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Effective Dosage and Administration Schedule of Oral Alendronate for Non-nociceptive Symptoms in Rats with Chronic Constriction Injury

We evaluated the efficacy of oral alendronate with different dosing regimens for non-nociceptive symptoms and osteoporosis in a sciatic nerve chronic constriction injury (CCI) model. Male Sprague-Dawley rats (n=60) were subdivided into sham control (SC) group and CCI groups, which were divided according to dosage and time of oral alendronate administration: no treatment (NT), low dosage early (LE), high dosage early (HE), low dosage late (LL) and high dosage late (HL). We measured the thickness and temperature of the hind paw, bone mineral density (BMD) of the tibia, along with tibia bone strength. On the 14th day post-CCI, the HE group showed significant reduction in thickness and temperature (P<0.001). On the 42nd day post-CCI, the HE group showed significant reduction in temperature compared to the NT group (P<0.001). Also, both HE and HL groups showed statistically significant increased tibia BMD (P<0.001), along with increase of tibia bone strength compared to the NT group. Based on these findings, early alendronate in high dosages is effective in the non-nociceptive symptoms; early and late alendronate in high dosages, are effective in preventing bone dystrophic changes in a CCI model.

Key Words : *Chronic Constriction; Edema; Temperature; Alendronate; Bone Density*

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INTRODUCTION

Complex regional pain syndrome (CRPS) refers to a chronic pain condition associated with autonomic disturbances of vasomotor and sudomotor origin (1), along with trophic skin changes and patchy demineralization of the bones (2). Although the mechanism for CRPS has not been elucidated, studies indicate that it is a complex disorder involving both the central and peripheral nervous systems (3, 4). This complex etiology of CRPS is manifested by its heterogeneous constellation of clinical symptoms. In the acute stages, hallmarks include mechanical hyperalgesia, edema, increased sweating skin temperature, and hair growth (5). After some time, CRPS symptoms progress to a cold stage, with decrease of skin temperature, formation of skin atrophy and bony osteoporotic changes (6). CRPS pathogenesis is heterogeneous and complex, which makes its treatment challenging.

Pharmacological therapy for CRPS includes a wide array from anti-inflammatory drugs, systemic corticosteroids (7), antidepressants, opioids (8) to bisphosphonate agents (9-12). However, there is yet no single pharmacological agent or treatment algorithm that can resolve all of its heterogenic features. The efficacy for most pharmacological agents remains largely empirical, with the exception of bisphosphonate agents, which are the only agents with proven efficacy for CRPS based on multiple controlled trials (8-13).

Bisphosphonates are antiresorptive agents, which are used in osteoporosis and other bone conditions such as tumor-induced hypercalcemia. The use of bishphosphonate agents in CRPS are widely supported for their efficacy of pain, but some clinical studies have also suggested efficacy for edema (9) and joint mobility (10). However, despite the extensive researches on bisphosphonate agents, there are no definite guidelines that recommend the routine use of bisphosphonate for CRPS. This is due to a lack of specific standardized treatment regimens and scant clinical data on the optimum dosage, frequency and duration of treatment to cover the heterogenic features of CRPS. Previous animal studies have shown that alendronate, a bisphosphonate agent, showed analgesic effects only with high dosage supplementation (14, 15).

To determine on the optimal dosage and administration schedule of bisphophonate in CRPS, further studies are warranted on the effective administration schedule and dosages of bisphophonate that target various symptoms, including autono-

mic and bone dystrophic changes. Therefore, in order to determine a proper dosing regimen of bisphosphonate agents in CRPS, this study was carried out with the following aims; first, to determine the efficacy of oral alendronate, a bisphosphonate agent, on non-nociceptive symptoms, namely, on edema, skin temperature and bone dystrophic changes, using a Sprague-Dawley rat model of chronic constrictive injury (CCI) to represent CRPS, and second, to determine if these efficacies varied with different dosing regimens and time of administration.

MATERIALS AND METHODS

Animals

Sixty male Sprague-Dawley rats with a mean weight of 347 g were used in the present study. Animals were purchased from the Orient Bio Company (Seongnam, Korea). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Catholic University (Approval number CUMC-2009-0096-01). Rats were randomly divided into 6 groups: a sham control group (SC) and 5 CCI groups. CCI groups were divided according to dosage and time of bisphosphonate administration: no treatment (NT), low dosage early treatment (LE), high dosage early treatment (HE), low dosage late treatment (LL) and high dosage late treatment (HL) bisphosphonate groups. Each group consisted of 10 rats.

