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High-dose zoledronic acid narrows the periodontal space in rats

Y. Okamoto[1,2], M. Hirota[1], Y. Monden[1], S. Murata[1], C. Koyama[1,2], K. Mitsudo[1], T. Iwai[1], Y. Ishikawa[2], I. Tohnai[1]

[1]Department of Oral and Maxillofacial Surgery, Yokohama City University Graduate School of Medicine, Kanagawa, Japan; [2]Department of Oral Surgery, Fujisawa City Hospital, Kanagawa, Japan

Y. Okamoto, M. Hirota, Y. Monden, S. Murata, C. Koyama, K. Mitsudo, T. Iwai, Y. Ishikawa, I. Tohnai: High-dose zoledronic acid narrows the periodontal space in rats. Int. J. Oral Maxillofac. Surg. 2013; 42: 627–631.

Abstract. The aim of this experiment was to evaluate the histological effects of zoledronic acid on the periodontal space in rats. 40 male Wistar rats were divided into three zoledronic acid groups and a control group. Zoledronic acid was injected subcutaneously at doses of 10, 50, or 500 μg/kg once a week for 3 weeks. The rats were killed 1 or 9 weeks after the last injection. Histological examination of the periodontal space around the incisor tooth revealed that zoledronic acid did not inhibit tooth development. In the rats killed 1 week after treatment discontinuation, the periodontal space gradually narrowed in response to increasing zoledronic acid doses, and the changes were statistically significant according to ANOVA but not according to ANOVA with *post hoc* tests. The changes persisted in the high-dose zoledronic acid group despite zoledronic acid discontinuation, with significant differences identified by ANOVA and ANOVA with *post hoc* tests. Therefore, although zoledronic acid had an insignificant effect on tooth development, it had a significant effect on the periodontal space when high doses were administered. The results of this experiment may provide useful information for future investigations on the role of zoledronic acid in the osteonecrosis of the jaw.

Key words: bisphosphonate; zoledronic acid; osteonecrosis of the jaw; periodontal space; rat.

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Intravenous bisphosphonates are widely used as the first choice of treatment for bony metastasis of cancer and hypercalcaemia of malignancies.[1,2] Bisphosphonate treatment is effective in decreasing bony pain and serum calcaemia symptoms. Conversely, osteonecrosis of the jaw (ONJ) related to bisphosphonate treatment has been reported since 2003.[2–4] ONJ is defined as exposed necrotic bone in the maxillofacial region that persists for more than 8 weeks in a patient with current or previous bisphosphonate treatment and no history of radiation therapy against cancer in the jaws.[5,6] The highest incidence of ONJ has been associated with zoledronic acid (ZA).[7,8] Marx et al. suggested that bisphosphonates are directly responsible for ONJ because of their anti-angiogenic effects.[1,4,6,9,10] The main event precipitating ONJ is dental extraction.[5,7,11] Histopathological examination revealed that bisphosphonates remarkably delayed wound healing after tooth extraction by inhibiting new bone formation.[2,5,12,13] Basi et al.[5] observed aberrant wound healing of the tooth extraction socket with decreased mineralization in a rat administered ZA and suggested that the pathogenesis of ONJ is related to high matrix metalloproteinase-9 expression and osteoclast dysfunction.

Although there are numerous clinical reports on ONJ, little information is available regarding the pathogenesis of ONJ and bony changes in the jaw after bisphosphonate treatment. Hoefert et al. reported that microcracks were present within the bones in approximately 54% of ONJ patients.[14] Takahashi et al. reported that the alveolar bone around the root of a tooth showed higher density on radiographs in ONJ patients than in age-matched

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controls.[15] Therefore, the present authors hypothesized that there are histological changes in the periodontal space, including the teeth and alveolar bone.

The aim of this study was to observe changes in the periodontal space of ZA-administered rats.

Materials and methods

40 male Wistar rats (Nihon SLC, Shizuoka, Japan: body weight 300–350 g; 10–12 weeks old) were used in the experiment. All rats were housed in cages with free access to food and water, and a 12 h light/dark cycle was maintained. All experiments were approved and performed in accordance with the guidelines for Animal Experiments Ethic Committee of Yokohama City University.

