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Histopathological Changes in the Periphery of the Sciatic Nerve of Rats after Knee Joint Immobilization

Shinya Yoshida, RPT, MS[1)*], Taro Matsuzaki, RPT, MS[2)], Akio Kamijo, RPT, MS[3)], Yoshitaka Araki, RPT, MS[4)], Makoto Sakamoto, RPT[5)], Shigenori Moriyama, RPT[1)], Masahiro Hoso, MD, PhD[2)]

[1)] *Division of Rehabilitation, Kanazawa University Hospital: 13-1 Takaramachi, Kanazawa-shi, Ishikawa 920-8641, Japan. TEL: +81 76-265-2013, FAX: +81 76-234-4308*
[2)] *Division of Health Sciences, Kanazawa University Graduate School of Medical Science*
[3)] *Department of Rehabilitation Medicine, Azumino Red Cross Hospital*
[4)] *Department of Rehabilitation Medicine, Kanazawa Cranial Nerve Surgery Hospital*
[5)] *Department of Rehabilitation Medicine, Bobath Memorial Hospital*

Abstract [Purpose] This study was performed to investigate the histological changes that occur in the periphery of the sciatic nerve in rats undergoing knee immobilization. [Subjects and Methods] 29 male 9-week-old Wistar rats were divided randomly into a control group (C group, n = 7) and an immobilized group (I group, n = 22). The animals in the I group had the left knee joint immobilized in maximal flexion with plaster casts for two weeks. After the experimental period, we obtained cross-sections of tissues from the center of the left thigh, and the periphery of the sciatic nerve was observed under an optical microscope after hematoxylin-eosin staining. [Results] In contrast to the rats of C group, the rats in I group showed adherence between the bundle of nerve fibers and perineurium, as well as thickening of the perineurium. These histological changes were statistically significant. [Conclusions] Immobilization of the knee joints of rats resulted in characteristic histological changes in the connective tissue around the sciatic nerve.
Key words: Contracture, Sciatic nerve, Perineurium

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INTRODUCTION

Joint contracture occurs when joint movements are reduced, such as by immobilization of a joint with a plaster cast, orthosis, or after prolonged bed rest. Several conditions limit the range of motion (ROM) of joints, including cerebrovascular diseases, neuromuscular diseases, bone and joint diseases, and various other diseases.

Contracture may be classified into three groups: arthrogenic, myogenic, and soft tissue[1)]. Arthrogenic contracture is caused by immobility caused by damage and inflammation of cartilage, synovial tissue, and joint capsule. Myogenic contracture may be due to intrinsic and/or extrinsic causes. Soft tissue contracture occurs because of pathological shortening of connective tissue, including the skin, subcutaneous tissue, tendons, and ligaments. Examples of intrinsic causes include trauma, inflammation, degenerative changes, and ischemia. Examples of extrinsic causes include spasticity, flaccid paralysis, malpositioning, and immobilization.

Many investigators have studied the pathogenesis of contracture in dogs, monkeys, rats, and other experimental animals. These studies provided evidence of a relationship between the period of immobilization and ROM limitation factors, and changes in joint components and soft tissue, including muscle and skin. In addition, decreases in mobility and plasticity of the nervous system are considered to cause ROM limitation[2)]. Nervous system mobilization is used as a physical therapeutic approach to improve such conditions. It uses palpation or joint movements to initiate physical or physiological changes in blood flow or the axonal flow of nerves, aiming to improve the plasticity of the nerve[3)]. In addition, nervous system mobilization has been reported to be effective at improving ROM limitation and pain, and induces the largest longitudinal excursion of the nerve relative to its surrounding structures without being associated with a potentially detrimental increase in nerve strain[4–6)]. On the other hand, there is no evidence that mobilization of neural tissue, independent of other anatomical structures, would be feasible in living people, or that nervous system mobilization is effective in the treatment of musculoskeletal dysfunction. Therefore, it is still unclear whether nervous system mobilization is effective[7–10)].

As stated above, nervous system mobilization is used to improve ROM limitation and other conditions, but the changes that it causes in the nerves and the periphery in contracture arising from secondary disabilities or pro-

*To whom correspondence should be addressed.
E-mail: yoshi511@med.kanazawa-u.ac.jp

longed bed rest remain unclear. In addition, there is no evidence that nervous system mobilization is effective. The present study was performed to determine changes in the tissue located in the periphery of the sciatic nerve during contracture in an animal model.

SUBJECTS AND METHODS

Male Wistar rats (9 weeks old, n = 29, weight 240 − 280 g) were divided randomly into a control group (C group, n = 7) and immobilized group (I group, n = 22), and were kept in plastic cages for 2 weeks. The rats had unlimited activity in the cage and free access to food and water. The animal room was maintained at a constant temperature of 22 °C, and the animals were maintained under a 12-h light/dark cycle to avoid effects arising from the biological rhythm of the rats. This experiment was performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Kanazawa University and the protocol for this experiment was approved by the Animal Research Committee of Kanazawa University.