Induction of CCI

All rats were anesthetized by injecting 1% ketamine (30 mg/kg body weight) and xylazine hydrochloride (4 mg/kg body weight). The temperature during surgery was maintained at 37±1℃ CCI was induced according to the procedure previously described by Bennett and Xie (16). Sham-operated rats that served as controls (SC) underwent the same surgical procedure with sciatic nerve exposure, but without sciatic nerve ligation. Animals were allowed to recover from anesthesia and surgery. One animal per cage was housed with free access to water and standard laboratory chow.

Drug administration

Alendronate sodium (FOSAMAX®, Merck & Co. Inc., Whitehouse Station, NJ, USA) was prepared in powder form, dissolved in saline, and was administered into the oral cavity once a day via a tube. Alendronate sodium was administered at the same time every day. The 2 early treatment groups (HE, LE) were treated immediately after surgery for 6 weeks. Late treatment groups (HL, LL) were treated starting at the 14th day after CCI for 4 weeks. We chose the 14th day to categorize those into early (HE, LE) and late (HL, LL) groups based on results from Suyama et al. (17) who showed that bone mineral density (BMD) significantly decreased from the second week after CCI.

For the low dosage groups (LE, LL), 0.1 mg/kg/day was administered (18). For the high dosage groups (HE, HL), 1 mg/kg/day was administered along with sufficient water (19, 20). When the other groups received alendronate, we provided to the SC and NT groups, the same amount of saline via the same route. Similarly, we provided to the LL and HL groups the same amount of saline via the same method during the first 2 weeks after CCI, when these 2 groups were not eligible to receive alendronate administration. In order to reduce potential bias, we ensured that the examiner who provided alendronate or saline was blinded to the procedures performed and amount of alendronate administered to the rats.

Evaluation of vasomotor and sudomotor symptoms, measurement of dorsal-ventral thickness of the hind paw and temperature of the hind paw dorsum

To confirm the aptness of the CCI model and to examine the effects of alendronate therapy on CRPS-related symptoms, sudomotor function was evaluated by temperature measurement of the right hind paw dorsum using a digital infrared thermometer (OPTEX Co., Shiga, Japan) according to a method previously described (21). To evaluate vasomotor function, edema of the dorsal-ventral thickness of the right hind paw was measured using a manual mobile caliper. During the experiment, all measurements were performed 3 times: on the day prior to, the 14th and 42nd day after CCI.

Evaluation of bone metabolism; BMD and bone strength

Prior to CCI, 14th and 42nd day after-CCI, BMD measurements were performed on the right tibia for all 6 groups using Dual Energy radiography Absortiometry (Hologic Inc., Bedford, MA, USA), which was equipped with a program to evaluate BMD for rats. BMD measurement was carried out according to the methods recommended by previous studies (22, 23).

After the rats were sacrificed on the 42nd day after surgery, we removed the right tibia and measured bone strength for all 6 groups. The measurement used a 3-point system and the strength against physical force was measured using an Imperial™ 1000 (Mecmesin Limited, Slinford, UK) according to the methods used in other studies (23).

Statistical analysis

We presented our results in means±standard deviations. We evaluated all data using the Kruskal-Wallis test followed by Mann-Whitney test with Bonferroni's correction using SPSS 11.0 for Windows. All tests were two-tailed, and P values <0.05 were considered statistically significant.

RESULTS

All rats survived to the completion of the experiment, except for 1 case in the SC group, which died during the procedure. Data from a total of 59 rats were analyzed. All data for hind paw thicknesses, skin temperature, BMD and bone strength measured prior to, and on 14th, 42nd day post-CCI are presented in Table 1.

General observations

The CCI rats exhibited typical pain-related pain behaviors and these were similar in nature to those previously described by Bennett and Xie (16). Pain-related behaviors, such as, limping, abnormal hindpaw clawing, and guarding at the CCI side were observed in a consistent manner in all CCI groups. None of the SC rats showed these abnormal pain related behaviors, and these were consistent to findings reported from other studies (16, 17).

