsule, and ligaments. The myogenic contracture is defined as the one caused by the muscle, tendon, and fascia [3, 4]. Some investigators insisted on an importance of the myogenic components as etiological factors [5], whereas others did on the arthrogenic components [3, 4, 6–9]. It is difficult to compare these reports because different animal species and different methods were used in these studies.

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Using the rat model, Trudel et al. reported a decrease in the length of the synovial intima after 4 weeks of immobilization [10]. In their model, the range of motion in extension remained restricted even after total extra-articular myotomies [4]. These reports suggest that the contribution of arthrogenic components is greater than the myogenic components. Among the arthrogenic components, the synovial membrane or the capsule is suggested to be a cause of joint contracture [14].

There have been no reports solely evaluating the effects of arthrogenic components on the range of motion. In all the previous reports, the arthrogenic components were evaluated together with the myogenic components in the first step, and then the arthrogenic components alone were evaluated in the second step after releasing the myogenic components [3, 4, 12]. The major drawback of this methodology is that there is a possibility that the first step of evaluation might have damaged the arthrogenic components, which were evaluated alone in the second step. In order to avoid this drawback, we measured an isolated effect of arthrogenic components on the range of motion in the present study by measuring the range of motion after releasing all the soft tissues except the arthrogenic components. Another drawback of the previous studies is that they used a goniometer to measure the joint angle. It seems difficult to obtain precise measurements of joint angles using a hand-held goniometer. We chose to use x-rays instead to measure precise angles between the femur and tibia. The purpose of this study, therefore, was to evaluate the effect of one of the arthrogenic components, the posterior capsule, on the limitation of joint extension.

Materials and Methods

Animals

The protocol for this experiment was approved by the Animal Research Committee of Tohoku University. Adult male Sprague-Dawley rats (CLEA Japan Inc., Tokyo, Japan) weighing from 380 to 400 g were used. Their knee joints were immobilized at 150 degrees in flexion by a rigid plastic plate (POM-N, Senko Med. Co., Japan) and metal screws (Stainless Steel, Morris, J. I., Co., USA) according to a previously described method [11]. The knee joint capsule and the joint itself were untouched. The surgery was performed under anesthesia with sodium pentobarbital (50 mg/kg) administered intraperitoneally. Sham operated animals had holes drilled in the femur and tibia and screws inserted but none of them were plated. The animals were allowed unlimited activity and free access to water and food. The immobilized animals and the sham operated animals made up the immobilized group and the control group, respectively. Seventy rats (1, 2, 4, 6, 8, 12, and 16 weeks) were prepared for measuring joint angle. Each group was composed of 35 immobilized and 35 control animals.

Influence of surgery on the body weight and bone growth was important. As the bone was elongated, the immobilized angle might increase. We checked the body

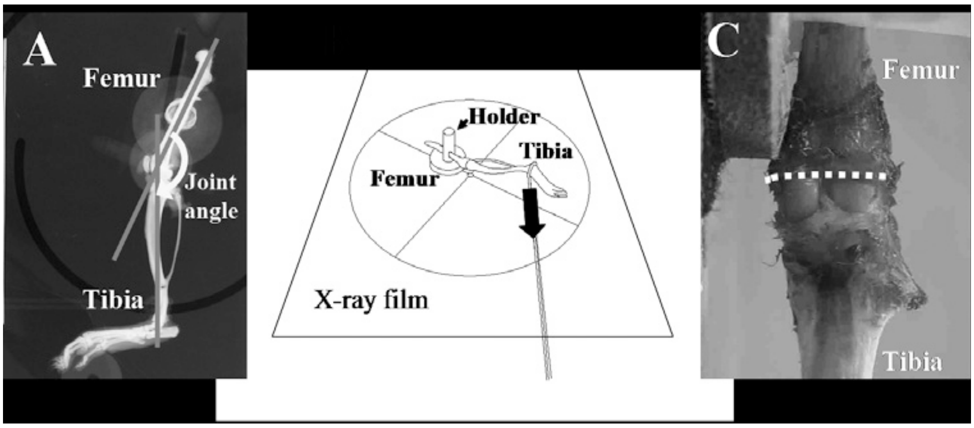

Figure 1. Methods of measuring the joint angle and releasing of the posterior capsule. A, The joint angle was defined as an angle between the longitudinal axis of the femur and a line passing through the center of the ankle joint and the center of the eminence of the tibia. B, Schematic illustration of an apparatus to fix the hind limb for taking x-rays. C, Photograph of the posterior capsule after release. The posterior capsule was incised at the insertion to the femoral condyle with a surgical knife. Bilateral femoral condyles appeared after releasing. (Dotted line indicates the cutting line of the posterior capsule).

weight of 16 weeks of immobilized group (N=10) and control group (N=10) every week after surgery.

