# Effects of Sciatic Neurectomy on Arthritis and Bone Loss in Rats with Collagen-Induced Arthritis

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**We investigated the effects of sciatic neurectomy on arthritis and bone mineral density (BMD) in 7-month-old female Sprague-Dawley rats with collagen-induced arthritis (CIA). After the animals obtained a uniform mean body weight, we divided them at random into 4 groups, and treated them with the following surgical manipulations: (i) sham group ($n$ = 8), sham surgery; (ii) CIA group ($n$ = 9), collagen sensitization and sham surgery; (iii) NTx group ($n$ = 9), sciatic neurectomy and (iv) CIA + NTx group ($n$ = 9), collagen sensitization and a sciatic neurectomy. Every 2 weeks up to 8 weeks after sensitization, the arthritis score for hind paw swelling was evaluated, and BMD of the cancellous and cortical bones in the proximal tibia was measured by peripheral quantitative computed tomography (pQCT). Both the hind paw swelling and arthritis score in the CIA + NTx group were significantly lower than those in the CIA group at 6 and 8 weeks after sensitization. There was no significant difference in the cancellous BMD between the CIA and CIA + NTx groups. The cortical BMD of the tibial metaphysis was significantly lower in the NTx and CIA + NTx groups than in the sham group at 4 and 8 weeks, and in the CIA + NTx group than in the CIA group at 4 weeks after sensitization. There was no significant difference between the CIA and sham groups as well as the CIA and CIA + NTx group at 8 weeks after sensitization. It was concluded that sciatic neurectomy suppressed the severity of arthritis, but did not affect the cancellous bone loss in adult CIA rats.**

**Key words:** bone mineral density; collagen-induced arthritis; peripheral quantitative computed tomography; rheumatoid arthritis; sciatic neurectomy

One of the common complications of rheumatoid arthritis (RA) is osteoporosis, which is categorized into generalized osteoporosis and localized osteoporosis. Periarticular osteoporosis, which is included in the latter category, is a lesion that develops early in RA and it is an important finding in the diagnosis of the disease (Alenfeld et al., 2000). In addition, RA patients have a 1.51-fold increase of risk for hip fracture and a 2.56-fold increased risk for pelvic fracture compared to

healthy individuals (Hooyman et al., 1984). Therefore, control of osteoporosis in RA patients is an important issue.

RA is a disease to which middle-aged to elderly persons are susceptible, similar to lifestyle related diseases. Paralytic diseases such as strokes, which are lifestyle related diseases, are known to cause disuse bone loss and to worsen osteoporosis (Takamoto et al., 1995). The risk for fracture may increase when RA patients have ac-

companying paralysis. On the other hand, neural paralysis is known to have a controlling effect on osteoarthritis and rheumatoid arthritis. For instance, it has been reported that the progression of osteoarthritis is suppressed in a leg paralyzed by stroke (Coste et al., 1935) and peripheral nerve palsy (McEwen, 1940). In addition, many studies have reported that the occurrence and progression of rheumatoid arthritis are suppressed in legs paralyzed by stroke (Bland et al., 1968) and poliomyelitis (Glick, 1967). Okumura et al. (1987) have reported that sciatic neurectomy performed on adult female rats caused osteoporosis in the paralyzed legs and worsened osteoporosis when it was performed on rats with oophorectomy. Weinreb et al. (1989) have reported that sciatic neurectomy caused cancellous bone loss in the proximal tibia. Thus, sciatic neurectomy is considered to be an appropriate method for studying the effects of neural paralysis on arthritis and osteoporosis.

There have so far been no reports on the effect of neural paralysis on osteoporosis associated with RA in either animals and humans. Collagen-induced arthritis (CIA) in rats has been used as a model for studying the pathology and therapy of RA. Enokida et al. (2001) have reported that adult CIA rats were more appropriate than young CIA rats for studying secondary osteoporosis associated with RA, because the bone loss in adult CIA rats occurred mainly in periarticular regions, which resembled secondary osteoporosis in the early stage of RA. The aim of this study was to investigate the effects of sciatic neurectomy on arthritis and periarticular osteoporosis in adult CIA rats.

