EFFECTS OF ALENDRONATE ON BONE MINERAL DENSITY AND NOCICEPTIVE PAIN IN CHRONIC PAIN MODEL RATS

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Abstract

Objective : Alendronate (ALN) is used to treat postmenopausal osteoporosis, and it is also considered to have analgesic effect. However, the effects of ALN on hyperalgesia in the postmenopausal osteoporosis and chronic pain model are still unclear. In this study, the effects of ALN on nociceptive pain, as well as bone mineral density (BMD), were evaluated by a chronic constriction injury (CCI) model in ovariectomized (OVX) rats.

Methods : Four-week-old, Wistar rats underwent OVX, and then the left sciatic nerve was ligated to make a CCI, at 8 weeks of age. ALN (0.15 mg/kg/week) or its vehicle was administered for 2 weeks starting on the 0^{th} day of CCI. Behavioral evaluations with the von Frey test and the hot plate test were performed on days 0 (8 weeks old) and 14 (10 weeks old). After evaluation, bilateral femora were harvested for BMD measurement.

Results : Two weeks of treatment with ALN showed no significant improvement of mechanical and thermal hypersensitivity in CCI limbs. ALN treatment significantly increased femoral BMD in the sham and CCI limbs compared with vehicle treatment ($p < 0.01$) at 10 weeks old.

Conclusion : Two weeks of treatment with ALN improved BMD in CCI limbs in OVX rats, but mechanical and thermal hypersensitivity were not improved by ALN treatment.

Key words : alendronate, bone mineral density, chronic pain model

Introduction

Ovarian hormones such as estrogen have been shown to alter nociceptive behaviors in a variety of animal models[1–3]. Clinically, postmenopausal osteoporotic patients sometimes complain of chronic pain, even without fragility fractures[4]. If these patients' activities are disturbed by the chronic pain, further decreases in bone mineral density (BMD) or body weight can occur, and lower BMD or body mass index (BMI) causes an additional risk of further fragility fractures[5,6]. Thus, alleviation of chronic pain and an increase in BMD of postmenopausal osteoporotic patients should be very important goals of treatment.

Alendronate (ALN), which is a second-generation bisphosphonate, is one of the candidate drugs to achieve the goals of treatment for postmenopausal osteoporosis. ALN increases BMD of osteoporotic patients by suppressing stimulated bone resorption[7,8]. Several previous studies have also shown that ALN has a kind of analgesic effect[4,9] in addition to its actions affecting bone resorption and BMD. However, the details of the effects of ALN on nociceptive pain and its relationships to BMD

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or body weight are still unclear.

To evaluate the analgesic effects of ALN, we have used a chronic constriction injury (CCI) model of the sciatic nerve as a chronic pain model[10] in ovariectomized (OVX) rats. A CCI of the sciatic nerve is used to induce neuropathic pain. The purpose of the present study was to evaluate the analgesic effects of ALN on nociceptive pain and thermal pain and the effects of ALN on BMD of the CCI limb in OVX rats.

Materials and Methods

Animals

Four-week-old, female Wistar rats (Japan SLC, Shizuoka, Japan) were housed in a controlled environment (temperature 23 ± 2°C, humidity 40% ± 20%) with a 12-h light/dark cycle. The rats were allowed ad libitum access to tap water and standard food (CE-2 ; Clea Japan, Tokyo, Japan) containing 1.14% calcium, 1.06% phosphorus, and 250 IU vitamin D3 per 100 g.

Experimental Design

General anesthesia was induced by intraperitoneal injection of xylazine hydrochloride (Sederac ; Nippon Zenyaku Kogyo, Fukushima, Japan) and ketamine hydrochloride (Ketalar ; Daiichi Sankyo Propharma, Tokyo, Japan). Rats were bilateral OVX under general anesthesia at 4 weeks of age as a model of estrogen deficiency. Four weeks after OVX, a CCI was also produced under general anesthesia by ligating the sciatic nerve of the left hindlimb with 4-0 silk suture to create a chronic pain model[11]. On the right hindlimb, a sham operation, involving only exposure of the sciatic nerve without ligation, was performed to create a control limb for the chronic pain model.