Measurement of a joint angle

We measured the angle between the longitudinal axis of the femur and a line passing through the center of the ankle joint and the center of the eminence of the tibia (Figure 1A). The plate and screws were removed before taking the x-ray such that we were able to avoid any chance of breaking the arthrogenic components. From our gross observation, the plate was covered with thick fibrous granulation tissues which might contribute to limitation in extension after 4 weeks in the immobilized group. A difference between the mean joint angle during extension in the control group and the one in the immobilized group was defined as a “contracture angle,” which represented the arthrogenic restriction in joint extension [3, 4]. We used the same angular velocity (3 degrees/sec) as previously described [4]. The measurements were done in room temperature. The lateral x-ray pictures were scanned with LP-9200 (EPSON, Tokyo, Japan) and the joint angle was measured with the aid of an ImageJ 1.36b (National Institutes of Health, Besthesda, MD, USA). As we immobilized the knees in hyper flexion (150 degree), the fibula easily touched the femur holder with a minimum flexion torque. From clinical point of view, if a joint is immobilized in hyper flexion, the problem is always in limited extension. Thus, we measured only the joint angle during extension in this study.

In order to evaluate the influence of the posterior capsule on knee joint motion, we measured the joint angle with three different extension torques before and after releasing the posterior capsule from the femoral condyle (Figure 1C). We defined an acquired angle as follow: joint angle after release – joint angle before release at the maximum torque. As the capsule was very thin and it was difficult to distinguish the synovial membrane from the capsule, we released the synovial membrane and the capsule together as a complex. All measurements were completed within 20 minutes after disarticulation at the hip joint.

Choice of the torques used

In our preliminary study in normal knees, the joint angle was over 170 degrees at a torque of 1,350 g-cm and the posterior capsule was completely stretched. Based on this preliminary data, we chose three different torques (450, 900, and 1,350 g-cm) to extend the knee joint in this study.

Statistics

Statistical analysis among groups was performed using the Kruskal-Wallis test, with Bonferroni/Dunn post-hoc multiple comparisons. Differences between the experimental and control groups were compared at each time point by Mann-Whitney's U test. Data were expressed as mean ± SD. A value of $P < 0.05$ was accepted as statistically significant.

Results

There were 6 specimens which showed fractures either at the tibial or at the femoral growth plate under the maximum torque. As a result, these specimens were excluded from the analyses (Table 1).

Table 1: The number of excluded specimens at the maximum torque (1,350 g-cm).

		before release		after release	
		Femur	Tibia	Femur	Tibia
1w	control				1
	immobilized		2		
2w	immobilized			1	2

Body weight and bone length

All the animals in the immobilized and the control groups gained weight over time. There was no statistical difference between the groups at any time point of immobilization (Figure 2A). Both the length of the femur and that of the tibia increased over time in both groups (Figure 2B and C). The femur of the immobilized group at 2 weeks was significantly shorter than that in the control group. The tibia of the immobilized group at 1 and 8 weeks were significantly longer than that in the control group. There were no statistical differences at other time points. The operative procedure had little influence on body weight and bone growth.

Bone length effect on immobilization angle

Though the bone length increased over time, there were no significant differences in joint angle at any time point of immobilization (Figure 3A).

Limitation of arthrogenic components in extension

Limitation in extension began to develop as early as 2 weeks and progressed over time (Figure 3B–D). The contracture angle progressed as immobilization period increased (Figure 4A). The rate of contracture progression showed that there were two phases of rapid progression in joint contracture: one phase was between 1 and

