Dexamethasone Prevents the Decrease of Bone Mineral Density in Type II Collagen-Induced Rat Arthritis Model

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ABSTRACT—This study demonstrated the decrease of bone mineral density (BMD) in the type II collagen (CII)-induced arthritis (CIA) model in rats and the relationship between BMD and paw edema and the effect of dexamethasone-21-phosphate (DEX). The paw swelling occurred on Day 10 and reached its peak on Day 18 after CII injection. BMD in the CII-injected group is lower than that in the control group. BMD in the proximal and distal regions of the femur largely decreased in comparison with that of the middle region. The oral administration of DEX (0.1 mg/kg) inhibited the swelling and decrease of BMD in all three regions of the femur.

Keywords: Collagen-induced arthritis, Bone mineral density, Dexamethasone

The rat adjuvant-induced arthritis (AIA) model and type II collagen (CII)-induced arthritis (CIA) model are commonly used as animal models of rheumatoid arthritis (RA). In particular, the CIA model has been widely used for the pharmacological evaluation of anti-rheumatic drugs (1). This model is useful for the study of rheumatoid arthritis because it has some of the clinical characteristics of RA; e.g., proliferation of synovial tissue, formation of subcutaneous nodules, and the destruction in cartilage and bone. Furthermore, anti-CII antibodies detected in the CIA model were also a common clinical feature of RA (2). Although much is known about the pathology of bone damage in this model, quantitative evaluation of bone damage is poorly defined. Earlier studies using the CIA model for the investigation of arthritic bone erosion are limited to histological and/or x-ray evaluation. These reports, however, presented little information on the quantitative changes in bone mineral density (BMD).

Joint destruction and osteoporosis are frequently found in RA patients. While most anti-rheumatic drugs can suppress inflammation and pain, very few drugs could inhibit the bone and cartilage erosion. In fact, long term treatment with some anti-rheumatic drugs have been shown to decrease BMD in RA patients (3). It is expected that a new generation of anti-rheumatic drugs would suppress the bone and cartilage erosion in addition to inhibition of inflammation. Therefore, it is important to study the changes in BMD in animal models for the development of new anti-rheumatic drugs.

The aim of this study is to investigate the changes in BMD during the development of CIA and the effect of dexamethasone-21-phosphate (DEX) on BMD using the non-invasive dual energy x-ray absorptiometry (DXA) technique for yielding useful information for the development of anti-rheumatic drugs.

Female Lewis rats (Charles River Japan, Kanagawa), 7-week-old at the time of experiment, were used. CIA was induced as follows: the CII and DEX groups were injected with an emulsion of Freund's incomplete adjuvant (Difco Laboratories, Detroit, MI, USA) and bovine CII solution (K-41; Koken, Tokyo) at 1.5 mg bovine CII per 1 ml of emulsion. The emulsion was administered to the back of rats by subcutaneous injection. The non-treated group was injected with an emulsion that contained 0.05 M acetic acid instead of the CIA solution. The severity of the edema was monitored by measuring changes in the volume of the two hind paws with a plethysmometer (TK-105; Unicom, Inc., Chiba). The paw volume measurements were made in triplicate for each of the rats, and the mean value was used. The edema was defined as the increase in paw volume on the day of experiment compared to Day 0. DEX (Sigma Chemical Co., St. Louis, MO, USA) was administered 0.1 mg/kg/day (oral administration) once a day during Day 0 to Day 23. BMD was measured by the bone densitometer (DSC-600; Aloka
Co., Ltd., Tokyo) using a procedure developed in our laboratory (4). Data were analyzed by the previously described method (5).

For the measurement of the plasma concentration (calcium, phosphorus, alkaline phosphatase, acid phosphatase), experiments were done using the same protocol for 21 days. The changes of BMD and paw volume were similar to those in the first experiment. The plasma parameters were measured by an automatic analyzer (model 7070; Hitachi, Tokyo) and prostaglandin (PG) E₂ was measured by an EIA kit (PerSeptive Biosystems, Boston, MA, USA) on Day 21.

All results were expressed as the mean ± standard error of the mean (S.E.M.). All data were evaluated statistically by ANOVA, and the statistical significance of difference was assessed by the unpaired Tukey-Kramer method; P values < 0.05 were considered significant.

Figure 1 shows the changes of paw volume. In the CII group, all animals showed inflammation on Day 10 as indicated by the increase in paw volume. On Day 18, the increase of paw volume peaked at 70% above the age-matched control and persisted until the end of the experiment. Both feet showed similar changes in paw volume. The administration of DEX inhibited the edema. The non-treated rats did not show any edema.

Figure 2 shows the changes of BMD in the femur. In proximal, middle and distal of femur of non-treated rats increased in a time-dependent manner. On the other hand, in the CII-injected group, the increase of BMD in all regions was suppressed. The BMD in the distal region decreased after Day 14, and the BMD on Day 24 was lower compared with that of Day 0. After Day 21, all three regions of the femur in the CII group showed significant decreases (P < 0.01) of BMD as compared with the non-treated group. The oral administration of DEX prevented from the decrease of BMD and those BMD values were as same as those of the non-treated group at all time points. The time course of the changes of BMD correlated with the suppression of paw edema.

