Histopathological changes in surrounding tissue of the sciatic nerve after spinal cord injury in rats

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Abstract.

[Purpose] The purpose of this study was to examine histopathological changes in tissue surrounding the sciatic nerve after spinal cord injury (SCI) in rats. [Subjects and Methods] Thirty adult, nine-week-old, female Wistar rats were used in this study. Fifteen experimental rats underwent spinal cord transection at the level of Th8-9 and the other fifteen control rats were raised normally. Animals were assessed at 1, 2, 4, 8, and 12 weeks following surgery. After the experimental period, we obtained tissue surrounding the sciatic nerve of the thigh after hematoxylin and eosin staining under a microscope. [Results] Adherence between the nerve bundle and perineural innermost layer was observed in tissue surrounding the sciatic nerve in the SCI group. Adherence among the inter-perineurium was evident at 2 weeks after SCI. It had declined at 4 weeks after SCI, but was still evident at 8 and 12 weeks after SCI. [Conclusion] Histopathological findings in the SCI model may be related to compression of the nerve bundle and neurogenic contracture of tissue surrounding the nerve bundle.

Key words: Spinal cord injury; Sciatic nerve; Perineurium

INTRODUCTION

Joint contracture inhibits movement or the activities of daily living and is one of the most frequent functional disorders treated. Joint contracture is classified into congenital contracture and acquired contracture. Acquired contracture is caused by immobilization and includes myogenic contracture, connective tissue contracture, arthrogenic contracture, neurogenic contracture, and dermal contracture [1]. Furthermore, although muscular factors cause decreasing ranges of joint motion in initial contracture, it is complicated by other factors such as articular factors which cause protracted immobilization [2-6].

Joint immobility is one of the hallmark consequences of a lack of mechanical stimulation, and may be caused by corrective immobilization, such as cast immobilization or external fixation, and by immobilization after neurological paralysis, such as spinal cord injury (SCI). Several studies have described pathological changes in muscle, connective tissue, and joint components after cast or splint immobilization [7-12]. Previous reports on the effect of immobilization of the hindlimb after SCI mainly focused on the neurological consequences and were based on morphometric studies, such as evaluation of the range of motion [13] or muscle fiber diameter [14] of the hindlimb. One study reported changes in joint cartilage thickness after SCI [15]. Moreover, as reported in our previous study, histopathological changes in knee joint components after SCI in rats were dilatation and congestion of microvasculature, lymphoid infiltration, villous proliferation in the synovial membrane, fibrous proliferation of membrane tissue in the surface layer of articular cartilage, and atrophy of adipose cells [16]. Although several studies have reported changes in muscle and joint components after SCI, a detailed histopathological investigation has not been performed on components other than muscle and joint components. Improvements in the range of joint motion are needed to obtain gliding of a nerve, in addition to flexibility of muscular or dermal tissue and tensibility of intra-articular soft tissue. Understanding more about extra-muscular or articular conditions has clinical relevance because rehabilitation approaches may need to differ in these conditions.

In this study, we examined histopathological changes in tissue surrounding the sciatic nerve at five

different time points after SCI in rats.

SUBJECTS AND METHODS

Thirty female Wistar rats aged 9 weeks old (body weight: 160-190 g), acclimatized for 1 week, were used in this study. Rats were individually raised in sterilized cages laid with floor chips, had free and easy access to food and tap water, and unlimited activity. The animal room was maintained at 20-26°C, on a 12-hour light dark cycle. This study was carried out in accordance with the guidelines of the Committee for Animal Experimentation of Kanazawa University (Approval no. 081147). Rats were divided randomly into 2 groups, fifteen in the experimental group and fifteen in the control group. The experimental SCI groups were examined at 1 week (SCI-1w), 2 weeks (SCI-2w), 4 weeks (SCI-4w), 8 weeks (SCI-8w), and 12 weeks (SCI-12w) after surgery (three rats, six limbs in each group); and the control groups (Con-1w, Con-2w, Con-4w, Con-8w, and Con-12w) were examined at the same times (three rats, six limbs in each group).

The 15 experimental rats were given an intraperitoneal administration of 50 mg/kg sodium pentobarbital after anesthetization with ethyl ether. After the back pelages of these rats were shaved at the thoracic vertebrae level, the shaved area was painted with povidone-iodine to prevent infection. With rats in the prone position, a median incision on the back area was performed. Paraspinal muscles were exposed along the bilateral side of the neural spine. After the spinal cord was exposed by laminectomy of the Th8-9 vertebrae, it was completely transected at the level of T8-9 using a scalpel blade. Finally, paraspinal muscles and skin were sutured.

Rats were observed for nutrition, excretion amount, pressure ulcers, and motor function of the hindlimb everyday throughout the experimental period. Bladders of the experimental animals were compressed manually twice daily [13, 15, 17].

