Study on changes in skin extensibility during the development of joint contracture due to joint immobilization in rats

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Abstract. [Purpose] The purpose of this study was to elucidate whether skin extensibility decreases when a contracture develops as a result of joint immobilization. [Subjects] This study was conducted on six female Wistar rats. [Methods] The rats were divided into two experimental groups. In the immobilized group, the right ankle joints were immobilized in complete plantar flexion by plaster casts for two weeks. In the control group, the left ankle joints had no intervention. On the final day, skin extensibility was determined from a length-tension curve by collecting skin from the posterior aspect of the ankle joint and using a tensile strength tester. [Results] Compared with the control group, the immobilized group showed a significant decrease in skin extensibility. [Conclusion] The results demonstrated that the extensibility of the skin itself decreases when joint contracture develops.

Key words: Joint contracture, Skin, Rat

INTRODUCTION

Joint contractures are one of the conditions for which physical therapists perform therapeutic interventions in clinical rehabilitation settings, and in order to conduct physical therapy for joint contractures, it is important to understand the tissues that are involved in causing joint contractures. Previous studies that created contracture models in animal experiments demonstrated that skin and muscle tissues are causative tissues of joint contractures. In addition, the degree to which skin and muscle are involved in joint contractures have been shown based on changes in range of motion obtained by measuring the range of motion (ROM) of the joint immediately after releasing joint immobilization, measuring the ROM again after removing the skin to eliminate the influence of the skin, and then measuring the ROM after removing the muscle.

On the other hand, changes in skeletal muscle extensibility have been shown to be functional changes in a causative tissue of joint contracture. When Tabary et al. investigated the passive length-tension curve of the soleus muscle of cats after immobilizing their ankle joints in full plantar flexion for 4 weeks, they found that it was less extensible than a normal muscle. However, there are no reports on the extensibility of the skin itself, which is also a causative tissue of joint contractures.

The purpose of this study was to investigate in an animal model whether skin extensibility decreases as a result of contractures that develop due to joint immobilization.

SUBJECTS AND METHODS

The animals used in this experiment were 8-week-old female Wistar rats (n = 6). The animals were housed in cages and given free access to food and water. The environment of the animal care room was maintained at a constant temperature of 23 °C with an air conditioner, and day and night were artificially regulated by setting the lights on for 12 h and off for 12 h each day. The animal experiment was conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and the experiment was carried out with the approval of the Research Ethics Committee of the Prefectural University of Hiroshima.

The duration of the experiment was two weeks. The rats were divided into two experimental groups. In the immobilized group (6 limbs), the right ankle joints were immobilized in complete plantar flexion by plaster casts,
and in the control group (6 limbs), the left ankle joints had no intervention. Joint immobilization was achieved by the method described in a report by Ono et al. Pentobarbital (40 mg/kg) was injected intraperitoneally (i.p.), and under anesthesia, the ankle joint was held in full plantar flexion and immobilized with a plaster cast from the thigh to the foot area. In addition, the cast was covered with a stainless steel net to prevent breaking and falling. Effects of edema as a result of immobilization were observed in the toes during the immobilization period, and the cast was reapplied whenever necessary. Skin extensibility was evaluated on the final day of the experiment by measuring it with a tensile strength tester (Autograph Tensile Tester model AG-50kNG, Shimadzu Corporation). First, the casts were removed from the rats under pentobarbital anesthesia (40 mg/kg, i.p.). Next, in preparation for producing a specimen for the tensile strength test, the ankle joint was placed in full plantar flexion, and the skin was marked on the posterior aspect of the lower leg at point A, a location 3 mm distal to the calcaneal region, and at point B, a location 10 mm proximal to point A. The rats were sacrificed by exsanguinating them by transecting the aorta in the abdomen, and the skin specimens were immediately collected by excision. The distal end of the skin that was collected was 5 mm distal to point A, and its proximal end was 5 mm proximal to point B, and the skin specimen was 20 mm in length. The width of the skin specimen was 4 mm. A hole was made at the point marked A and the point marked B in the skin that was collected, and after passing a stainless steel nonstretchable wire through each hole, the ends of each wire were attached to a tensile strength tester with clamps. The skin specimen was adjusted with calipers so that the locations of the 2 places where the wires had been inserted would be 10 mm apart at the start of the test, the same distance as when the marks were made. The tension test was conducted by setting the distension distance at 0 mm and tension at 0 N. Based on a report by Ono et al., the extensibility measurement parameter was used as the distension distance when 0.3 N of distension force was applied at the start of the test.

