

Change of Bone Mechanical Strength in Rats after Spinal Cord Injury over a Short Term

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ABSTRACT

This study investigated the time-course of changes in bone mechanical strength in rats with spinal cord injury (SCI). Sixty-four male Wistar rats underwent spinal cord transection at the thoracic nerve. Control rats underwent a sham procedure (SHAM). Animals were sacrificed at day 1, 4, 7 and 14 after operation. The mechanical strength of the left femur and tibia was measured by the three-point bending strength test. The bones were dried, weighed and burned to ash. A specimen of right tibia was prepared and examined under a microscope.

Bone mechanical strength, dry bone weight, and ash content of the femur and tibia in SCI rats were significantly lower than those in SHAM animals. Dry bone weight and ash content began to decrease from the 4th day after SCI and reached their lowest at day 7 after operation. Bone mechanical strength had reduced significantly by the 14th day. Gaps and spaces were observed in the trabecular area at the same time. After SCI, calcified cartilage decreased and the reduction of bone mass occurred rapidly. Moreover, a decline of bone mechanical strength is caused within 2 weeks. Thus, SCI led to the atrophy of bone and caused the reduction of mechanical strength at an early stage. It is thus necessary to prevent bone loss after SCI immediately.

Key words: Spinal cord injury, Bone mechanical strength, Bone mass

Immobilization is one of the risk factors in decreasing bone mass. Bone loss results from the removal of mechanical stress. Bone loss in post-menopausal women is 2–4% in a year, whereas bone mass in long bed rest patients declines 1% in a week and 10–20% in a few months⁵⁾. This suggests that loading and exercise in daily life are closely related to the maintenance of bone mass. Osteoporosis after spinal cord injury (SCI) is reported in human and animal studies^{1,2,8,11)}. Paralysis like hemiplegia, paraplegia and tetraplegia causes bone loss. Such bone loss occurs mainly in trabecular bone. An osteopenic animal model with neurectomy of a peripheral nerve is known and used widely. However, there have been few basal studies of bone mechanical strength in a SCI model. These studies investigated the change of bone over a month^{2,8)}, but no experiment dealt with the acute change of bone in SCI animals. Since in the SCI model the hind limbs and pelvis are paralyzed completely, the model is useful for

ascertaining the effect of immobilization on bone loss. Therefore, this study investigated the changes in bone, particularly its mechanical strength, at the acute stage from 1 to 14 days after SCI in rats.

MATERIALS AND METHODS

Animal Care

Sixty-four male Wistar rats (6-weeks old) with an average body weight of 157.4 g were used. They either underwent spinal cord transection at the middle thoracic level or were sham-operated (SHAM) under pentobarbital sodium (1ml/kg). Paraplegia was identified by a sense of pain reaction in both hind limbs. After this, they were kept in separate cages under the following conditions: temperature, $23 \pm 1^\circ\text{C}$, humidity, $50 \pm 5\%$ and a 12 hour day-night cycle. All the rats were allowed ad libitum feeding (normal laboratory food) and drinking water. Urination was facilitated by an induction of pressing the bladder. This study was

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carried out in accordance with the Guide for Animal Experimentation, Hiroshima University and the Committee of Research Facilities of Laboratory Animal Science, Hiroshima University School of Medicine.

Mechanical Testing

The rats were sacrificed at day 1, 4, 7 and 14 after surgery. The femur and tibia were dissected out and soft tissue was removed. A materials testing machine (computer control system autograph AGS-1000A, SHIMADZU, Co, Japan) was used to measure the three-point bending strength of the left femur and tibia. The compression head loaded the center of the bone at a constant vertical velocity of 0.5 mm/min. Measured values were calculated at the ratio of body weight. The femur and tibia were dried, weighed and burned at 600°C for 7 hours to ash. The right femur and tibia were demineralized and embedded in paraffin for morphology study. Sections (3–4 μm) were prepared and stained by Hematoxylin-Eosin. The sections were observed under a microscope at a magnification of 500.

Statistic Analysis

An unpaired t-test was used to find the differences between SCI and SHAM rats on each day. One-way ANOVA and post hoc tests were used to determine time-course changes of bone properties in each group. p less than 0.05 was considered significant. All values were expressed as means \pm standard deviation (SD).