After animals had been sacrificed under ethyl ether anesthesia, bilateral hindlimbs were transected as expeditiously as practicable from the hip joint. Excised hindlimbs were denuded of skin and fixed in 10%

neutral buffered formalin for 72 hours. The specimens were then washed with running water, and decalcified with Plank-Rychlo's solution at 4°C for 72 hours. Decalcified tissue specimens were perpendicularly cut from the intermediate part of the thigh at the long axis of the femur. Tissue specimens were set in a cassette for paraffin embedding and neutralized with 5% anhydrous sodium sulphate for 72 hours. Neutralized tissue specimens were washed with running water for 15-30 minutes, and were then defatted in 100% alcohol for about 2 hours. After defatting, tissue specimens were dehydrated and embedded in paraffin using an automated tissue processor TEK III (TISSUE-TEK, Japan). Paraffin-embedded tissues were sliced at a thickness of 3 μm in the sagittal plane using a rotary microtome SM-2000R (LEICA, Germany). Thin sections were stretched in distilled water at 38-40°C using a paraffin stretching plate PS-M (Sakura Finetek Japan, Japan) for about 10 minutes. After stretching, sections were fixed on microscope slide glasses. The slide glasses were dried at 37°C for 24 hours, then stained with hematoxylin-eosin and sealed. The surrounding tissues of the sciatic nerve of the thigh were then examined under an optical microscope BX51 (OLYMPUS, Japan).

RESULTS

The space between the nerve bundle and perineurium around the sciatic nerve was observed in the hindlimbs of all control rats. The perineurium showed a multilayer structure in a concentric ring fashion. Adherence between the nerve bundle and perineural innermost layer was more evident in surrounding tissue of the sciatic nerve in the SCI groups (SCI-1w: 4/6 limbs, SCI-2w: 6/6 limbs, SCI-4w: 6/6 limbs, SCI-8w: 5/6 limbs, SCI-12w: 5/6 limbs) than in the control groups. Adherence between the nerve bundle and perineural innermost layer was observed at 2, 4, 8, and 12 weeks after SCI. Adherence among the inter-perineurium was observed from 2 weeks after SCI (3/6 limbs) (Fig. 1). Adherence among the inter-perineurium in the SCI group was evident at 2 weeks after SCI. It had declined at 4 weeks after SCI, but was still evident at 8 and 12 weeks after SCI (Fig. 2).

DISCUSSION

The peripheral nerve consists of a plural nerve bundle. Nerve fibers in the nerve bundle are surrounded by the endoneurium with Schwann cells. Several nerve bundles are bundled by the perineurium. The perineurium is wrapped in seven or eight-layers of perineural cells. A perineural cell is a fibrocyte like an endothelial cell. Moreover, tight junctions between perineural cells establish the perineural tube [18-21]. The perineural tube communicates with the subarachnoid space because perineural cells continue in arachnoid barrier cells in the spinal nerve root [22, 23]. Furthermore, Hashimoto *et al.* [18-20] suggested that spinal fluid was passed from the perineural tube into tissue fluid by the barrier of perineural cells. However, there is no anatomical description of the space between the perineurium and the nerve bundle, even though perineural invasion has been identified in carcinoma. This space attracts attention as the perineural space [24, 25]. From the results of experimental and clinical studies, it has become generally accepted that the perineural space does not communicate with the lymphatic system [24, 25]. Although, it is evident that the perineural space exists between the perineurium and nerve bundle of autonomic nerves, research regarding the existence of the perineural space in somatic nerves has not been performed. Thus, the physiological meaning of the perineural space has still not been identified. In this study, the space between the nerve bundle and perineurium around the sciatic nerve was observed in the hindlimbs of control rats. The unctuous tissue may increase contractile force by defatting. Therefore, we suggest the nerve bundle that consists of a myelin sheath high in lipids [26, 27] is contracted more than other tissues. In contrast, the perineurium consists of collagen fibers high in extracellular matrix protein, and the contraction factor is lower than that lipids. Thus, we suggest that such spaces seen in control rats may be artifacts created in the preparation of the specimens (e.g. defatting operation).

In contrast to the control rats, adherence between the nerve bundle and the perineural innermost layer was observed in the SCI rats. The tendency to adhere among the perineurium in the SCI rat persisted, mildly,

and the concentric layer of the perineurium was maintained. Thus, we suggest that although it is necessary to assess the adhesion factor with other immunostaining methods, the surrounding tissue of the sciatic nerve in the SCI rats may not be affected by preparation of the specimen. Yoshida *et al.* [28] reported that adherence between the bundle of nerve fibers and the perineurium, and thickening of the perineurium was observed in knee joint immobility after a plaster cast in the posture of maximum flexion for 2 weeks. Thus, we suggest that immobilization of the joint induced palsy that affected the periphery of the nerve tissue. The perineurium acts as a mechanical barrier against external force [29] and is the structure most resistant to tensional force [30]. Histopathological findings in the SCI model may be related to compression of the nerve bundle, such as typical disturbances in the peripheral nerve (e.g. allodynia), and neurogenic contracture, such as disturbances in the perineurium, declines in the perineurium-mediated buffer, gliding, and flexibility functions in the tissues surrounding the nerve bundle.

