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Histopathological Changes in Knee Joint Components after Spinal Cord Injury in Rats

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Abstract. [Purpose] The purpose of this study was to examine the histopathological changes in knee joint components after spinal cord injury (SCI) in rats. [Subjects and Methods] Eighteen adult, nine-week-old female Wistar rats were used in this study. Nine experimental rats underwent a spinal cord transection at the level of Th8-9 and the other nine control rats were raised normally. The animals were assessed at 1, 2 and 4 weeks after surgery. Formalin fixed sections from knee joints were morphologically examined after hematoxylin and eosin staining. Alterations of knee joint components were evaluated at four regions: the synovial membrane within the posterior articular capsule region, the cartilage apposite the femur, the cartilage apposite the tibia, and the fat pad under the patellar ligament region. [Results] Dilatation and congestion of the microvasculature and lymphoid infiltration were observed in the synovial membrane in the SCI group. These findings are similar to those found in early osteoarthritis. The surface layer of the articular cartilage in the SCI group showed fibrous proliferation. [Conclusion] The histopathological changes appear not to be progressive and may be related to spasticity of the hindlimb or a disorder in the autonomic function.
Key words: Spinal cord injury, Knee joint components, Histopathological changes

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INTRODUCTION

Joint immobility is one of the hallmark consequences of lack of mechanical stimulation. The duration of joint immobility causes secondary disability such as joint contracture, bone atrophy and amyotrophy. Joint immobility may be caused by cast immobilization (CI), external fixation, or after neurological paralysis. Variable and somewhat controversial morphological alterations in the knee joint components resulting from immobilization have been described, and the changes include, increased thickness and decreased area of elastic fibers of the joint capsule[1], increased surface irregularity[2–4], increased or decreased or unchanged thickness of cartilage[5–8], decreased synovial intima length[9], augmentation of levels of type I collagen in the synovial intima[10], synoviocyte proliferation[11, 12] and decreased cross-sectional areas of myocytes[13].

Several studies have described the histopathological changes in joint components after CI[1,9–12,14]. Previous reports on the effect of immobilization of the hindlimb after spinal cord injury (SCI) have mainly focused on its neurological consequences and were based on morphometric studies such as evaluation of the range of motion[15] or muscle fiber diameter[16] of the hindlimb. Although one study reported the findings of changes in the joint cartilage thickness after SCI[17], a detailed histopathological investigation was not performed.

Several lines of evidence suggest that the mechanisms that cause joint immobilization after neurological paralysis differ from those caused by CI. Understanding more about these differing processes has clinical relevance because physical therapy approaches may need to be different for these situations. The purpose of this study was to establish SCI model rats and to examine the histopathological changes in their knee joint components.

SUBJECTS AND METHODS

Eighteen female Wistar rats aged 9 weeks old (body weight: 160–190 g) acclimatized for 1 week were used in this study. The rats were individually raised in sterilized

cages laid with floor chips and 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, nine in the experimental group and nine in the control group. The experimental SCI groups were examined at 1 week (SCI-1w), 2 weeks (SCI-2w), and 4 weeks (SCI-4w) after surgery (three rats, six limbs in each group); and the control groups (Con-1w, Con-2w, and Con-4w) were examined as the same times (three rats, six limbs in each group).

Nine experimental rats were anesthetized by intraperitoneal administration of 50mg/kg sodium pentobarbital after using ethyl ether. After the back pelages of these rats were shaved at thoracic vertebra 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. The 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, the paraspinal muscles and skin were sewed up.

Rats were observed for nutrition, excretion amount, pressure ulcer, and the motor function of the hindlimb everyday throughout the experimental period. The bladders of the experimental animals were compressed manually twice daily [15,17,18].

After animals had been sacrificed using ethyl ether, their bilateral hindlimbs were transected as expeditiously as practicable from the hip joint. The excised hindlimbs were denuded of skin and fixed in 10% neutral buffered formalin for 72 hours. Then, these specimens were washed with running water, and decalcified with Plank-Rychlo's solution at 4°C for 72 hours. The decalcified tissue specimens were cut in the sagittal plane. Next, the tissue specimens were set in a cassette for paraffin embedding and neutralized 5% anhydrous sodium sulphate for 72 hours. The neutralized tissue specimens were washed with running water for 15–30 minutes, and then they were defatted 100% alcohol for about 2 hours. After defatting, the tissue specimens were dehydrated and embedded in paraffin using an automated tissue processor TEK III (TISSUE-TEK, Japan). The paraffin-embedded tissues were sliced to a thickness of 3μm in the sagittal plane using a rotary microtome SM-2000R (LEICA, Germany). The thin sections were stretched on distilled water at 38–40°C using a paraffin stretching plate PS-M (Sakura Finetek Japan, Japan) for about 10 minutes. After stretching, the sections were fixed on micro slide glasses. These slide glasses were dried at 37°C for 24 hours, then stained with hematoxylin-eosin and sealed. Lastly, synovial membrane, articular cartilage and fat pad of the knee joint were examined under an optical microscope BX51 (OLYMPUS, Japan). Alterations of knee joint components were evaluated at four regions: the synovial membrane within the posterior articular capsule region, the cartilage apposite the femur, the cartilage apposite the tibia, and the fat pad under the patellar ligament region.