Experimental protocol

After these procedures, the rats were divided into the following two groups : 1) an ALN group (n = 5), administered 0.15 mg/kg/week of ALN (Wako Chemical, Osaka, Japan) subcutaneously ; and a 2) Vehicle group (n = 5), administered 0.2 ml of saline solution subcutaneously. These treatments were continued for 2 weeks, and the following evaluations were performed on the day of CCI (8 weeks old) or 2 weeks after the CCI (10 weeks old). Behavioral evaluations were performed 6 hours after CCI under general anesthesia at the day 0. After 2 weeks of evaluations, the rats were sacrificed, and bilateral femora were harvested. All animal experiments were approved by the ''Guidelines for Animal Experiments'' of our institute (IACUC number : a-1-2513).

Body weight measurement

The rats' body weights were measured at the CCI (8 weeks old) and at sacrifice (10 weeks old) (Keimaiko ; Yamato-scale, Hyogo, Japan). Body weight was compared between the groups at each time point.

Behavioral Analyses

To evaluate the responses to nociceptive stimulation, the von Frey test and the hot plate test, as shown below, were performed on the day of CCI (8 weeks old) and at sacrifice, 2 weeks after CCI was performed (10 weeks old).

To assess sensitivity to a tactile stimulus causing mechanical hypersensitivity, hindlimb withdrawal in response to a tactile stimulus was measured using von Frey filaments (Aesthesio, DanMic Global, San Jose, CA, USA) with 0.16-g bending force. The test was performed and evaluated according to a previous report[12]. Briefly, the filament was applied to the plantar surface of the hindlimbs for 3 seconds, and this was repeated three times with an interval of more than 3 minutes between the measurements. Each of the hindlimbs was tested individually. Hindlimb withdrawal in response to a stimulus was evaluated by scoring as follows : 0, no response ; 1, a slow and/or slight response to the stimulus ; 2, a quick withdrawal response away from the stimulus without flinching or licking ; and 3, an intense response to the stimulus with brisk flinching and/or licking. The hindlimb was assessed alternating between right and left sides with an interval of greater than 3 minutes between the measurements. The test was carried out 3 times for each limb, and the mean was calculated as the pain score.

To quantify the sensitivity to thermal stimulation leading to thermal hypersensitivity, each of the hindlimbs of the rats was tested individually using a hot plate

technique[13]. The rat under observation was placed on a smooth metal surface kept at 52°C. The hind-limb withdrawal latency (HWL), which was the time it took for the rat to withdraw the hindlimb from the plate, was measured. The examination was carried out 3 times, and the mean was calculated as the HWL.

BMD measurement

BMD of the entire excised femur was measured by dual-energy X-ray absorptiometry (DXA, Hologic QDR-4500 ; Hologic, Methuen, MA, USA) in the anterior plane. Bones were scanned in the "small animal" scan mode, with the "regional high-resolution" scan option. Total femoral BMD was measured.

Statistical analyses

All numerical values are expressed as means ± standard deviation (SD). Statistical analysis was performed using the unpaired *t*-test to compare the differences between the ALN and Vehicle groups, and the paired *t*-test was used to compare the differences between the CCI limb and the sham limb or between 0 and 2 weeks after treatment. Values of $p < 0.05$ were considered significant.

Results

Body weight (Table 1 and Figure 1)

The body weight (mean ± SD, g) was significantly higher at 10 weeks old (275 ± 17 and 280 ± 20, respectively) than at 8 weeks old (186 ± 8 and 180 ± 6, respectively) in both the ALN and vehicle groups (both $p < 0.01$), but there were no significant differences between the groups at the two time points.

Table 1. Body weight (g)

	ALN ($n = 5$)	Vehicle ($n = 5$)
8 weeks old	186 ± 8	180 ± 6
10 weeks old	275 ± 17*	280 ± 20*

Values are means ± SD. * : $p < 0.01$ vs. at 8 weeks old by paired *t*-test.
SD : standard deviation, ALN : alendronate

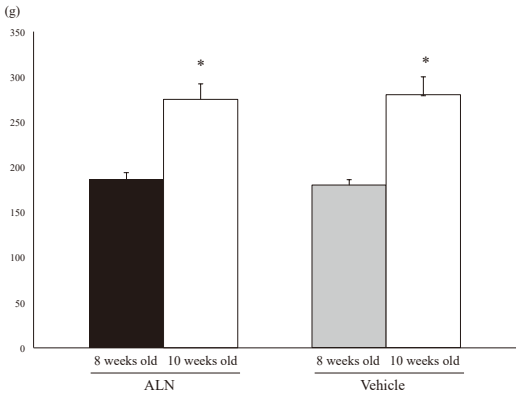

Figure 1. Body weight (g)
Values are means ± SD. * : $p < 0.01$ vs. at 8 weeks old by paired *t*-test. SD : standard deviation, ALN : alendronate