Rats in I group had the left knee joint immobilized for 2 weeks in the posture of maximum flexion with a plaster cast of our own making and aluminum wire netting. The casts were applied under deep anesthesia induced by intraperitoneal injection of pentobarbital sodium (40 mg/kg) (Fig. 1). Care was taken to avoid disturbance of blood flow to the hip and ankle joints due to pressure from the cast. During the immobilization period, wounds and edema were prevented and if the plaster cast came off immobilization was repeated again as soon as possible. Animals in C group were given food ad libitum, and had no restrictions placed on their mobility.

At the end of the immobilization period, the rats were euthanized with diethyl ether and both hip joints were disarticulated immediately. The joint tissue was fixed with 10% formalin neutral buffer solution for 72 h decalcified in EDTA at 4 °C for 72 h. After decalcification, the specimens were cut vertically at the center of the femur and neutralized with 5% sodium sulfate for 72 h, and then embedded in paraffin. The embedded paraffin blocks were cut into sections at a thickness of 3 μm. The tissue sections were stained with hematoxylin-eosin (HE).

We examined the periphery of the sciatic nerve under an optical microscope (BX51; Olympus). The histopathological findings were independently confirmed by three investigators.

Data for adherence and thickening were analyzed using Fisher's exact test. The software program JMP7 (SAS Institute Japan Inc.) was used for statistical analyses, and values of $p < 0.05$ were accepted as statistically significant.

RESULTS

In C group, the bundle of nerve fibers tended to separate from the perineurium, and a space was clearly observed between the bundle of nerve fibers and the perineurium (Fig. 2 and Table 1). Conversely, in the rats of I group, strong adherence between the bundle of nerve fibers and perineurium was observed in 19 of 22 animals (Fig. 3 and Table 1), and thickening of the perineurium was detected in 20 of 22 of these rats (Fig. 3 and Table 2). These histological changes were statistically significant ($p < 0.01$).

DISCUSSION

Generally, peripheral nerves consist of several bundles

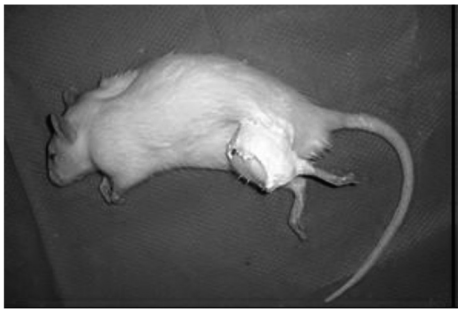

Fig. 1. Immobilization model

Table 1. Adherence between the bundle of nerve fibers and perineurium

	No. adherence	No. non-adherence
C group (n =7)	0	7
I group (n = 22)	19	3

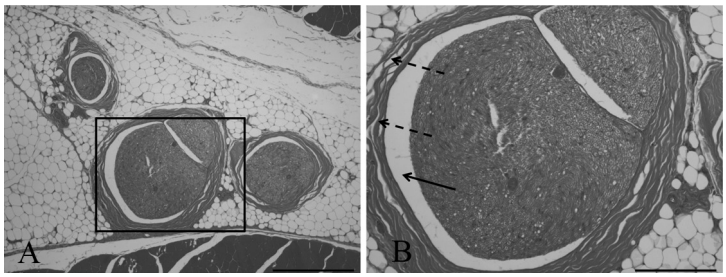

Fig. 2. Sciatic nerve in C group. The perineural space (black arrows) can be seen between the bundle of nerve fibers and the perineurium (dotted arrows). Scale bar: A: 500 μm, B: 200 μm.

of nerve fibers. In these bundles of nerve fibers, each nerve fiber, including the Schwann cells enveloping it, is surrounded by endoneurium that consists of areolar connective tissues containing blood vessels. Each bundle of nerve fibers is covered by perineurium, which is a dense layer composed of collagenous connective tissue enveloped by 7 or 8 layers of squamous epithelial-like cells[11]. There is a space between the perineurium and the bundle of nerve fibers, but there have been no previous anatomical descriptions of this space. Peripheral nerves consist of more than one bundle of nerve fibers and the epineurium consisting of areolar connective tissues. The bundles of nerve fibers are combined with each other and form a nerve trunk, with a cylindrical strong connective tissue sheath[12].

The sciatic nerve is the largest nerve in humans and represents most of the nerve fibers forming the sacral plexus. The sciatic nerve is derived from spinal nerves L4 through S3. This nerve descends into the posterior compartment of the thigh from the gluteal region through the greater sciatic foramen. Proximal to the knee, and sometimes within the pelvis, the sciatic nerve divides into its two terminal branches, the tibial nerve and the common fibular nerve. It innervates all muscles in the posterior compartment of the thigh, lower leg, and foot, as well as most of the skin of the lower leg and foot[13–15]. The spinal nerves in the rat consist of 34 pairs of nerves: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral, and 3 caudal. Typical plexuses are formed in the cervical, brachial, and lumbosacral regions. The sacral plexus in the rat is more limited in the extent of its origin than the human one. It is formed by the fifth, and the adjacent parts of the fourth and sixth lumbar nerves. The sciatic nerve divides into its two terminal components, the common fibular nerve and the tibial nerve, which in their course through the thigh cross the obturator externus, quadratus femoris, and adductor magnus, lying between these muscles and biceps femoris, until they reach the popliteal fossa where they separate. As described above, the sciatic nerve tract in the rat is almost the same as in humans[16].

