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Effect of a cathepsin K inhibitor on arthritis and bone mineral density in ovariectomized rats with collagen-induced arthritis

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ABSTRACT

Objectives: Cathepsin K is expressed by osteoclasts and synovial fibroblasts and degrades key components of bone and cartilage. Inhibition of cathepsin K protease activity may be beneficial for the prevention of bone erosion and cartilage degradation in rheumatoid arthritis (RA). The collagen-induced arthritis (CIA) rat model is well established for studying the pathology and treatment of RA. We investigated the effect of ONO-KK1-300-01, a cathepsin K inhibitor (CKI), on arthritis and bone mineral density (BMD) in rats with CIA.

Methods: Seven-month-old female Sprague Dawley rats were divided into 5 groups: rats without CIA (CNT); CIA rats that underwent ovariectomy (OVX) and were treated with CKI; CIA rats that underwent OVX and were treated with vehicle (Veh); CIA rats that underwent sham surgery and were treated with CKI; and CIA rats that underwent sham surgery and were treated with Veh. CKI was orally administered at a dose of 15 mg/kg, thus initiating collagen sensitization, until death at 4 weeks. We evaluated hind paw thickness and the arthritis score every week until death. Radiographs of the resected left foot were obtained with a soft X-ray apparatus. Destruction of bone and cartilage was classified and scored as previously described by Engelhardt et al. BMD was measured by bone densitometry at the halfway point between the distal metaphysis and the diaphysis of the resected right femur. We also performed histomorphometry of the proximal left tibia, histological evaluation of arthritis, and a bone strength test.

Results: CKI administration significantly reduced hind paw thickness and the arthritis score, and prevented a decrease in BMD. The radiographic score was significantly lower in the CKI group than in the Veh group. In the histomorphometric analysis, bone-resorption parameters were significantly lower in the CKI groups than in the Veh groups. CKI significantly inhibited synovial proliferation in the CIA rats. In the bone strength test, the ultimate stress was significantly higher in the CKI groups than in the Veh groups.

Conclusion: Our findings indicate that cathepsin K inhibitors may inhibit systemic and local bone loss, ameliorate arthritis, and attenuate the decrease of bone strength in an animal model of arthritis.

1. Introduction

Cathepsin K, which is expressed by osteoclasts and synovial fibroblasts, degrades key components of bone and cartilage, such as type I and type II collagen, osteonectin, and aggrecan (Salminen-Mankonen et al., 2007). Since cathepsin K inhibitors (CKIs) selectively inhibit bone resorption with a minor effect on bone formation (Ochi et al., 2011), CKIs have been used to treat osteoporosis in previous studies. Cathepsin K is highly expressed in synovial fibroblasts and macrophages in rheumatoid arthritic joints (Hou et al., 2001; Hummel et al., 1998). A positive correlation has been observed between the extent of radiological destruction and serum levels of cathepsin K (Skoumal et al., 2005). Inhibition of cathepsin K protease activity may be beneficial for the prevention of bone erosion and cartilage degradation in rheumatoid arthritis (RA) (Salminen-Mankonen et al., 2007; Weidauer et al., 2007; Yasuda et al., 2005).

Osteoporosis is often a complication of RA, resulting in an increased risk of fracture. Furthermore, osteoporosis is exacerbated by estrogen deficiency (Saville and Kharmosh, 1967; Teshima et al., 1987; Reid et al., 1982). In our previous studies, we evaluated the effects of estrogen replacement therapy on arthritis severity and bone mineral density (BMD) in ovariectomized rats with collagen-induced arthritis

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Table 1
Arthritis scores, radiographic scores, and bone mineral density.

	CIA			
	OVX		Sham	
	CKI	Vehicle	CKI	Vehicle
Incidence of arthritis (%)				
3 weeks	45.5	63.6	54.5	63.6
4 weeks	90.9	72.7	90.9	81.8
Arthritis score (points)[a]				
3 weeks*	2.0 (0.5, 3.5)	4.5 (3.0, 6.0)	1.5 (0, 3.0)	4.0 (1.5, 4.75)
4 weeks*	4.5 (3.0, 6.5)[b]	7.0 (5.0, 8.0)[b]	4.0 (2.0, 4.0)[c]	6.0 (5.25, 6.75)[c]
Radiographic score (points)[a]				
4 weeks*	3.88 (3.25, 4.0)	5.63 (4.25, 6.25)	2.63 (2.375, 2.875)[d]	4.50 (3.0, 6.25)[d]
BMD (g/cm^2)	0.155 ± 0.016	0.148 ± 0.014	0.169 ± 0.019[e]	0.148 ± 0.006[e]