## Materials and Methods

### Animals

Seven-month-old female Sprague-Dawley rats weighing 290 to 350 g (Simizu Laboratory Supply, Kyoto, Japan) were used in the present study. The study was carried out in accordance with the Guidelines for Animal Experimentation of Tottori University Faculty of Medicine. The animals were given regular chow (CE-2, Clea Japan, Tokyo, Japan; 1.18 g/100 g Ca, 250 IU/100 g vitamin $D_3$) and tap water ad libitum. The animals were maintained at a temperature of 24°C with 12 h lighting (7:00 AM to 7:00 PM). After acclimatization for 2 weeks, the animals obtained a uniform mean body weight. We divided the animals at random into 4 groups, and treated them with the following surgical manipulations: (i) sham group ($n = 8$), sham surgery; (ii) CIA group ($n = 9$), collagen sensitization and sham surgery; (iii) NTx group ($n = 9$), sciatic neurectomy and (iv) CIA + NTx group ($n = 9$), collagen sensitization and sciatic neurectomy.

### Preparation of the CIA model

After intraperitoneal anesthesia with 50 mg/kg of ketamine hydrochloride and 10 mg/kg body weight of xylazine, 1 mL of emulsion containing 500 μg of bovine type II collagen (0.3% acetic acid solution, k-41; Cosmo-Bio, Tokyo) and 500 μg of incomplete adjuvant (521-00021, Difco Laboratories, Detroit, MI) was intracutaneously injected at 3 sites on the back of each rat. For additional sensitization, 0.5 mL of the same emulsion was intracutaneously injected into the posterior of both hip joints 1 week after the initial sensitization (Trentham, 1977). Physiological saline was intracutaneously injected by the same procedure in the sham and NTx groups.

### Sciatic neurectomy

Under anesthesia with the same method, a dorsolateral incision was made on the bilateral hips at the same time of the initial sensitization. The sciatic nerves were exposed, and 0.5 cm sections were excised. The muscle and skin were then sutured.

### Gastrocunemius muscle weight

All animals were anesthetized with 50 mg/kg ketamine injected intraperitoneally together with 10 mg/kg xylazine, and killed by exsanguination at

8 weeks after the sensitization. The bilateral gastrocunemius muscles were removed, and immediately weighed, using a Sartorius balance (Northern Balance and Scale, Blooming, MN).

### Evaluation of arthritis

Hind paw swelling was quantified by measuring the ankle width from the medial malleolus to the lateral malleolus with a constant-tension caliper at 2 week intervals for 8 weeks. Soft X-ray images of both hind paws were taken with a soft X-ray radiographic apparatus (SPO-M50; Sofron, Tokyo), operating at 40 kV peak and 2 mA at an exposure time of 30 s at 2 week intervals for 8 weeks. The radiologic arthritis score of the right ankle and subtalar joints was assessed according to Engelhardt's method (Engelhardt et al., 1995) using X-ray images. The body weight, hind paw swelling and radiologic arthritis score were measured every 2 weeks for 8 weeks.

### Bone mineral density

The bone mineral density (BMD) in the proximal metaphysis (2 mm distant from the epiphyseal line) of the left tibia was measured every 2 weeks for 8 weeks after the initial sensitization by peripheral quantitative computed tomography (pQCT; Model XCT-960, Norland Stratec, Prorzhem, Germany). Under the anesthesia just described, the animals were restrained so that the bone axis of the proximal metaphysis was vertical to the scan beam. Voxel size and slice thickness were set to 0.295 mm and 1 mm, respectively. The metaphysis was identified and BMD was measured. The analysis parameters were set to contmode 2, peelmode 2 and cortmode 1, and the measurement parameters were set to bone area (square millimeters), bone mineral content, and BMD (milligrams per cubic centimeter) for total bone (total BMD), trabecular bone (trabecular BMD) and cortical bone (intra-cortical BMD). The threshold value was set to 0.65 for cortical bone. The coefficient of variation for the measurement of BMD by pQCT was 1.7% without repositioning and 4.6% with repositioning. The BMD value at the time of initial sensitization (week 0) was regarded as 100 (baseline), and the BMD values at 4 and 8 weeks after sensitization in each group were presented as the percentage to the value at week 0.

### Statistical analysis

All data values are presented as the mean and SE. Dunn's test was used for comparisons among multiple groups, and Dunnet's test was used for comparisons between the sham and other groups. The effects of neurectomy and arthritis on BMD were analyzed by the two-way analysis of variance. All statistical analyses were performed using Stat View J-5.0 (SAS Institute, Cary, NC) on Windows. A significant level of $P < 0.05$ was used for all comparisons.