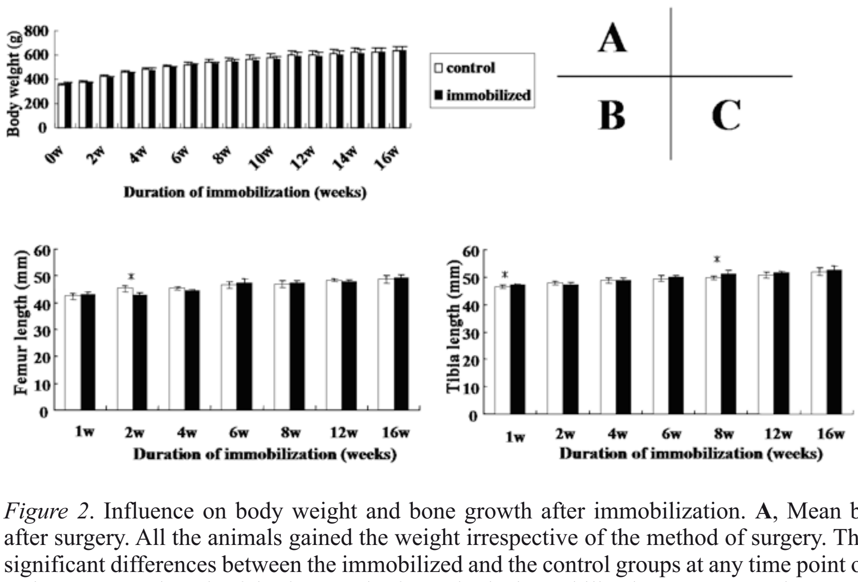

Figure 2. Influence on body weight and bone growth after immobilization. **A**, Mean body weight after surgery. All the animals gained the weight irrespective of the method of surgery. There were no significant differences between the immobilized and the control groups at any time point of immobilization. **B**, Bone length of the femur. The femur in the immobilized group at 2 weeks was significantly shorter than that in the control group. There were no significant differences at other time points. **C**, Bone length of the tibia. The tibia in the immobilized group at 1 and 8 weeks was significantly longer than that in the control group. There were no significant differences at other time points. The immobilization had little influence on body weight and bone length. (N= 10, *p<0.05 v.s. control)

2 weeks and the other between 4 and 6 weeks (Figure 4B). During the rest of the time, the contracture progression was relatively slow.

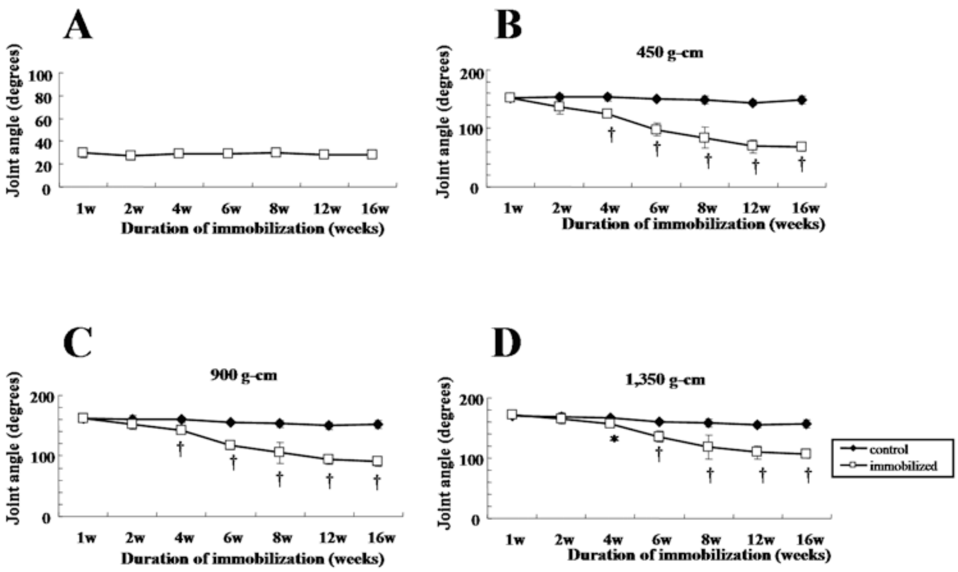

Figure 3: Joint angle changes after immobilization. **A**, Joint angle at the end of immobilization. The angle was kept at approximately 30 degrees at any time point of immobilization. There were no significant differences among the different time points in the immobilized group. **B,** 450 g-cm. **C**, 900 g-cm. **D**, 1,350 g-cm. Limitation in extension began to develop as early as 2 weeks and progressed over time. The joint angle at the torque of 450 g-cm was significantly smaller than the control group after 2 weeks of immobilization. The joint angle at the torques of 900 and 1,350 g-cm were significantly smaller than the control group after 4 weeks of immobilization. (N=3: 1 week of immobilized group, 1,350 g-cm, *p<0.05 v.s. control, †p<0.01 v.s. control).

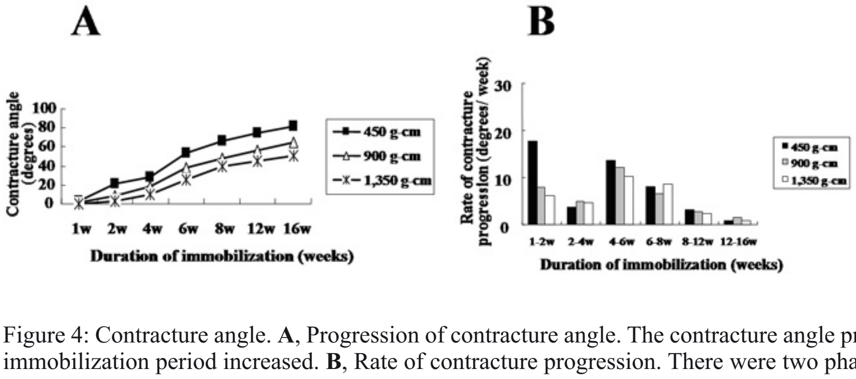

Figure 4: Contracture angle. **A**, Progression of contracture angle. The contracture angle progressed as immobilization period increased. **B**, Rate of contracture progression. There were two phases of rapid progression in joint contracture: one phase was between 1 and 2 weeks and the other between 4 and 6 weeks. (N=3: 1 week of immobilized group, 1,350 g-cm).