RESULTS

Table 1 and Figs. 1 through 3 show the change of measured properties over time. All measured properties except for bone mechanical strength of the femur and tibia in SCI and SHAM groups showed significant differences during the experiment, though the bone mechanical strength of the femur and tibia in SCI rats decreased through the term. The final body weight of SHAM rats increased faster than that of SCI rats, and tended to be heavier with term (Table 1). The bone length of the femur and tibia was independent of SCI and

became longer with growth (Table 1). The bone mechanical strength of the femur and tibia in SCI rats declined significantly, compared with SHAM rats at day 14 after the operation (Fig. 1). The dry bone weight and ash content of both bones were significantly different between SCI and SHAM on all days except day 1 after the operation (Figs. 2, 3).

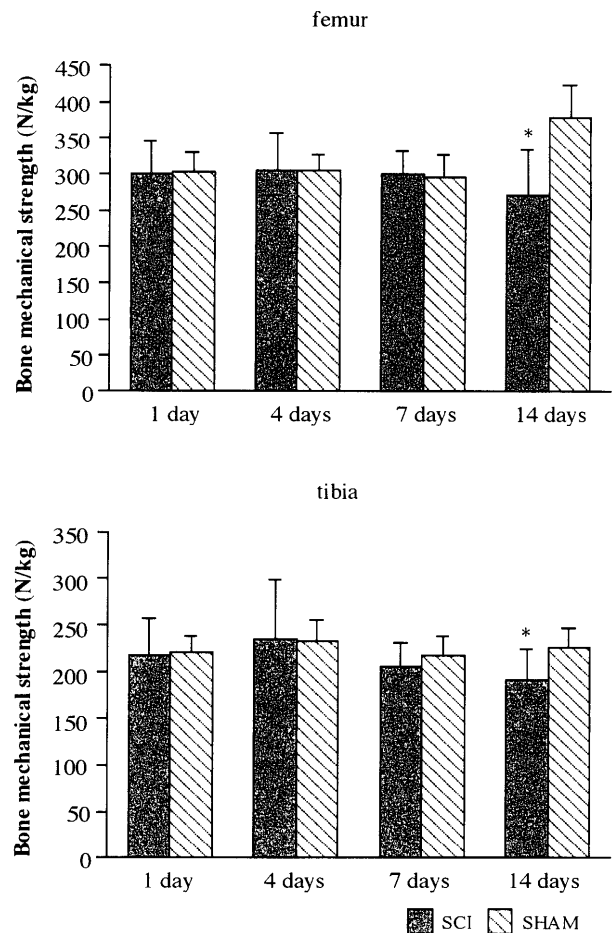


Fig. 1. Bone mechanical strength of femur and tibia in both groups. SCI group decreased after operation whereas SHAM group increased.

* Significantly different from SHAM group ($p < 0.05$).

Table 1. Results of final body weight, rate of body weight gain, and bone length

groups		final body weight (g)	rate of body weight gain (%)	bone length (mm)	
				femur	tibia
1 day	SCI	158.8 \pm 6.4	96.4 \pm 7.9	25.5 \pm 0.3	29.8 \pm 1.1
	SHAM	161.9 \pm 4.6	100.0 \pm 1.7	25.8 \pm 0.4	29.3 \pm 0.8
4 days	SCI	153.8 \pm 20.0*	96.6 \pm 14.9*	26.5 \pm 0.5	30.7 \pm 1.1
	SHAM	193.1 \pm 8.4	119.5 \pm 9.3	27.3 \pm 0.5	30.1 \pm 0.6
1 week	SCI	155.0 \pm 11.0*	105.5 \pm 8.1*	27.1 \pm 0.8	30.4 \pm 0.9
	SHAM	206.9 \pm 8.4	131.2 \pm 10.5	27.4 \pm 0.9	31.3 \pm 0.9
2 weeks	SCI	193.8 \pm 24.0	128.6 \pm 15.2*	28.6 \pm 1.4	33.7 \pm 1.1
	SHAM	211.3 \pm 23.0	139.9 \pm 8.1	29.9 \pm 0.9	34.0 \pm 0.4

(mean \pm S.D.)