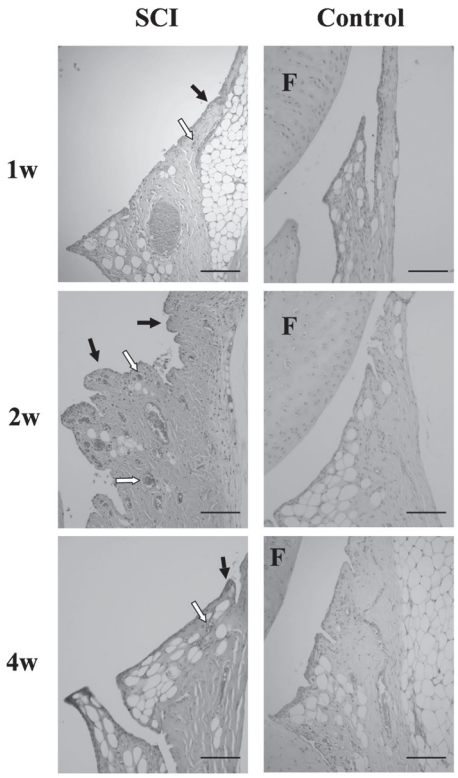

Fig. 1. Synovial membrane within the posterior articular capsule region
Dilatation and congestion of the microvasculature (white arrows) and villous proliferation (black arrows) were observed in the SCI group. Scale bar: 100 μm, SCI: Spinal cord injury group, Control: Control group, F: Femur.

RESULTS

None of the hindlimbs in the control group had paralysis or external injury, and all performed normally. All of the hindlimbs in the SCI group had flaccid paralysis from 1 day after surgery; the gait pattern of these rats showed dragging of the hindlimbs. All of the hindlimbs in the SCI group had developed abnormal behavior like spasticity in response to contact stimuli by 2 weeks after SCI.

Dilatation and congestion of the microvasculature, and villous proliferation were observed in the synovial membrane within the posterior articular capsule region in the SCI-1w group (6 / 6 limbs), compared with the Con-1w group (Fig. 1). Lymphoid infiltration was observed in the SCI-2w group (5 / 6 limbs) (Fig. 3-ab). These changes were more evident at 2 weeks after SCI but had declined at 4 weeks after SCI.

A membranous tissue covering the surface of the articular cartilage was observed in the 5 limbs of the SCI-

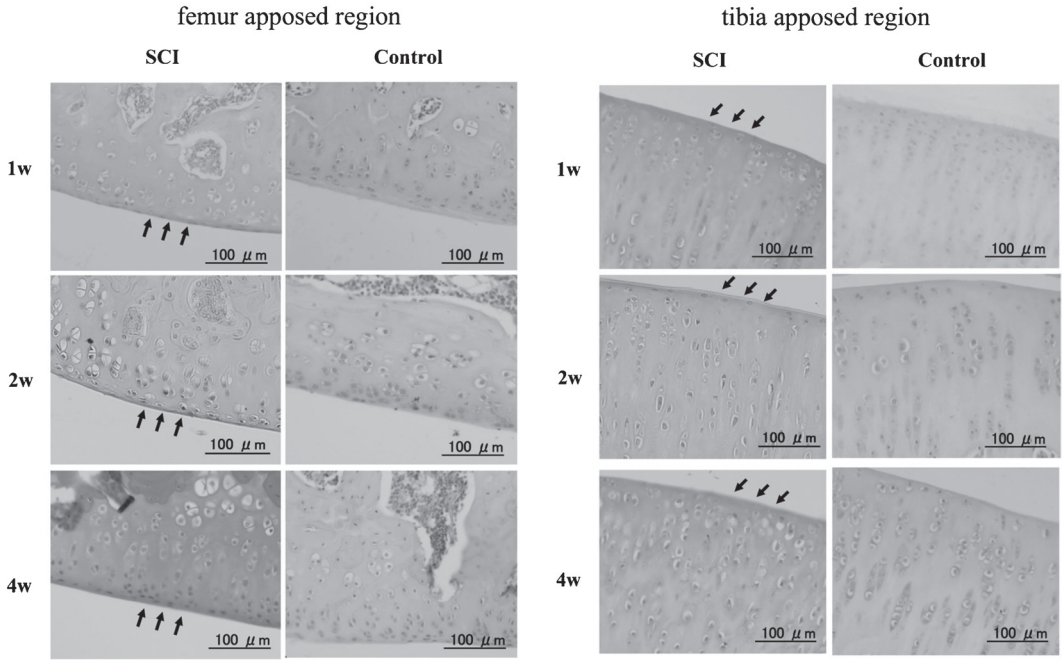

Fig. 2. Articular cartilage in the region apposite the femur
A membranous tissue covering the surface of the articular cartilage was observed in the SCI group that showed fibrous proliferation (black arrows). Scale bar: 100 μm, SCI: Spinal cord injury group, Control: Control group.