Dorsal-ventral thickness of right hind paws

Prior to CCI, no significant differences of hindpaw thickness were detected between groups (Fig. 1). Fourteen days after CCI, the thicknesses of the hind paws for 5 CCI groups were significantly thicker than that of the SC group. Thicknesses for the HE and SC group were significantly reduced than that of the NT group ($P<0.001$). Forty two days after CCI, only the SC group showed significantly reduced thickness than that of the NT group. Also, thicknesses of hind paws for the 2 low dosage treatment (LE and LL) groups and NT were significantly thicker than that of the SC group. The 2

Table 1. Hindpaw dorsal ventral thickness, skin temperature, and tibia BMD from Sprague-Dawley rats, measured prior to, and 14th and 42nd days after CCI. All data are presented as mean±standard deviation

Hindpaw dorsal ventral thickness (mm)				Skin Temperature (°C)			BMD tibia (g/cm²)		
CCI	Prior	Post 14th day	Post 42nd day	Prior	Post 14th day	Post 42nd day	Prior	Post 14th day	Post 42nd day
SC	3.6±0.2	3.8±0.2*	4.1±0.4*	33.1±3.0	28.7±1.0*	31.1±2.2*	0.079 ±0.004	0.088±0.006	0.091±0.004*
NT	3.5±0.2	4.9±0.3†	5.2±1.3†	33.8±2.0	32.0±1.4†	33.6±0.7†	0.083±0.003	0.084±0.003	0.078±0.006†
LE	3.5±0.1	5.9±1.3†	5.2±0.9†	33.3±1.0	34.0±1.8†	33.6±1.0†	0.082±0.006	0.082±0.008	0.089±0.007
HE	3.7±0.2	4.4±0.2*,†	4.9±1.0	34.1±1.6	29.8±1.1*	32.2±1.1*	0.078±0.008	0.085±0.006	0.097±0.012*
LL	3.6±0.1	4.9±0.3†	5.0±0.6†	34.1±0.7	34.5±0.6†	34.8±0.6†	0.084±0.003	0.083±0.004	0.078±0.008†
HL	3.6±0.2	5.2±0.2†	4.6±0.4	33.6±1.6	34.6±1.2†	31.9±1.8	0.081±0.004	0.087±0.005	0.089±0.011*

*$P<0.001$ as compared with the NT group; †$P<0.001$ as compared with the SC group.
BMD, bone mineral density; CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment.

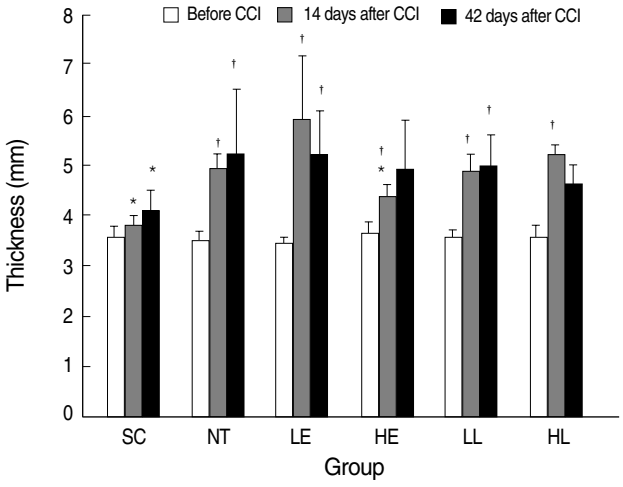

Fig. 1. Efficacy of oral alendronate in different dosage and time of administration in dorsal-ventral thicknesses of the affected hind-paw from Sprague-Dawley rats.
*$P<0.001$ as compared with NT group; †$P<0.001$ as compared with SC group.
CCI, chronic constriction injury; SC, sham control; NT, no treatment.