* Significant difference between SCI and SHAM group ($p < 0.05$).

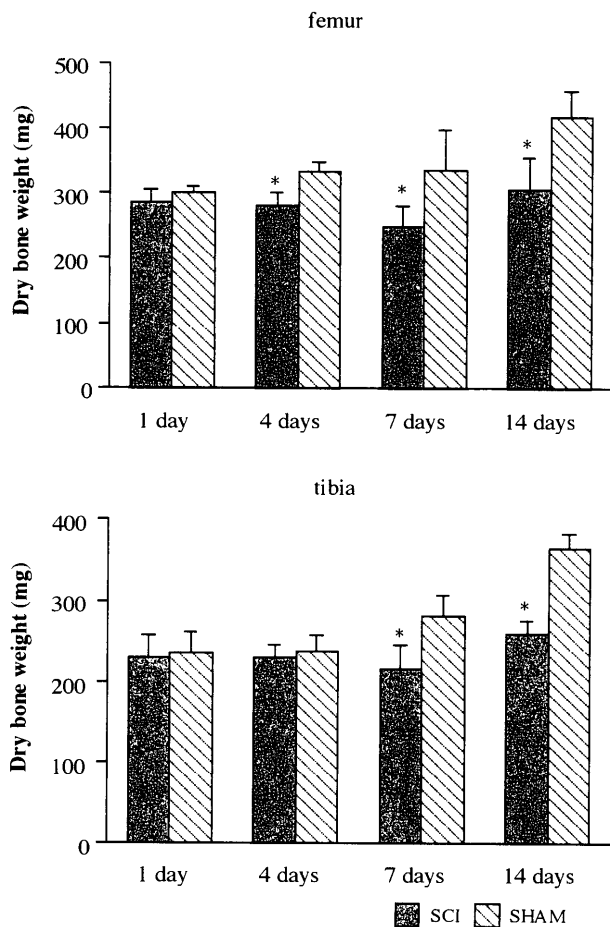


Fig. 2. Dry bone weight of femur and tibia in both groups. SCI group decreased after operation, compared with SHAM group.
* Significantly different from SHAM group ($p < 0.05$).

The dry bone weight and ash content of SCI rats decreased significantly from the 4th day to the 14th day, whereas SHAM animals increased with growth. The bone mechanical strength of the femur and tibia in SCI group reduced significantly at day 14 after the operation, compared with SHAM group. All the measured properties of SHAM rats increased with growth, but those of SCI animals decreased and remained at a low level during the study. Thus, the measured properties related to bone mass were reduced by SCI in this experiment.

The border of the growth plate and metaphyseal trabecular bone of the upper tibia was examined under a microscope. Gaps and spaces were seen in the trabecular area of sections of SCI rats at days 4, 7 and 14 (Fig. 4).

DISCUSSION

The bone mechanical strength, dry bone weight, and ash content of the femur and tibia in SCI rats were significantly lower than those in SHAM rats. It is reported that bone loss with SCI is related to the degree of paralysis, time since injury, and age^{9,11}.