Adherence among the inter-perineurium in the SCI group was more evident 2 weeks after SCI, but had declined 4 weeks after SCI. Chronic, high frequency, flexion-extension movement in knee and ankle joints simultaneously occurring in combination with hyperflexion of the hip joint was often observed from 2 weeks after SCI [13, 31]. van de Meent *et al.* [31] suggested that chronic jerking of the hindlimbs was “kick movement”. Our SCI model rats showed functional similarities to the animals described in previous studies [16]. The hindlimb after SCI performed involuntary articular movements because immobilization of the hindlimb after SCI was not inhibited by immobilization cast or splint as reported in previous studies [2-7, 9]. In our previous study about knee joint components after SCI, although we observed chronic inflammatory responses in the synovial membrane, fibrous proliferation of membrane tissue in the surface layer of the articular cartilage, and atrophy of adipose cells, these changes were more evident at 2 weeks after SCI, and had declined at 4 weeks after SCI [16]. In this study, histopathological changes in the surrounding tissue of the sciatic nerve after SCI were not progressive, similar to the histopathological changes of joint components after SCI. Articular movement needs gliding of the nerve in addition to intra-articular

flexibility and tensibility of soft tissues, such as muscles and tendons. Thus, we suggest that these non-progressive changes could be related to involuntary movements such as the SCI-specific spasticity observed in the hindlimb from SCI-2w.

In clinical practice, several papers have advocated that early rehabilitation treatment is crucial for patients with SCI because it improves activities of daily living and functional independence after injury [32, 33]. Physical medicine such as range of motion exercises may need to take into account compression of the peripheral nerve, through myogenic, arthrogenic or neurogenic contracture factors.

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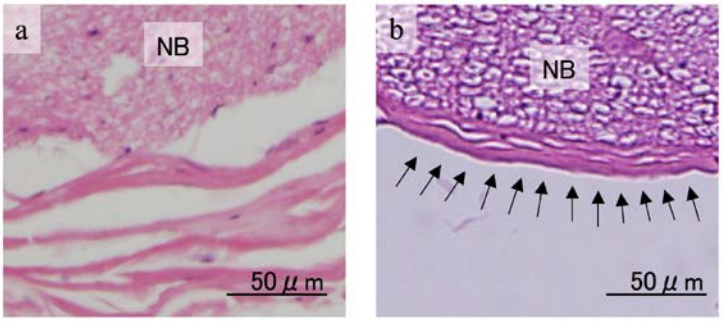

Fig. 1. Surrounding tissue of the sciatic nerve in the Con-2w and SCI-2w rat

A space between the nerve bundle and perineurium around the sciatic nerve was observed in the hindlimbs of the Con rats. Adherence between the nerve bundle and perineural innermost layer was more evident in surrounding tissue of the sciatic nerve in the SCI rats. Adherence among inter-perineurium was observed from 2 weeks after SCI (black arrows). NB: the nerve bundle, Scale bar: 50μm. a) Con-2w rat, b) SCI-2w rat

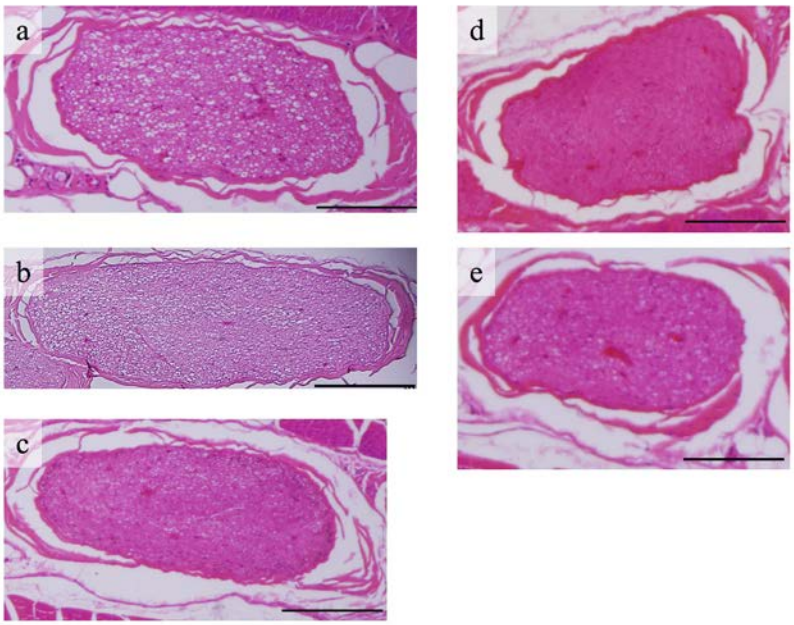

Fig. 2. Surrounding tissue of the sciatic nerve in the SCI group

Adherence between the nerve bundle and perineural innermost layer was observed at 2, 4, 8, and 12 weeks after SCI. Adherence among inter-perineurium in the SCI group was more evident at 2 weeks after SCI. It had declined at 4 weeks after SCI, but remained visible at 8 and 12 weeks after SCI. Scale bar: 200μm. a) SCI-1w, b) SCI-2w, c) SCI-4w, d) SCI-8w, e) SCI-12w