The statistical analysis was performed with software. The nonparametric Wilcoxon test was used to compare skin extensibility. A significant difference was concluded to exist at a probability value of 5% in all of the statistical tests.

RESULTS

The results for skin extensibility are shown in Table 1. Skin extensibility in the immobilized group was lower than that in the control group. Compared with the control group, the immobilized group showed a significant decrease in skin extensibility.

DISCUSSION

In physical therapy research, animal studies have suggested that skin contractures develop as a result of joint immobilization. Ichihashi et al. observed the development of joint contractures after immobilizing the knee joint of rats in the fully flexed position for 30 days, but they stated that when they removed the skin and measured the range of extension of the knee, they observed expansion of the ROM. In our previous study, the range of dorsiflexion was compared between a group in which the skin was removed and a group in which the skin was not removed, and an improvement in mobility was observed in the former group. Based on these reports, skin has been shown to have an impact on the contractures that developed as a result of joint immobilization. These previous studies showed that the presence or absence of skin was related to joint contractures. However, no change in skin extensibility was demonstrated.

The results of the present study demonstrated that the extensibility of the skin on the posterior aspect of the ankle joint decreases as a result of immobilizing the ankle joint in plantar flexion. In the past, the mechanical properties of skin have been investigated by methods that compressed, twisted, suctioned, etc., the skin, and suction is said to be the method most often used. Suction is a method of investigating the mechanical properties of the skin by applying a probe to the skin surface and suctioning the skin into it by exerting negative pressure. The Cutometer has been mentioned as a representative instrument for this purpose, and several studies have reported the extensibility of the skin using a Cutometer. When Dobrev measured the skin extensibility of psoriasis patients with a Cutometer, he found significantly lower values than in healthy subjects of the same age. In addition, Braham et al. measured the skin extensibility of 13 acromegaly patients during treatment and healthy subjects with a Cutometer, and they reported significantly greater skin extensibility in the acromegaly patients. However, because their research methods measured skin extensibility in vivo by suctioning the skin with a Cutometer, the influence of the fascia and muscle in layers under the skin may have been reflected in its extensibility. The present study reviewed their methods, and skin extensibility was obtained by collection of skin in vivo and measurement with a tensile strength tester. This method made it possible to demonstrate the extensibility of the skin alone because it eliminated the influence of the subcutaneous tissues.

Next, when the skin that had been collected at the end of the immobilization period was subjected to the tension test, the results revealed that the skin extensibility in the immobilized group was lower than that in the control group. According to a report by Okamoto et al., who investigated changes in the caustic tissues of contractures associated with joint immobilization in animal experiments, the percentage of involvement of the skin in ROM limitation was 13.1% after 2 weeks of immobilization. The results of the present study showed that the percentage decrease in skin extensibility was 45.4% after 2 weeks of immobilization, and the values differed from those of the study of Okamoto et al. In their study,

<table>
<thead>
<tr>
<th>Group</th>
<th>Distension distance (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.1±0.7</td>
</tr>
<tr>
<td>Immobilized</td>
<td>2.8±0.7*</td>
</tr>
</tbody>
</table>

Values are means ±SD. *Significant decrease compared with the control group (p<0.05)
the percentage of involvement of skin in joint contracture was investigated on the basis of the ROM values before and after removing the skin. However, because skin extensibility was investigated by conducting tension tests on skin that had been collected, differences in the skin contribution rate arose, and the results suggested that extensibility tends to decrease. The present method consisted of focusing on the skin and performing tension tests. As a result, it was able to demonstrate for the first time the impact of the skin alone on joint contractures from the standpoint of skin extensibility.

Clinically, when immobilization in a plaster cast is considered necessary because of conservative treatment or surgical treatment after a fracture, skin extensibility may decrease. In the future, it will be necessary to determine whether stretching exercises to improve skin extensibility are effective and whether joint ROM improves at the same time.

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