Table 2. Thickening of the perineurium

	No. thickening	No. non-thickening
C group (n = 7)	0	7
I group (n = 22)	20	2

The nervous system adapts to lengthening in two ways, "movement" and "elongation"[2, 3]. Movement may be considered as gross movement or movement occurring intraneurally between the connective tissues and the neural tissues. Gross movements refer to movement of the system as a whole in relation to the connective tissue interface. A peripheral nerve sliding through a tunnel, such as the median nerve, is a clear example of this type of gross movement. Intraneural movement refers to movement of the neural tissue elements in relation to the connective tissue interfaces. The brain or the spinal cord can move in relation to the dura mater and a fascicle can slide in relation to another fascicle in peripheral nerves. On the other hand, elongation is like elastic gum and the nervous system adapts to joint movement by increasing intraneural pressure or intradural pressure. The looseness of nerve fibers, epineurium, and perineurium decrease as the nerve is elongated. The epineurium is only slightly involved in resisting elongation of the nerve, and resistance is mainly offered by the perineurium. Reductions in these adaptations induce limitation of ROM, pain, and neurological symptoms. Generally, nervous system mobilization can increase the mobility of the nerve by improving the motility and extensibility of the nervous system when movement and elongation are reduced due to the influence of various factors, but there is no histological evidence of such an effect.

In this study, a space was observed between the bundle of nerve fibers and the perineurium of the sciatic nerve in the C group. It is possible that this space is an artificial histopathological image caused by differences in the rate of contraction between the bundle of nerve fibers and perineurium tissue in the process of specimen preparation. However, in I group this space was not observed, so, we considered it unlikely that this was an artificial image appearing only in I group.

There have been no anatomical descriptions of this space and it has not been the focus of prior study. However, in pancreatic cancer, gastric cancer, and prostate cancer, the invasion of cancer tissue into this space (perineural invasion) has been seen; therefore, this space has attracted attention and is known as the "perineural space." Watanabe[17] reported that cancer cells do not invade bundles of nerve fibers, but scatter along loose connective tissue in the perineurium. The results of experimental and clinical studies

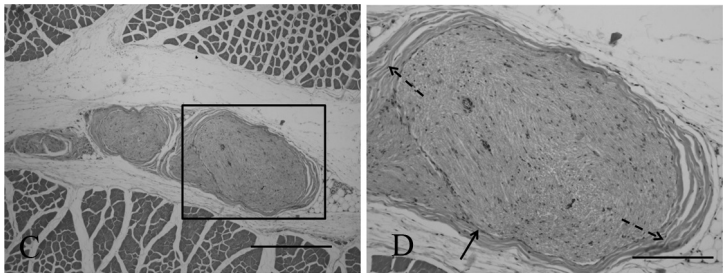

Fig. 3. Sciatic nerve in I group. Adherence between the bundle of nerve fibers and the perineurium (black arrows), and thickening of the perineurium (dotted arrows) were observed. Scale bar: C: 500 μm, D: 200 μm.

indicate that the perineural space is independent of the lymphatic system and there is no traffic between the two[18, 19]. In addition, Miyazaki[20] reported that perineural invasion shows continuous tumor spread, unlike lymphatic invasion and vein invasion, and there is no flow in the perineural space, unlike lymphatic and blood vessels. As mentioned above, it is clear that there is a space between the bundle of nerve fibers and the perineurium of autonomic nerves. However, there have been no previous reports of its presence in somatic nerves and its physiological significance remains unknown. Further studies and descriptions of the perineural space have not been reported to date.

In C group, the bundle of nerve fibers separated from the perineurium physiologically, and there was a perineural space in the periphery of not only the autonomic nerve, but also the somatic nerve. On the other hand, there was no perineural space in I group: the bundle of nerve fibers tended to make contact with the perineurium, and there was the possibility of adhesion between the two. With regard to this adhesion, if the perineurium functions as a buffer between the bundle of nerve fibers and the peripheral tissue of the nerve, it is possible that this adhesion decreased the mobility and plasticity of the bundle of nerve fibers. Thomas[21] reported that the perineurium acts as a mechanical barrier to external forces, and Sunderland[22] reported that it was the structure most resistant to tensile forces. We suggest that thickening disturbs these functions of the perineurium, causing a decrease in the buffer function and the mobility and plasticity of the nerve. This is the first study to examine the changes in the periphery of nerve tissue after joint immobilization with histopathological observations.

In conclusion, abnormal histopathological images were observed in not only the joint components and soft tissue, but also in the peripheral tissue of nerves in the rat knee joint contracture model indicating joint immobilization may affect the peripheral tissue of nerves.

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