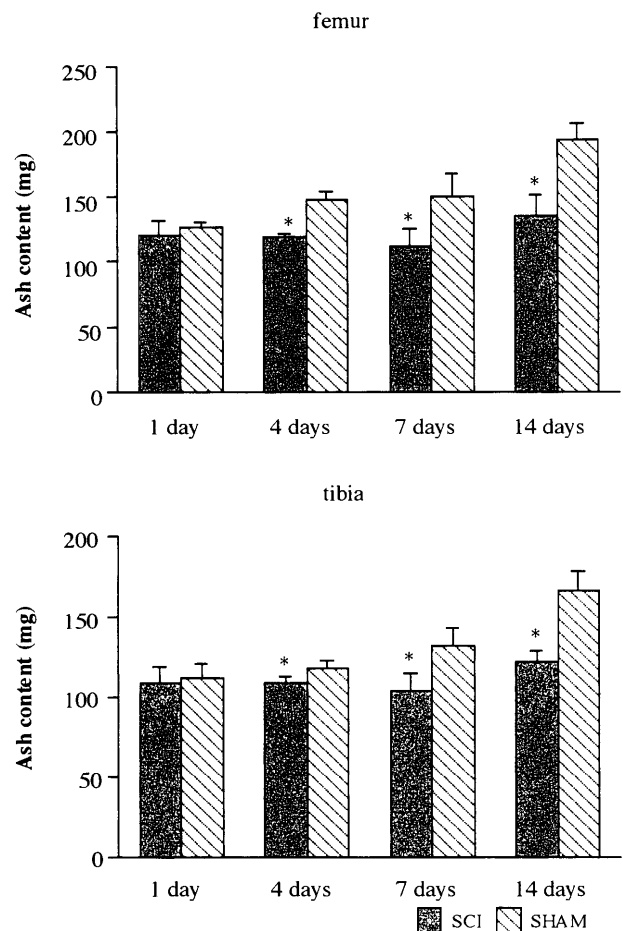


Fig. 3. Ash content of femur and tibia in both groups. SCI group tended to decrease after operation whereas SHAM group increased.
* Significantly different from SHAM group ($p < 0.05$).

In the experiment, bone mechanical strength showed a significant difference between SHAM rats and SCI rats over 14 days. Moreover, the bone mass of SCI rats decreased to its lowest level within a week after the operation. This indicated that bone loss occurred at an early stage after SCI as in the case of ovariectomy (OVX) and other immobilization models. Almost all studies of bone loss with SCI animals investigated changes of bone properties over more than a month^{2,8}. The present study investigated the change of bone mechanical strength with acute SCI animals. Markers of bone resorption showed a significant rise after acute SCI⁷, and immobilization secondary to SCI is associated with marked and rapid atrophy of trabecular bone¹. In addition, the cortical bone area and rates of formation declined². Reduction of mechanical stress thus inhibits osteoblast-mediated bone formation and accelerates osteoclast-mediated bone resorption. This was the reason SCI caused rapid bone loss. In fact, rapid changes were seen in measured properties, and a reduction of calcified cartilage was observed on the 4th day (Fig. 4) in this study. Moreover, it was reported that the reduction of bone formation