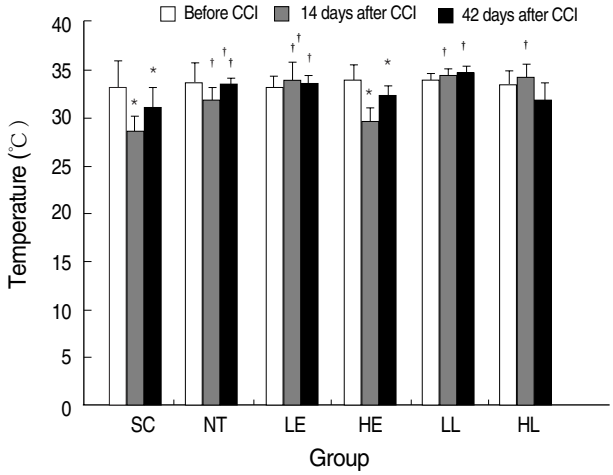

Fig. 2. Efficacy of oral alendronate in different dosage and time of administration in skin temperature of the affected hind-paw from Sprague-Dawley rats.
*$P<0.001$ as compared with NT group; †$P<0.001$ as compared with SC group.
CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment.

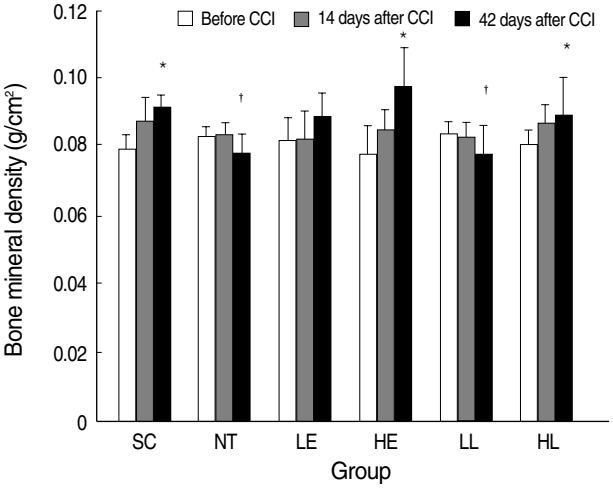

Fig. 3. Efficacy of oral alendronate in different dosage and time of administration in BMD of the affected tibia from Sprague-Dawley rats.
*P<0.001 as compared with the NT group; †P<0.001 as compared with the SC group.
BMD, bone mineral density; CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment.

high dosage groups (HE, HL) did not show statistically significant difference of thickness (Table 1) in comparison to NT group.

Skin temperature of right hind paws

Prior to CCI, no significant difference of right hindpaw skin temperature was detected between groups (Fig. 2). Fourteen days after CCI, the temperatures of all CCI groups, except the HE group, were significantly elevated than that of the SC group (P<0.001). Temperatures of HE and SC group were significantly reduced than that of the NT group (P<0.001). Similarly, forty two days after CCI, the temperatures of the HE and SC group were significantly reduced than that of the NT group (P<0.001); also the temperatures of the LE, LL and NT groups were significantly elevated versus the SC group (P<0.001) (Table 1).

Bone metabolism

Prior to, and 14 days after CCI, tibia BMD showed no significant differences between groups (Fig. 3). Forty two days after CCI, tibia BMDs of the 2 high dosage and SC group were higher than that of the NT group (P<0.001). BMDs of tibia for the NT and LL group were significantly reduced than that of the SC group (P<0.001) (Table 1).

Bone strengths were measured after sacrificing the rats on the 42nd day post-CCI, and these values for the SC, NT, LE, HE, LL and HL groups were 69.7±9.6N, 62.8±4.2N, 69.2±10.0N, 70.7±8.1N, 64.2±6.4N, and 70.8±5.8N, respectively. Bone strength values of the 2 high dosage groups (i.e., HE and HL) were significantly higher than that of the NT group (P<0.05) (Fig. 4).

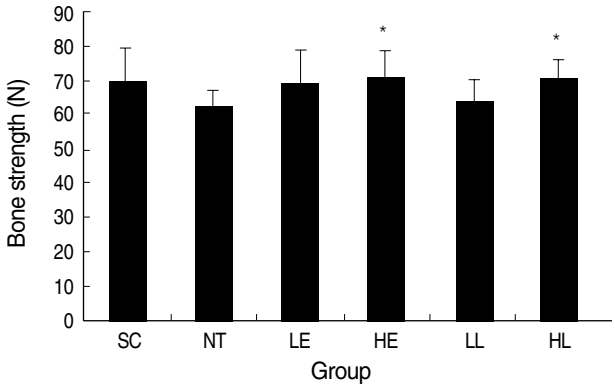

Fig. 4. Efficacy of oral alendronate in different dosage and time of administration in bone strength of the right tibia from Sprague-Dawley rats, obtained after the rats were sacrificed.
*P<0.05 as compared with the NT group.
CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment.