## Results

### Body weight and gastrocnemius muscle weight

The mean body weight decreased gradually in the CIA, NTx and CIA + NTx groups. After the initial sensitization, the body weight loss was significant at 6 and 8 weeks in the CIA group, and at 8 weeks in the NTx and CIA + NTx groups in comparison with the sham group (Fig. 1A). The weight of gastrocnemius muscle at 8 weeks after the initial sensitization was significantly lower in the CIA, NTx and CIA + NTx groups than in the sham group (Fig. 1B), and was also significantly lower in the NTx and CIA+NTx groups than in the CIA group.

### Changes in hind paw swelling

The incidence of arthritis was 100% in both the CIA group and CIA + NTx group. Significant increase of swelling of the hind paws was noted in the CIA groups at 4, 6 and 8 weeks after the initial sensitization compared with the sham and NTx groups. In the CIA + NTx group, the swell-

ing was significantly higher than in the sham and NTx groups, and was significantly lower than in the CIA group at 6 and 8 weeks (Fig. 2A).

### Changes in the radiologic arthritis score of the hind paw

The radiologic arthritis score of the right hind paws was significantly higher in the CIA than in the sham and NTx groups at 4, 6 and 8 weeks after the initial sensitization. In the CIA + NTx group, the score was significantly higher than in the sham and NTx groups at 4, 6 and 8 weeks, and was lower than in the CIA group at 6 and

8 weeks after the initial sensitization (Fig. 2B). Typical X-ray photographs of the ankle and subtalar joints in CIA and CIA + NTx groups (Fig. 3) showed that arthritis was less severe in the CIA + NTx group (Fig. 3B) than in the CIA group (Fig. 3A).

### Changes in the BMD

The total BMD in the CIA, NTx and CIA + NTx groups was significantly lower in the NTx and CIA + NTx groups at 4 and 8 weeks, and in the CIA group at 8 weeks after the initial sensitization than in the sham group. However, there was no significant difference among the CIA, NTx and CIA + NTx groups (Fig. 4A). The cancellous
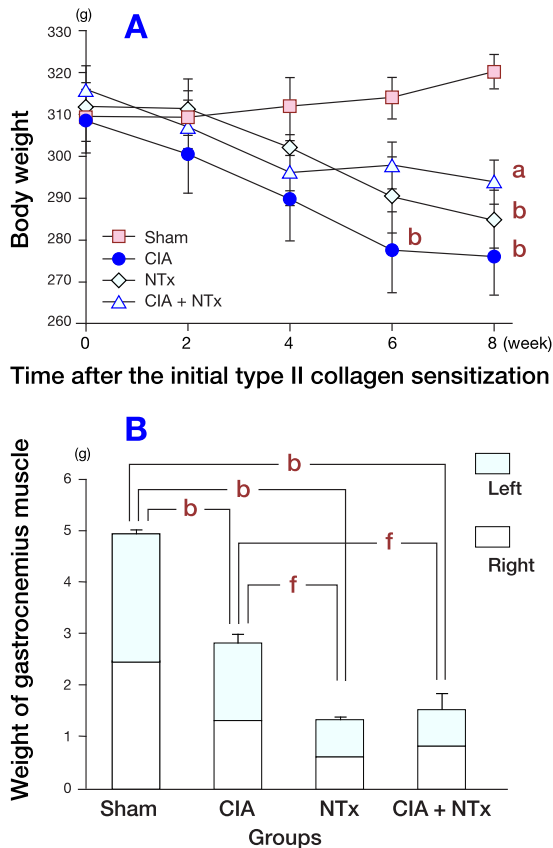