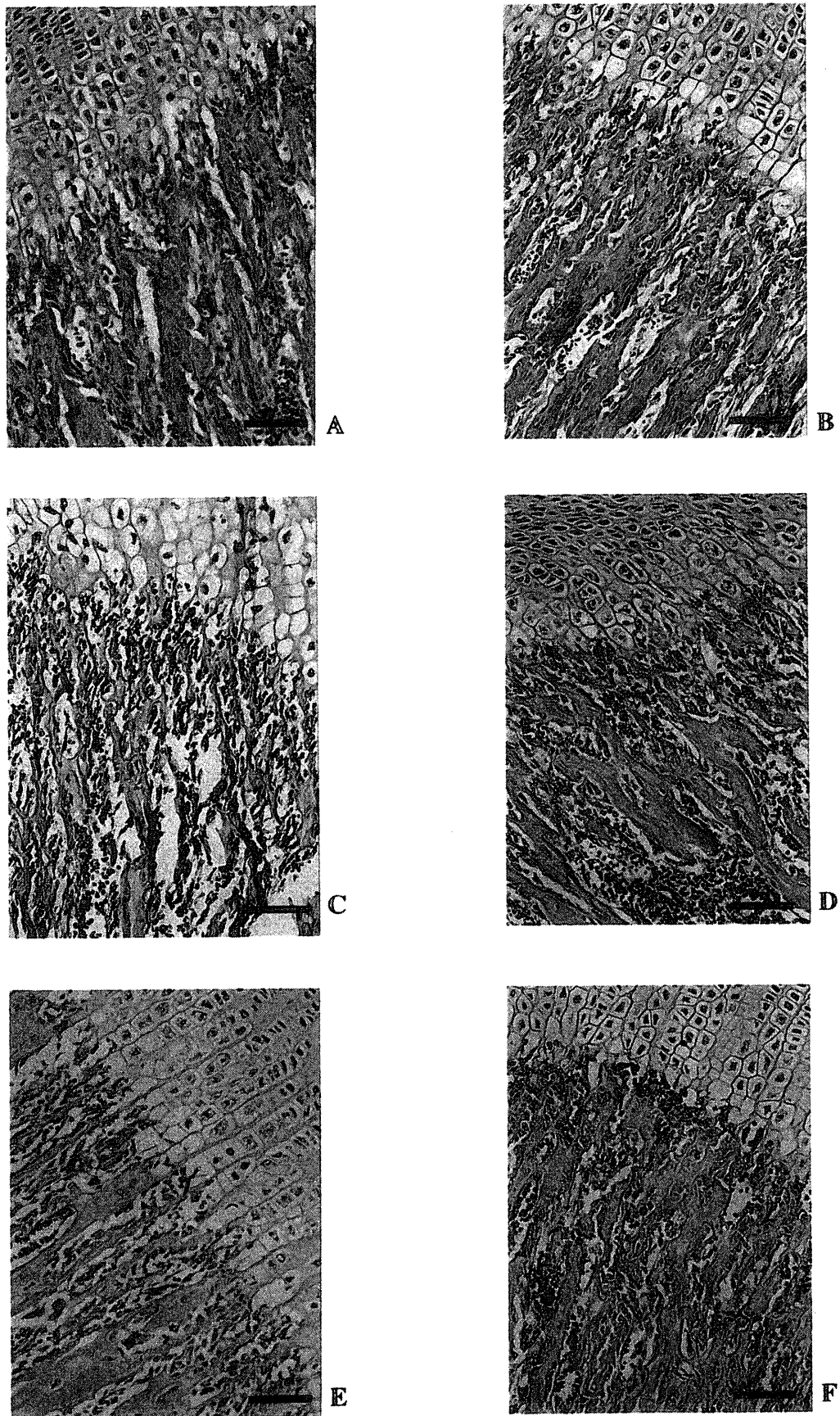


Fig. 4. Growth plate (upper side) and metaphyseal trabecular bone of upper tibia (Hematoxylin-Eosin stain). A: 1 day SCI, B: 1 day SHAM, C: 4 days SCI, D: 7 days SCI, E: 14 days SCI, F: 14 days SHAM. Gaps and spaces were seen in trabecular area on and after 4 days. But there were few gaps and spaces in sham rats. bar = 0.01mm

reached a peak at about day 5 after bed rest, and the inhibition of bone formation continued⁶). The bone mass of SCI patients declined remarkably in the year after injuries¹⁰). The femoral neck, trochanter and Ward's triangle are the sites where bone mass decreases most¹⁰). Moreover, the thickness of cortical bone becomes thinner in the trabecular bone area after SCI.

Bone properties in SCI group barely increased at all, and maintained a low level during the experiment. It was inferred that the bone mass began to decrease at the acute stage and this reduction continued. After surgery, cortical bone decreased following the decline of trabecular bone. This continuous reduction of trabecular and cortical bone leads to a low level of bone mass. Moreover, gaps and spaces in the trabecular area were observed at day 4 after SCI under the microscope (Fig. 4). In addition, trabecular bone was narrowed and cut off for two weeks. This was the reduction of mineralization in the border of the growth plate of the tibia. This demonstrated that mineralization and bone formation were disturbed by SCI. On the other hand, the trabecular after of SHAM rats retained its mineralization. It was considered that immobilization by SCI influenced bone mass from an early stage. That is, bone responds to the decline of mechanical stress, and bone formation decreases as a reaction. A reduction of calcified cartilage occurs immediately after SCI, and this leads to a decrease of bone mass. In consequence, bone mechanical strength declines. This phenomenon takes place at both the trabecular and cortical area over a short period. Bone length was not affected by SCI, though the transient acceleration of bone length was reported in the case of OVX^{3,4}). Deficiency of estrogen leads to bone loss with the acceleration of bone absorption and its main target is trabecular bone. On the other hand, immobilization causes bone loss with the inhibition of bone formation, and its main target is both trabecular and cortical bone. It was considered that the effects of bone mechanical strength, dry bone weight and ash content over a short time differed with mechanism of bone loss.

Thus, SCI decreases bone mass and mechanical strength rapidly after operation. Therefore, the prevention of bone loss is needed to cope with the decrease of fracture risk as early as possible.

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