Figure 3 shows the histological changes in the tibia-talus joint from collagen injected animals and the effect of DEX administration. In the CII group, signs of inflammation such as the pannus formations and bone erosion were clearly observed. In particular, erosion and fragmentation of the trabecular bone were visible. The administration of DEX prevented these changes.

The plasma concentrations of alkaline phosphatase, acid phosphatase and calcium were not significantly different among the 3 groups (data not shown) on Day 21. A significant increase (P < 0.05) of plasma phosphorus was shown in the CII group, and the administration of DEX prevented this increase (Control group, 23.8 ± 0.74; CII group, 26.4 ± 0.24; DEX group, 23.8 ± 0.65 IU/dl; n = 5). There were no significant changes of plasma PGE₂ concentration in the 3 groups (Control group, 7.01 ± 1.19; CII group, 7.57 ± 1.42; DEX group, 5.81 ± 0.82 pg/ml; n = 5).

In the present study using the CIA model, we showed the significant decreases in BMD in the distal, middle and proximal regions of the femur. Decreases in BMD in these three regions of the femur were statistically significant. The magnitude of the decrease differed from each other in these regions: the decrease rate in BMD in the middle region was smaller than those of the ends of the femur.

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**Fig. 1.** Induction of paw edema and effect of dexamethasone treatment (0.1 mg/kg) on type II collagen-induced arthritic rats (n = 6). Non-treated (○), CII (■) and DEX (▲). **P < 0.01:** significantly different from CII group.
The decrease in BMD in the middle region of long bone was already reported in RA patients (6), and we also observed such a decrease in adjuvant arthritic rat (4). One possible reason for the decrease in BMD in the middle region of long bone may be because this region has a bone structure different from those of both end regions. The middle region of the femur is composed of cortical bone that usually showed a slower rate of decrease in BMD than the trabecular bone found at the joint region (7). These findings indicate that the decrease in BMD in the CIA model may be related to the difference in bone structure (e.g., trabecular vs cortical bone).

In the CII group, substantial bone erosion and synovial

Fig. 2. Time course of bone mineral density change in the proximal (A), middle (B) and distal (C) regions of femur in type II collagen-induced arthritic rats (n=6). Non-treated (○), CII (●) and DEX (■). **P<0.01: significantly different from CII group.

Fig. 3. Histological changes in the rat tibia-talus joint on Day 24 (Hematoxylin and Eosin staining, 50×). A: Normal; B: CII-injected, pannus formation (♦) and fragmentation of trabecular bone (▼); C: the treatment of DEX.
hyperplasia were observed in the tibia-talus joint. In particular, the talus or tibia bone loss with the formation of pannus was shown very clear in this region. Similar findings were reported by Imazumi et al. (8) who showed that three were more severe bone damages in the ankle joint than the knee joint in the mouse CIA model.

BMD is physiologically regulated by calcitropic factors such as parathyroid hormone, calcitonin or vitamin D. It, however, has already been reported that generalized bone loss associated with inflammation occurred independent of regulation by these factors in rats (9). This implies that the production of local factors caused by inflammation plays an important role in the decrease in BMD. Since CIA is a model of systemic autoimmune disease, changes in cytokine levels could also be an important factor to the development of inflammatory response. We have already reported that blood interleukin (IL)-6 level was elevated in this model (10). This elevation might be derived from an overproduction of IL-6 in the arthritic joints. The concentration of PGE₂ was increased slightly, so part of the BMD decrease was caused by these factors, because the inflammatory cytokines such as IL-1, IL-6, tumor necrosis factor (TNF)-α and PGE₂ are potent inducers of bone resorption (11, 12). The overproduction of these factors can cause the decrease of BMD by increasing bone resorption in this model. On the other hand, Tanaka et al. showed that the plasma level of osteocalcin, an index of bone formation, decreased in CIA (13). Thus, it is possible that the changes of BMD in this model are caused by the disproportion of the bone resorption and the bone formation.

In this model, the significant decrease in BMD was observed on Day 21 after the initiation of foot swelling (Day 10). The foot swelling is a result of edema and is an indication of the inflammatory response associated with CIA injection (1). This suggests that the decrease in BMD could be a result of the inflammatory response caused by CIA injection. This relationship is further supported by the observation in the DEX group. Treatment of the CIA group with DEX suppressed the development of edema and also inhibited the decrease in BMD with a close association between these two events. We already reported that DEX suppressed activated nuclear factor-κB (NF-κB) expression and synovial hyperplasia in vivo (14), and we confirmed these points in this model (data not shown). Various proinflammatory cytokines are also shown to be regulated by NF-κB (15). Therefore, inhibition of cytokine overproduction could be responsible for both the suppression of foot swelling and inhibition of the decrease in BMD. In addition, it is possible that the changes of body weight and the immobilization due to CIA could also aggravate the decrease in BMD.

In conclusion, the measurement of BMD changes in the CIA model could yield useful information that gives insight into the development of potential anti-rheumatic drugs.

REFERENCES