DISCUSSION

The results from the present study show that efficacy of alendronate in non-nociceptive symptoms varies with different doses and times of administration in a rat model of CCI. The results of the present are in accordance to those from previous findings (24) that bisphosphonates are effective in vasomotor, sudomotor and bone dystrophic changes. In addition, our results provide new information on the dosage and time administration that are effective for these non-nociceptive symptoms. For hindpaw thickness and temperature, we detected significant efficacy of early and high dosage alendronate. For BMD and bone strength, we detected significant efficacy of high dosage alendronate, with both early and late administration. In sharp contrast, we were not able to detect these significant efficacies with low dosage alendronate administration. From these results, we recommend that high dosage oral alendorate administered in the early stages is effective to cover the heterogenic features of CRPS in a CCI-rat model.

Previous clinical studies showed that bisphophonates helped to relieve some symptoms related to edema. Adami et al. (9) showed that intravenous alendronate resulted in improvements for edema, as well as pain, although their results were limited by a short follow-up period. Another study (10) showed that high dosage oral alendronate resulted in statistically significant reductions of swelling at 4 weeks. But, similar to our results, this efficacy was not sustained at later periods. In the

early stages of clinical CRPS, cutaneous vasodilatation is particularly prominent. However in later stages, as CRPS progresses to a cold stage, vasoconstriction predominates and swelling becomes less prominent. This typical cold stage progression should offer a possible hypothesis on why, despite the high dosage, we did not observe statistically significant reduction of skin thickness in the late administration groups and why we detected these changes only on the 14th day post-CCI in the HE group. Based on our results, we postulate that as CRPS progressed to a cold stage, efficacy of early high dosage administration of oral alendronate was not sustained in these late stages and that efficacy of late alendronate administration, regardless of dosage, was limited.

In contrast, for temperature, we observed that the positive effects of early high dosage alendronate were sustained with follow-up observation on the 42nd day post-CCI. Unfortunately, based on the present results alone, we were unable to come up with a suitable explanation for these different responses in temperature and we agreed that this was a topic that calls for future researches. Nevertheless, our results indicated that early high dosage alendronate supplementation should help alleviate the swelling and elevated temperature which predominated the acute stages of CRPS. The use of glucocorticoid therapy could be an alternative method to compensate for the limited efficacy of alendronate for the autonomic symptoms in the later stages of CRPS. Glucocorticoid therapy was reported to show sustained efficacy after long term follow-up by modulating de novo protein biosynthesis (25).

The exact action of alendronate for CRPS, especially for swelling and increased temperature, is still under debate, but a recent study of ibandronate, a potent bisphosphonate agent, showed that its effects were chiefly mediated by modulating substance P (24). Substance P is a neuropeptide that binds to NK1 receptors of postcapillary venules and causes albumin and protein extravasations. Schinkel et al. (26) pointed that the increased activity of this neuropeptide, which was elevated in serum samples in CRPS patients, was responsible for the increase of skin temperature and edema. We postulate that the mechanism of high dosage alendronate to control swelling and increased temperature was through modulation of this substance P. Nevertheless, the results of the present study could be supported by direct quantification of substance P and other neuropeptides.

Patchy demineralization with bone loss and osteoporosis in the affected limb are postulated to occur due to increased osteoclast activities. Also alendronate administration has been shown to be effective to reverse this CCI-induced bone loss. Whereas edema and vasomotor symptoms are predominant features of acute CRPS, bone loss is a predominant feature of chronic CRPS. BMD in CCI rats show significant decrease from two weeks after CCI (17), also this loss persists for at least 20 weeks after sciatic nerve resection (27). Likewise, bone density loss progresses over several months after injury in clinical CRPS. We suggest that it was due to this reason, that we detected significant efficacy in bone metabolism for the high dosage group even with late administration, and that we detected this efficacy with further follow-up on the 42nd day post-CCI. This sustained efficacy of the high dosage groups was again confirmed through our measurements of the tibia strength, which, similar to the results for BMD, showed preserved values after the rats were sacrificed.