**Fig. 1.** Changes in body weight of rats in each group (**A**) and weight of the gastrocunemius muscle at 8 weeks after the initial sensitization (**B**). [a]$P < 0.05$ and [b]$P < 0.01$ compared with sham rats (Dunnett's test). [f]$P < 0.01$ compared with CIA rats (Dunn's test). Values expressed as mean ± SE. CIA, collagen sensitization; NTx, sciatic neurectomy.
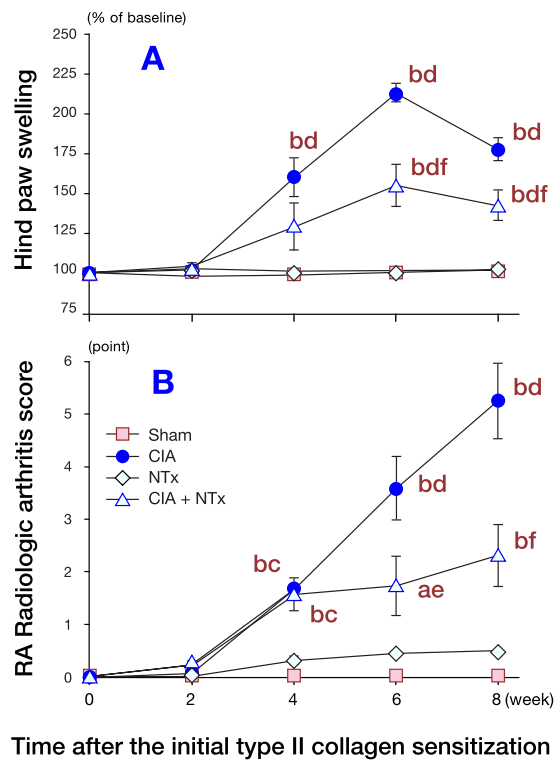
**Fig. 2.** Changes in hind paw swelling (**A**) and radiological arthritis score (**B**) in rats of each group. [a]$P < 0.05$ and [b]$P < 0.01$ compared with sham rats (Dunnett's test). [c]$P < 0.05$ and [d]$P < 0.01$ compared with NTx rats (Dunn's test). [e]$P < 0.05$ and [f]$P < 0.01$ compared with CIA rats (Dunn's test). Values expressed as mean ± SE. CIA, collagen sensitization; NTx , sciatic neurectomy.

**Fig. 3.** Lateral hind paw radiographs of a rat of the CIA group (**A**) and another of the CIA + NTx group (**B**) at 6 weeks after the initial sensitization. Proliferation of radioresistant tissue and bone destruction in the ankle and subtalar joints (arrows) is less prominent in the CIA+ NTx group than the CIA group. CIA, collagen sensitization; NTx, sciatic neurectomy.



**Fig. 4.** Changes in total BMD (**A**), cancellous BMD (**B**) and cortical BMD (**C**) of tibial metaphysis. [a]$P < 0.05$ and [b]$P < 0.01$ compared with sham rats (Dunnett's test). [e]$P < 0.05$ compared with CIA rats (Dunn's test). Values expressed as mean ± SE. BMD, bone mineral density; CIA, collagen sensitization; NTx, sciatic neurectomy.

BMD was significantly lower in the CIA, NTx and CIA + NTx groups than in the sham group at 4 and 8 weeks after the initial sensitization, but there was no significant difference among the NTx, CIA + NTx and CIA groups (Fig. 4B). The cortical BMD gradually increased in the sham group but gradually decreased in the NTx and CIA + NTx groups. It was significantly lower in the NTx and CIA + NTx groups than in the sham group at 4 and 8 weeks, and in the CIA+NTx group than in the CIA group at 4 weeks after the initial sensitization (Fig. 4C).
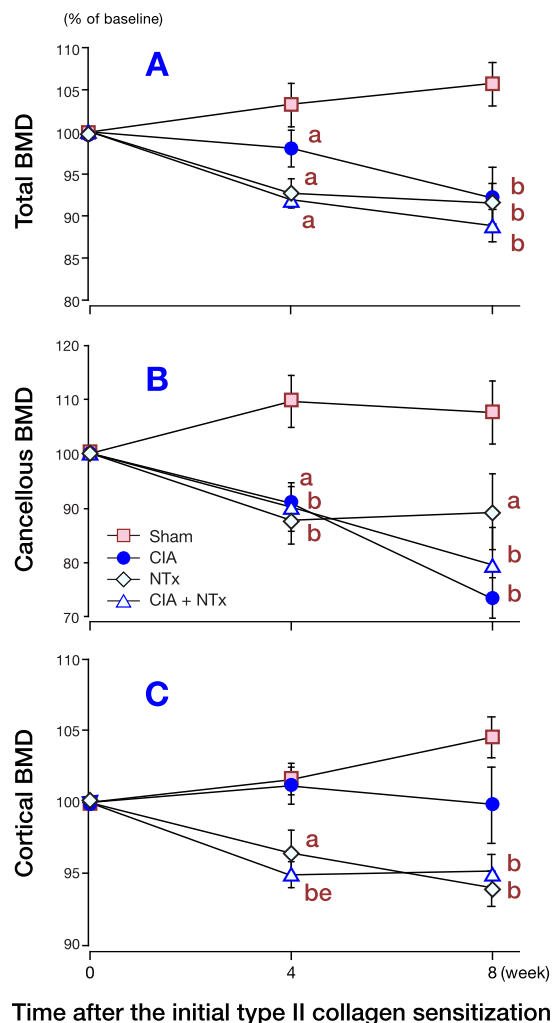
## Discussion