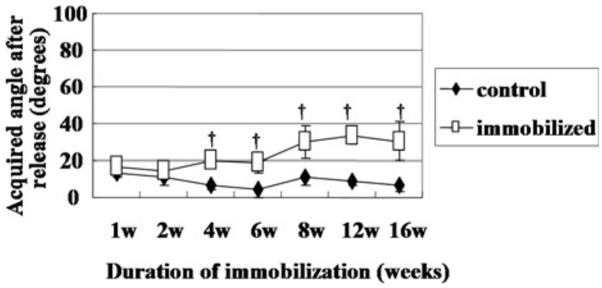

Figure 5: Posterior capsular release and the joint angle. Both the immobilized and the control group gained the joint angle. The acquired angle was greater in the immobilized group than in the control group after 4 weeks. (N=3: 1 week of the immobilized group, N=2: 2 weeks of the immobilized group, N=4: 2 weeks of the control group. †$p<0.01$ v.s. control)

Influence of the posterior capsule on limitation in extension

After release of the posterior capsule, both the immobilized and the control groups showed increases in the joint angle. The acquired angle after release was significantly greater in the immobilized group than the control group after 4 weeks and more (Figure 5). The acquired angle became plateau after 8 weeks in the immobilized group. During the cutting procedure of the posterior capsule, we noticed slight adhesion of the synovial membrane to the articular cartilage of the femoral condyle after 8 weeks in the immobilized group. We also noticed that the resistance to cut the posterior capsule increased after 6 weeks in immobilized group. In contrast, no adhesion of the synovial membrane to the articular cartilage was observed in the control group.

Discussion

Measurement of the range of motion is simple but indispensable process to evaluate joint contracture progression. In the same model as ours but immobilized up to 32 weeks, the arthrogenic components were evaluated after evaluating the myogenic components in the same rats [3, 4]. They concluded that the myogenic restriction decreased and the arthrogenic restriction increased over time. In respect of saving animals, the two components could be measured in the same animals. However, there still remains a possibility of tearing or damaging the arthrogenic components while measuring the myogenic components. Furthermore, the connective tissues around the plastic plate might have some influence on the myogenic restriction, and thus, management of these tissues was very critical in assessing the myogenic restriction. This is the reason why we focused on the arthrogenic components in the limited range of motion after immobilization in the present study.

The arthrogenic components were considered as an important factor of joint contracture after prolonged immobilization [3, 4, 8, 10, 13]. In the same rat model as ours but immobilized up to 32 weeks, a significant decrease in the length of synovial intima was observed in the posterior capsule after 4 weeks [10]. They concluded that mutual adhesions of synovial villi were the major pathophysiological changes. Among the arthrogenic components, we reported that the elasticity of the posterior capsule detected by scanning acoustic microscopy increased after 8 weeks of immobilization [11]. Histological and structural changes of the posterior capsule were implicated in this pathologic process.

After releasing the posterior capsule, the joint angle significantly increased. This implies that the posterior capsule contributed to the limitation in extension. The acquired angle was significantly greater after 4 weeks and became plateau after 8 weeks. This result suggests that the posterior capsule was a cause of limitation in extension until 8 weeks, but some other factors might have contributed to the limitation in extension after 8 weeks. These unknown factors need to be clarified in the future study. This was the first report to evaluate the true contribution of arthrogenic component to joint motion restriction.

Theoretically, the longer the bone, the greater the immobilization angle at the end of the immobilization as previously reported [18]. In the previous report, the immobilization angle increased to approximately 150 degrees at 16 weeks after immobilization, but it remained the same between 16 weeks and 32 weeks [18]. On the other hand, the angle in our study was constantly kept at 150 degrees (the joint angle = 30 degrees). The differences between the previous report and ours were: 1) the immobilized angle (150 degrees) was greater in our study than that in the previous report (135 degrees), 2) the animal body weight used in our study (380-400g) was greater than that in the previous one (340g), and 3) the method of measuring the joint angle was different (x-ray versus goniometer). The immobilized bone grew more than that in the control group in the previous report [18]. However, this was not the case in the present study. Our method seems to have less influence on bone growth and the joint angle.

Though the number of animals in this study was small, 2 out of 5 at 1 week of immobilization before release and 3 out of 5 at 2 weeks of immobilization after release had fractures at the growth plate. No fracture was observed after 4 weeks of immobilization. This may indicate that immobilization has some influence on the mechanical properties of the growth plate at the early phase of immobilization although the bone growth itself was not affected. The effect of immobilization on the mechanical properties of the growth plate needs to be studied in the future.

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