Behavioral Analyses (Table 2, Figure 2A and 2B)

The pain scores of the CCI limbs tended to be higher than those of the sham limbs at 8 weeks old and at 10

Table 2. Behavioral analyses

		ALN ($n = 5$)		Vehicle ($n = 5$)	
		Sham limb	CCI limb	Sham limb	CCI limb
Pain score	8 weeks old	0.4±0.5	0.9±0.8	0.3±0.4	0.6±0.4
	10 weeks old	0.1±0.3	0.3±0.4	0.3±0.4	0.5±1.3
HWL (seconds)	8 weeks old	31.0±3.5	58.6±29.0	29.3±9.0	91.0±13.6**
	10 weeks old	18.6±14.8	55.8±41.2	12.9±2.0*	80.0±22.3**

Values are means±SD. Pain score by the von Frey test and hindlimb withdrawal latency by the hot plate test.
* : $p < 0.01$ vs. 8 weeks old by the paired *t*-test. ** : $p < 0.01$ vs. sham limb in each group 8 weeks old and 10 weeks old by the paired *t*-test.
SD : standard deviation, HWL : hindlimb withdrawal latency, ALN : alendronate, CCI : chronic constriction injury

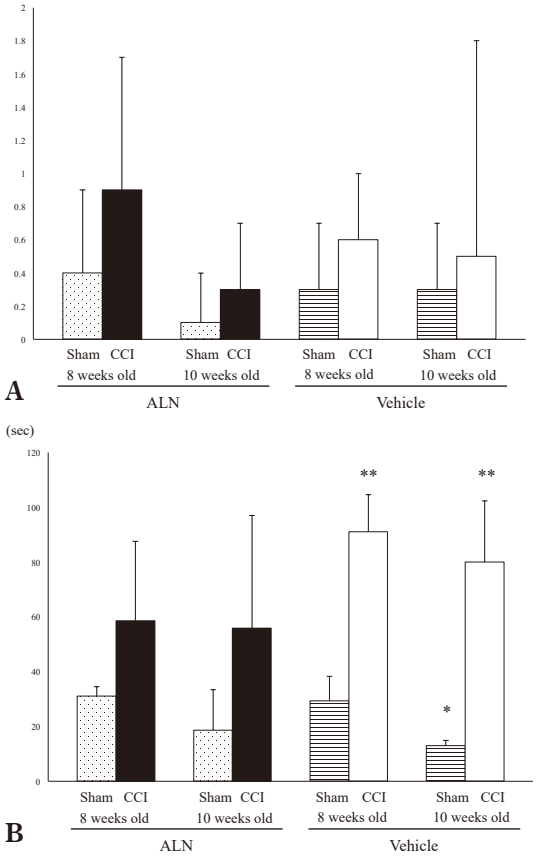

Figure 2. Behavioral analyses
A : Pain score by the von Frey test. Values are means ± SD. SD : standard deviation, ALN : alendronate, CCI : chronic constriction injury
B : Hindlimb withdrawal latency by the hot plate test. Values are means ± SD.
* : $p < 0.01$ vs. 8 weeks old by the paired t-test.
** : $p < 0.01$ vs. sham limb in each group 8 weeks old and 10 weeks old by the paired t-test.
SD : standard deviation, HWL : hindlimb withdrawal latency, ALN : alendronate, CCI : chronic constriction injury

weeks old, but there were no significant differences between the sham and CCI limbs. Two weeks of treatment with ALN showed no significant improvement of mechanical hypersensitivity in CCI limbs.

HWL (mean ± SD, seconds) of the CCI limbs in the Vehicle group (91.0 ± 13.6 at 8 weeks old and 80.0 ± 22.3 at 10 weeks old) was significantly longer than that of the sham limbs at each time point (29.3 ± 9.0 and 12.9 ± 2.0, respectively) ($p < 0.01$). In addition, HWL of the sham limbs was significantly shorter at 10 weeks old than at 8 weeks old only in the Vehicle group ($p < 0.01$).