Previous animal studies have shown that alendronate had analgesic effects only with high dosage (14, 15). The present results were in accordance with those studies, and showed statistically significant responses in non-nociceptive symptoms only in the high dosage groups. In addition, this is the first report, according to our knowledge, that has associated variable efficacy of a bisphopshonate agent with different dosages and time of administration across various symptoms in a CCI model. However, in order to translate our findings to clinical practice, there is a need to define a corresponding dose for humans that correlates to the high dosage used in this study. The 1.0 mg/kg/day dosage administered to the high dosage groups is equal to that used in previous studies (19, 20), but is approximately 5-6 times higher than standard clinical dosages. Thus, the high dosage used in our study poses potential problems for direct administration to humans. Continuous clinical studies to determine an appropriate human dosage are needed.

Some limitations of this study need to be considered. First, previous studies (14, 15) showed that alendronate shows nociceptive effects only at high dosages. However, pain was not directly assessed in our CCI model, because the main focus of this paper was to assess alendronate efficacy in non-nociceptive symptoms. Nevertheless, we indirectly observed some pain-related abnormal behavior patterns in the CCI groups as previously reported (3, 17). In addition, we observed that the high dosage alendronate groups recovered from these pain-related behaviors sooner than the NT or low dosage groups. However, we were unable to determine from these observations if these pain responses differed between those with early or late alendronate administrations. The results of this study could be supplemented with future studies that relate these autonomic symptoms to pain. Second, oral bisphosphonate agents have low absorption rates and their analgesic efficacy is questionable when compared to intravenous bisphosphonate agents with higher potencies. However, oral administration may be preferred in clinical practice because of its low cost (10) and safe side effect profile. High dosage intravenous bisphosphonate administration has been associated with some severe side effects. Moreover, Manicourt et al. (10) have shown that oral alendronate in high dosages was effective for CRPS-related hyperalgesia. Third, although we observed some variations within the groups across the follow-up time interval, we did not compare these changes to the contralateral side since the main scope of this study was to make comparison between different doses and administration groups and determine which group shows the best efficacy. The variation of

temperature within the groups across the followup interval could have been affected by the environment setting; temperature measurement is sensitive to environmental fluctuations. Therefore, in order to further prove the efficacies shown in the present study and exclude any environmental factor, further parameter measurements of the contralateral side at each follow-up assessment would be warranted in future studies. Finally, various other models (28, 29) have been introduced to be representative animal models for CPRS and some may question the validity of the CCI model used in this study. However, many studies (4, 30) showed that the CCI model, first introduced by Bennett and Xie (16), is reliable for the investigation of CRPS pathophysiology, and that it reproduced well the increase of skin flow and temperature typically seen in CRPS. Daemen et al. (3, 30) showed the role of inflammatory reactions of CRPS with a CCI model, while others used to this model to show the osteopathic changes of CRPS (17). For these reasons, the authors considered that the CCI model was adequate to represent CPRS for this study.

Previous studies (8-13) failed to report if analgesic effects differed between early and late alendronate administrations. However, they have noted that patients show heterogeneous responses, with large inter-individual responses, depending upon disease duration. According to our results, non-nociceptive symptoms responded to alendronate therapy other than hyperalgesia in a variable manner, and these positive effects were most apparent at a period when these symptoms are clinically most profound in CRPS. Our results have important clinical implications in the treatment of CPRS because they show that high dosage bisphosphonate treatment was effective to control the increased swelling, temperature and bone dystrophic changes of CRPS. Also, our results showed that different efficacies could be expected with early and late alendronate therapy. Whereas only early high dosage was effective to cover the autonomic features, high dosage alendronate was effective to cover the bone dystrophic changes, regardless of time of administration. As stated earlier, there are yet no established guidelines on the appropriate dosage and time of drug administration to cover the heterogenic features of CRPS. The clinical relevance of our present study should be supported with further clinical studies of alendronate.

In summary, our results showed that oral alendronate in high dosages was effective in non-nociceptive CRPS symptoms and that variable efficacy was observed with different time of administration. These results may help guide future clinical studies to establish the optimal dose and treatment regimens that guarantee the best therapeutic responses of bisphosphonate agents across the heterogenic clinical features of CRPS.

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