The 40 rats were randomly divided into four groups. Groups A, B, and C received ZA at doses of 10, 50, and 500 μg/kg, respectively. The rats in the control group received injections of saline instead of ZA. The time schedule of drug administration was designed according to the literature[2,13,16] with slight modifications to ensure long-term release after ZA discontinuation. Regarding the administration dosage, the dose of ZA for adult cancer patients weighing 50–80 kg was referenced.[9,17] As these patients receive 50–80 μg/kg ZA in one administration, 50 μg/kg was selected as the middle dose for use in this experiment. 10 μg/kg (a dose fivefold smaller than the middle dose) was selected as the low dose and 500 μg/kg as the high dose.

All rats received subcutaneous injections weekly for 3 weeks. All four groups were each randomly divided into short-term and long-term groups according to the length of the observation period. The rats in the short-term groups were killed 1 week after the last injection, and the rats in the long-term groups were killed 9 weeks after the last injection (Fig. 1). To distinguish these groups, the short-term groups were designated As, Bs, Cs, and Ctls for the A, B, C, and control groups, respectively, and the corresponding long-term groups were designated Al, Bl, Cl, and Ctll, respectively.

To evaluate the effect of ZA alone, no dental procedure or pharmacological therapy was performed.

Histological analysis

After the rats were killed, their mandibles were resected. Excess soft tissues were trimmed, and the remaining mandibular bones were fixed in 4% formalin.

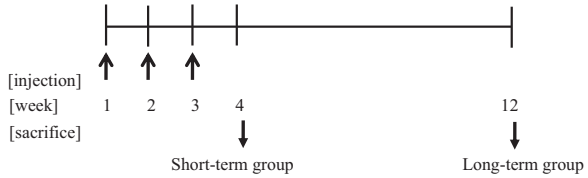

Fig. 1. Experimental design. Rats received injections of ZA or saline every Monday (↑: injection). Rats in the short-term groups were killed on the Monday of week 4, and those in the long-term groups were killed on the Monday of week 12.

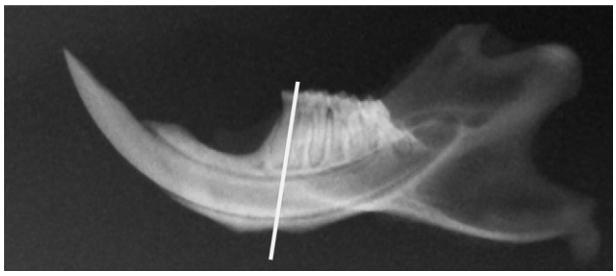

Fig. 2. To measure the periodontal space of the mandibular incisor at the same position for all specimens, cross-sections of the central portion of the first molar were used.

Following fixation, the samples were embedded in methyl methacrylate resin for histological evaluation. The embedded samples were sectioned with a microtome (30 μm thick) and stained with toluidine blue.

To measure the periodontal space of the mandibular incisor at the same position for all specimens, cross-sections of the central portion of the first molar were used (Fig. 2). On these sections, the areas of the incisor socket and the incisor were measured using MacromaX GOKO measurement software (GOKO camera Kawasaki, Japan) to calculate their cross-sectional areas (Fig. 3). To observe changes in the periodontal space, the ratio of the area of the incisor socket to that of the incisor (RSI) on the cross-section was calculated (Fig. 4).

Statistical analysis

For mean value comparisons of the incisor area and RSI between groups, ANOVA followed by Bonferroni's *post hoc* analysis for multiple comparisons was used. $P < 0.05$ indicated statistical significance.

Results

The experiment was performed without any complications, and no infection was

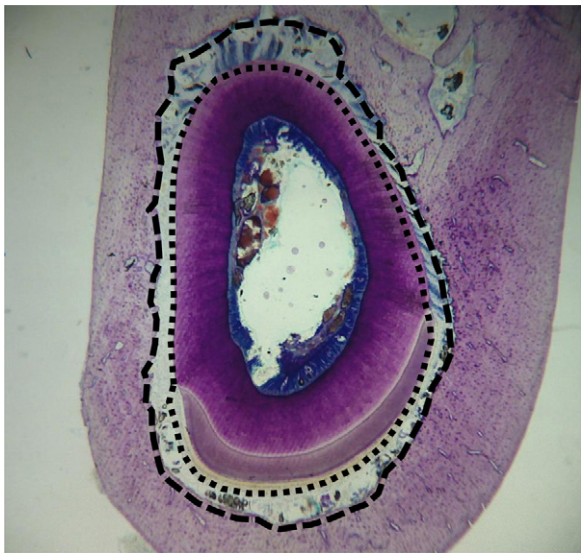

Fig. 3. The external length of the periodontal space (dotted line) and the circumference of the incisor (broken line) were measured for the statistical analysis.