1w group at the regions apposite the femur and tibia (Fig. 2). A surface layer of articular cartilage in the SCI-2w group showed more fibrous proliferation than the SCI-1w group. This surface layer of articular cartilage initially showed fibrous proliferation, but this alteration was absent in the SCI-4w group (Fig. 3-ab).

At 1 week after surgery, adipose tissue under the patellar ligament region of the SCI rats was unchanged compared with the control group (Fig. 4). However, at 2 weeks after SCI, remarkable atrophy and fibrosis of adipose tissue in the fat pad were observed, particularly under the patellar ligament region (6 / 6 limbs). Of note, adipose cells showed a trend toward improvement in the grade of atrophy 4 weeks after surgery indicating that those changes were transient.

DISCUSSION

The hindlimbs of SCI rats demonstrated flaccid paralysis from 1 day after surgery, and exhibited abnormal behavior like spasticity from 2 weeks after SCI. Moriyama et al.[15] indicated that the response to stimuli disappeared, and animals demonstrated complete flaccid paraplegia during the first few days after SCI. Moreover, clonic, high frequency, flexion-extension movement in the knee and ankle joints simultaneously occurring in combination with hyperflexion of the hip joint was often observed from 2 weeks after SCI[15,19]. van de Meent et al.[19] suggested the clonic jerking of hindlimbs was "kick movements". Our SCI rats showed functional similarities to the animals described in these previous studies.

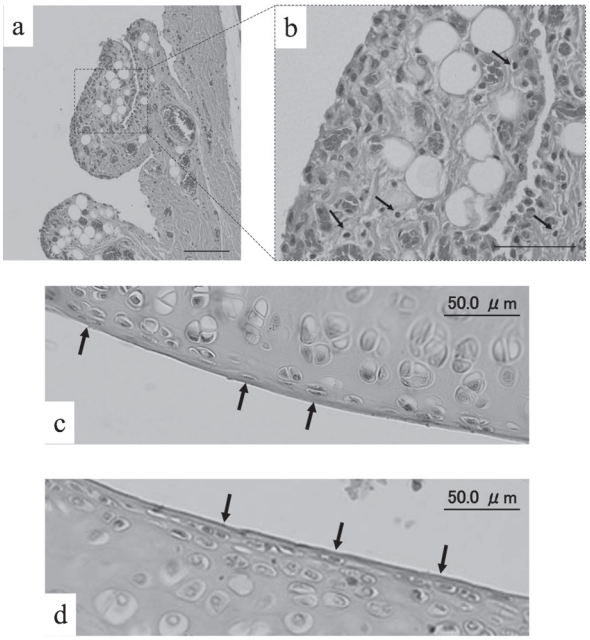

Fig. 3. Synovial membrane and articular cartilage in the SCI-2w group
Synovial membrane: Lymphoid infiltration was observed in the SCI-2w group (black arrows). Scale bar: a: 100 μm, b: 50 μm
Articular cartilage: A membranous tissue covering the surface of the articular cartilage was observed in the SCI-2w group which showed fibrous proliferation (black arrows). Scale bar: 50.0 μm (c: Surface layer of the femur, d: Surface layer of the tibia).

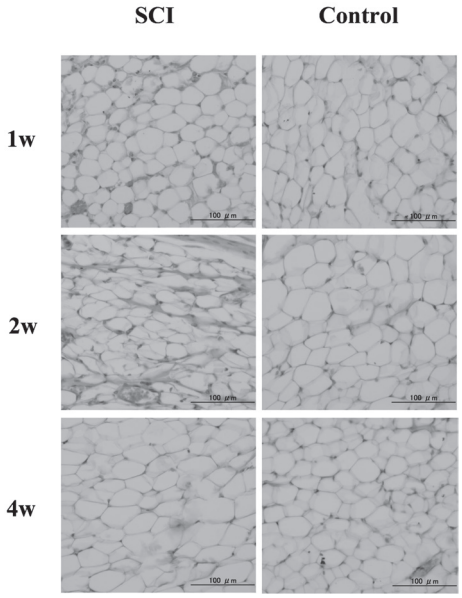

Fig. 4. Fat pad under the patellar ligament region
At 2 weeks after SCI, remarkable atrophy and fibrosis of adipose tissue in the fat pad was observed. The adipose cells showed a trend toward improvement in the grade of atrophy in the SCI-4w group indicating that the changes were transient. Scale bar: 100 μm, SCI: Spinal cord injury group, Control; Control group.