BMD (Table 3 and Figure 3)

Total femoral BMD (mean ± SD, mg/cm^2) was significantly higher in the ALN group (0.240 ± 0.001 and 0.215 ± 0.007, respectively) than in the Vehicle group (0.203 ± 0.003 and 0.179 ± 0.005, respectively) both in the sham and CCI limbs ($p < 0.01$). BMD was significantly lower of CCI limbs than of sham limbs both in the ALN and the Vehicle groups ($p < 0.05$ and $p < 0.01$, respectively).

Table 3. Total femoral BMD (mg/cm^2)

	ALN ($n = 5$)	Vehicle ($n = 5$)
Sham limb	0.240±0.001[a]	0.203±0.003
CCI limb	0.215±0.007*[a]	0.179±0.005**

Values are means±SD.
* : $p < 0.05$, and ** : $p < 0.01$ vs. sham limb by the paired t-test. a : $p < 0.01$ vs. vehicle group by the unpaired t-test.
SD : standard deviation, ALN : alendronate, CCI : chronic constriction injury

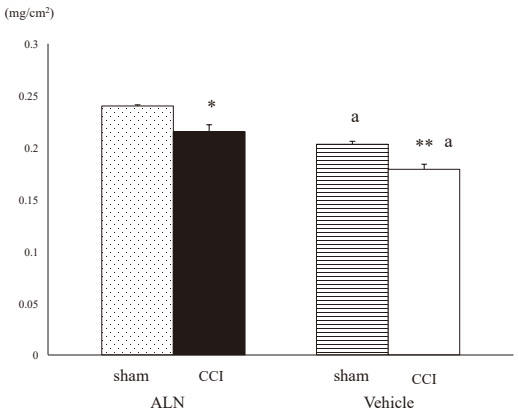

Figure 3. Total femoral BMD (mg/cm^2)
Values are means ± SD. * : $p < 0.05$, and ** : $p < 0.01$ vs. sham limb by the paired t-test. a : $p < 0.01$ vs. vehicle group by the unpaired t-test.
SD : standard deviation, ALN : alendronate, CCI : chronic constriction injury

Discussion

OVX is a well-known model of postmenopausal osteoporosis. On the other hand, tibial BMD of male rats showed no significant change with CCI in a previous study[14]. There are no previous reports of BMD changes by OVX and CCI. Total femoral BMD of the CCI limbs of OVX rats was significantly lower, and two weeks of treatment with ALN significantly recovered total femoral BMD of the CCI limbs in 2 weeks of 6-week OVX rats in the present study. However, the recovered BMD was still lower in the CCI limbs than in the sham limbs. Naruse *et al*. reported that, regardless of OVX, ALN was ineffective against the deterioration of cortical bone strength caused by inactivity (sitting), even though trabecular BMD was increased[15]. ALN treatment may require activity or recovery of damaged neurons to exert enough of an effect on decreased BMD or bone strength in the CCI limbs of OVX rats.

Several previous studies have demonstrated that OVX causes hypersensitivity to mechanical or thermal pain[16,17]. CCI also induced hyperalgesia in rats[18]. It is important to treat such hypersensitivity or hyperalgesia in patients with postmenopausal osteoporosis and inactivity or neural diseases. Previous studies have reported that ALN prevented ovariectomy-induced mechanical hyperalgesia in hindlimbs[17] and increased the pain threshold value in OVX animals[16]. Im *et al*. reported that ALN is effective for non-nociceptive symptoms in a rat model of CCI[14]. However, ALN treatment in the present study did not show significant improvement in the hypersensitivity to mechanical or thermal pain in the CCI limbs of OVX rats. OVX and CCI may cause further hypersensitivity to mechanical and thermal pain ; thus there were no significant effects of ALN on these nociceptive pains in the present study.

ALN treatment did not have significant effects on body weight during the experimental period in the present study. It was hypothesized that if ALN were to have some analgesic effect on nociceptive pain caused by a CCI, rats' activity or their appetite might change and result in a change in body weight.

In conclusion, two weeks of ALN treatment improved total femoral BMD in the CCI limbs of OVX rats, but it did not exert significant analgesic effects on the hypersensitivity to nociceptive and thermal pain caused by a CCI in OVX rats.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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