CIA = collagen-induced arthritis, OVX = ovariectomy, CKI = cathepsin K inhibitor, BMD = bone mineral density.
BMD was measured at the halfway point of the distal resected right femur. Values of BMD represent means ± SD. BMD was significantly higher in the CKI groups (2-way ANOVA, $p = 0.006$). Post hoc analyses with Fisher's PLSD test were performed among the 4 groups.
* $p < 0.05$ (by Kruskal-Wallis test).
[a] Data are represented as follows: median (75th percentile, 25th percentile).
[b] $p < 0.05$ (CIA + OVX + CKI vs. CIA + OVX + Vehicle by Scheffe's test).
[c] $p < 0.05$ (CIA + Sham + CKI vs. CIA + Sham + Vehicle by Scheffe's test).
[d] $p < 0.05$ (CIA + Sham + CKI vs. CIA + Sham + Vehicle by Scheffe's test).
[e] $p < 0.05$ (CIA + Sham + CKI vs. CIA + Sham + Vehicle).

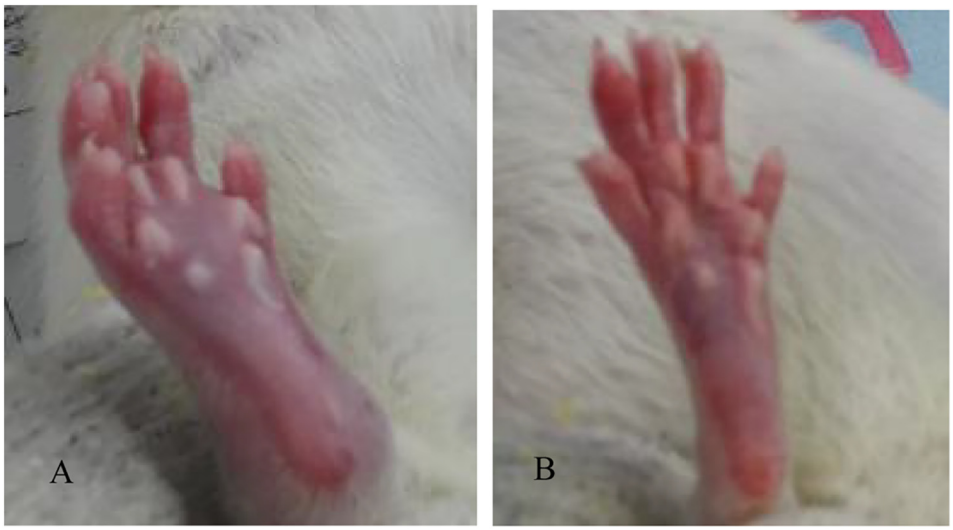

Fig. 1. Typical photographs of the hind paw.
A, swollen hind paw (CIA group). B, normal hind paw (Control group).
Hind paw thickness was evaluated by measuring the ankle width from the medial malleolus to the lateral malleolus using a constant-tension caliper.
CIA = collagen-induced arthritis.

(CIA), an established model for studying the pathology and treatment of RA (Fukata et al., 2004; Yamane et al., 2003; Yamasaki et al., 2001; Yoshioka et al., 2008). In these studies, OVX in CIA rats worsened arthritis severity and bone loss.

Two previous studies examined the effects of CKIs on arthritis, but both assessed only arthritis symptoms (Asagiri et al., 2008; Svelander et al., 2009). This is the first study to investigate the effect of a CKI not only on arthritis but also on BMD, bone histomorphometry, and bone strength. The aim of this study was to evaluate the effect of ONO-KK1-300-01, a CKI, on arthritis and BMD in CIA rats.

2. Materials and methods

2.1. Animals

Seven-month-old female Sprague-Dawley rats (retired breeder animals with a body weight of 278–410 g; Shimizu Laboratory Supplies, Kyoto, Japan) were used. This experiment was conducted at the animal research facilities of Tottori University, with approval by the Animal Experiment Ethical Committee of Tottori University. Animals were given tap water and solid food (calcium content 1.18 g/100 g, phosphorus content 1.09 g/100 g, vitamin D3 content 250 IU/100 g) (CE-2; CLEA Japan, Tokyo, Japan) ad libitum. Animals were maintained in an animal room, which was illuminated for 12 h daily (07:00–19:00), at a temperature of 24 °C. Animals were used in experiments after a 4-week acclimation period.

Animals were divided into the following 5 groups, with mean body weight equalized across groups during randomization: injection of saline only + vehicle administration (CNT; n = 11); collagen sensitization + ovariectomy (OVX) + CKI (CIA + OVX + CKI; n = 11); collagen sensitization + OVX + vehicle administration (CIA + OVX + Veh; n = 11); collagen sensitization + sham surgery + CKI administration