Synovial membrane is one of the joint components. It produces the joint fluid, and supplies nourishment to the articular cartilage[20]. Therefore, the synovial membrane plays an important role in articular motion. Dilatation and congestion of the microvasculature, villous proliferation and lymphoid infiltration were observed in the synovial membrane in the SCI-2w rats. This finding resemble the histopathology of initial osteoarthritis (OA) which presents with a chronic inflammatory response such as microvillus formation, growth and fibrosis of capillaries[21,22]. In a previous study of the histopathological changes in the synovial membrane after joint immobility, Evans et al.[12] reported that the joint cavity was narrowed by outgrowth of the connective tissue under the synovial membrane underneath the patellar ligament 15 days after CI. Thus, the histology of the synovial membrane after SCI was different from that after CI. In patients with SCI, the disturbance of proprioception, such as joint position sensibility, was evoked with motor and sensory disturbances below the level of the damaged spinal cord. Finsterbush et al.[23] indicated that cartilage cells were degenerated after transection of the sensory nerve to the knee joint. Generally, Charcot's joint[24] is known as an articular disorder coexisting with the proprioception disturbances induced by SCI. Moreover, the inflammation of synovial tissue in OA is a reactive inflammation accompanying injury[21,22]. Thus, we propose that the synovial tissue inflammation found in SCI rats is similar to OA-like synovial tissue inflammation induced by recurrent tissue damage with disturbances in proprioception. Additionally, the histopathological changes were nonprogressive change unlike other histopathological changes reported in CI models. Therefore, we suggest that the progression in the OA-like changes were inhibited by the spastic movements of the hindlimbs.

Cartilage membrane has pressure susceptibility. Mechanical stress on the articular cartilage activates chondrocytes, inducing the synthesis of proteoglycan[25], and increasing the amounts of cAMP and cGMP[26]. Furthermore, the invasion of the subchondral bone is inhibited by transient fluid pressures such as periodic stress related to muscle contraction around the joint occurring in deep layers of the cartilage[27, 28]. Therefore, the mechanical stresses provide by articular movement and loads are important for the restoration of articular cartilage[29,30].

In this study, a membranous tissue covering the surface of the articular cartilage was observed in the hindlimbs of SCI rats. This finding resembles the change observed in joint immobility after CI reported by Watanabe et al[1]. We suggest that decrease in synovial perfusion and abnormal muscle contraction arising from loss of load on the cartilage may be possible causes of this change. Generally, the mechanical load on the cartilage surface, such as transient articular load, promotes nutrient supply to the articular cartilage by the synovia secreted from the synovial membrane[20]. Therefore, cartilage tissue in the absence of mechanical load may lack nutrition or become hypoxic[31], resulting in the development of membranous tissue on the articular cartilage surface. This change progressed until 2 weeks after SCI, but not thereafter. The changes in cartilage and hindlimb movement, appearance of spasticity, occurred around the same time. We suggest that the progression of changes in the cartilage were inhibited, because the spastic "kick movement" of the hindlimbs loaded the cartilage.

Synovial membrane between the patellar ligament and femur consists of fatty synovial membrane covered by adipose cells in two- or three-layered synoviocytes. In a previous study of the histopathological changes in fatty synovial membrane after 2-week knee joint immobilization in rats, adipose cells were atrophied[1]. In the present study, atrophy and the fibrosis of the adipose cells were observed in the fat pad around the synovial membrane, in particular under the patellar ligament region in the SCI-2w group. The changes resemble the histopathological changes after two-week knee joint immobilization in rats. A shared mechanism may exist between the pathological condition of joint immobilization by SCI and by cast. However, the fat pads in SCI-4w rat showed a trend toward normalization. This normalization may have been caused by the motor function of the hindlimb. Generally, disturbance of autonomic function is evoked by SCI, and the vagal nerves become predominant. The autonomic nerve[32] and vagal nerve[33] are involved in the metabolism of adipose calls. Therefore, we suggest that the normalization of the fat pad is the result of an interaction between fat metabolism and autonomic function.

In conclusion, the histopathological changes in knee

joint components after SCI in rats were chronic inflammatory response in the synovial membrane, fibrous proliferation of the membrane tissue in the surface layer of articular cartilage and the atrophy of adipose cells. We suggest that these histopathological changes are not progressive and may be related to SCI-specific spasticity in the hindlimbs or a disorder in the autonomic function.

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