Several studies have been reported on arthritis and periarticular bone loss in animal models of RA (Hanyu et al., 1999; Enokida et al., 2001; Hoshino et al., 2001; Yamasaki et al., 2001), but few on arthritis and paralysis. It has been shown that sciatic neurectomy causes bone loss in the lower limbs of the animal models (Okumura et al., 1987; Murakami et al., 1994; Iwamoto et al.,

2003). Okumura et al. (1987) have reported a reduction in the BMD of ovariectomized rats, which deteriorated after sciatic neurectomy. We assumed in the present study that periarticular bone loss deteriorated in CIA + NTx rats as well. To evaluate this hypothesis, we examined the effects of sciatic neurectomy on arthritis and the BMD in CIA and CIA+NTx rats.

Kozina et al. (1977) have reported the effects of peripheral nerve and spinal lesions on arthritis in adjuvant arthritis rats. They found that peripheral nerve and spinal lesions suppressed hind paw swelling and suggested that the effect was due to hind paw paralysis. In our study, sciatic neurectomy reduced hind paw swelling and ankle and subtalar joint destruction in CIA rats. Many reports have described that paralysis suppressed RA, osteoarthritis and arthritis and radiological joint destruction caused by gout in humans (Coste et al., 1935; Bland et al., 1968; Pattrick et al., 1984). Bland et al. (1968) and Pattrick et al. (1984) stated that the restriction of movement due to paralysis may reduce mechanical stress, resulting in the suppression of arthritis. In many cases of osteoarthritis and RA, arthritis is severer in the dominant hands than in the non-dominant hands, suggesting that exercise stress has a significant effect on the severity of arthritis.

The occurrence of periarticular bone loss in arthritis is suggested to be related to synovitis associated with arthritis (Suzuki et al., 1995) and bone marrow lesions in the peripheral bones (Fujimoto et al., 1992; Enokida et al., 2001). In immobilized tibia after sciatic neurectomy in mice, a temporary increase in bone resorption and a continuing decrease in bone formation caused rapid cancellous bone loss (Sakai A et al., 1996). We assumed that immobilization due to sciatic neurectomy might deteriorate cancellous bone loss in the CIA+NTx group; however, there was no significant difference in the degree of cancellous bone loss between the CIA and the CIA+NTx groups. The reason for this may be that arthritis was suppressed by sciatic neurectomy in the CIA+NTx group, which reduced the degree of synovitis due to arthritis.

Enokida et al. (2001) have reported that the decreased proximal tibial cortical BMD in adult CIA rats might be related to changes of the periosteal bone surface and the intracortical bone. Hayashida et al. (1993) have histologically examined the cortical bone adjacent to the affected joint in adjuvant arthritis rats, and reported that both the bone resorption and inflammatory cell infiltration were increased around the intracortical blood vessels, which was similar to histological findings of juxtaarticular osteoporosis in patients with RA. The immobilization-related decrease in the cortical BMD is considered to be associated with a disorder of periosteal modeling-dependent bone gain and an enhancement of endocortical and intracortical bone remodeling-dependent bone loss (Li et al., 1991; Jee et al., 1992; Maeda et al., 1993). Yonezu et al. (2004) have reported that sciatic neurectomy decreased mechanical stress on the bone around paralyzed muscles and reduced periosteal bone formation, thus causing the decrease in the cortical BMD. Since the cortical bone loss mechanism in CIA rats may be different from that linked to immobilization, it is speculated that the cortical bone loss in the CIA+NTx group was greater than in the CIA group.

In summary, the present study revealed that sciatic neurectomy reduced the severity of arthritis, but did not affect the cancellous bone loss in adult CIA rats. It is speculated that limitations of movement due to paralysis reduces exercise stress, which thus ultimately suppresses arthritis.

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