Effect of Alendronate on Osteoclast Differentiation and Bone Volume in Transplanted Bone

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Abstract: Alendronate, one of the bisphosphonates, is known to have an inhibitory effect on bone resorption. The purpose of this study was to investigate the effects of alendronate on ectopic bone graft resorption and to determine the optimal dose in the mouse. The grafted bone in the control group disappeared due to resorption by osteoclasts within 5 weeks. In the experimental groups, the area of bone tissue decreased by only 20–40% at 5 weeks post-operatively. At 8 and 9 weeks after surgery, the decreased area of bone structure was significantly less in all the 10⁻⁴ M injected alendronate-immersed groups than in the 10⁻⁴ M non-injected alendronate-immersed. At 9 weeks after surgery, the number of osteoclasts were significantly less in the 10⁻⁴ M injected alendronate-treated groups than in the 10⁻⁴ M non-injected alendronate-treated groups. These results suggest that alendronate inhibits resorption of ectopic bone graft at concentrations of 10⁻⁴ and 10⁻⁶ M.

Key words: Alendronate, Osteoclast, Transplanted bone volume

Regeneration of bone in osseous defects resulting from maxillary alveolar clefts, as well as in craniofacial bone clefts of the palate, is of paramount importance in the restoration of lost form and function. At present, the most reliable bone regenerative material for maxillary alveolar clefts and cleft palate is fresh autogeneous graft, but it requires increased surgical duration and may cause ill effects at the donor site such as excessive blood loss, pneumothorax, wound infection, chronic pain, parapneumothorax, paresthesia, unsightly scars, and a potential disturbance of iliac development in the young [8]. Bisphosphonates suppress osteoclastic bone resorption and are used to treat bone disorders including metastatic bone disease and osteoporosis [7]. Although the molecular mechanism of the action remains to be elucidated, it is currently thought that their anti-resorptive action is through suppression of the formation of osteoclasts from hemopoietic precursors that originate in the bone marrow [14]. In a previous study, we reported the effects of alendronate on ectopic bone graft resorption [10]. Furthermore, we considered the influence on the bone by the concentration of alendronate in detail [10].

The relative contribution of these mechanisms to the
action of bisphosphonates *in vivo* is not known and may not be the same for every individual compound. In previous studies, the influence of immersing transplanted bone in, and injecting with bisphosphonates in bisphosphonates was not considered. This study investigated the effects of injected alendronate on ectopic bone graft resorption and determined the optimal dose.

C57BL/6J male and female mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). Newborn male mice were kept in metal cages (22 × 32 × 11cm) with autoclaved wood chips for bedding in an animal room (temperature 24 ± 2°C; relative humidity 50 ± 5%). They were kept in cages under an alternating 12 h light and dark cycle, and fed either a solid or granulated diet (CE2; Clea Japan, Tokyo, Japan) and water *ad libitum*. Newborn mice were weaned at 20 days after birth. One hundred and thirty-five mice were first divided into five groups (Table 1). This study was approved by the Animal Use Committee, Hiroshima University, and the animals were maintained in accordance with the guidelines issued by Hiroshima University for the care and use of laboratory animals.

In each of the experimental and control groups, three mice weighing about 30 g each were used. Tarsal bone, 1 mg in weight, was chopped and dipped in saline or alendronate (Banyu Pharmaceutical Co., Tokyo, Japan) solution before autograft. The mice were divided into three groups: 10⁻⁴ M alendronate immersed group (n=6), 10⁻⁶ M alendronate immersed group (n=6) and saline group (n=3). Alendronate was dissolved in physiological saline and diluted at a dose of 10⁻⁴ and 10⁻⁶ M, according to a previous finding that these doses stimulated transplanted bone formation in normally loaded bone at 5 weeks post-operation. Bone material was grafted into the subcutaneous layer under general anesthesia (Fig. 1). These mice were single injected 0.15 mg of purified alendronate into the abdominopelvic cavity 5 weeks after the transplant. Dorsoventral and lateral X-rays of each mouse were taken at 1, 2, 3, 4, 5, 6, 7, 8 and 9 weeks after the transplant. The cephalometric X-ray apparatus for rats and mice (Asahi Roentogen Ind. Co., Kyoto, Japan) was used at 20–25 kV and 6 mA with an exposure time of 3.0 s for Kodak Dental Ultraspeed film® (Eastman Kodak Co., Rochester, New York, USA). The transplanted bone volume was analyzed on the radiograph using an image-analysis program (NIH Image 1.59; National Institutes of Health, Bethesda, MD, USA).

Transplanted bone removed from the hypoderm was fixed in 4% formaldehyde for 12 h at 4°C, decalcified in 5% ethylenediamine tetraacetic acid (EDTA) (pH 7.4) for one week, embedded in paraffin, and cut into sections 7 µm thick. The sections were stained with hematoxylin and eosin (HE) and with tartrate-resistant

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Fig. 1. Bone material autografted into the subcutaneous layer under general anesthesia. An open arrow indicates tarsal bone fragment. A white arrow indicates the subcutaneous layer where the bone fragment should be transplanted.
acid phosphatase (TRAP), which is generally acknowledged as a cytochemical marker for osteoclasts, and finally counter-stained with hematoxylin. The number of positively stained cells in sections of the median portion of whole transplanted bone was enumerated for each group. ANOVA was carried out, followed by Scheffe’s tests to detect pairwise differences among groups.

There were no significant differences in body weights among the five groups throughout the whole experimental period (Fig. 2). It was thus confirmed that experimental or surgical invasion did not exert any substantial influence on the general growth of the mice.

In the control group, the grafted bone had disappeared by resorption by 5 weeks after surgery (Fig. 3). At 2 and 5 weeks after surgery, the decreased area of the bone structure was significantly less in all the alendronate-immersed groups than in the controls (Figs. 3 and 4). In experimental groups grafted with bones immersed in 10^{-4} M and 10^{-6} M alendronate, the areas of bone tissue were decreased only by about 20% 2 weeks after surgery (Figs. 3 and 4). Thereafter, the area decreased only slightly, and maintained sufficient bone volume until 9 weeks (Fig. 3). The bone volume appeared highest in the 10^{-4} M group showing a tendency toward dose-dependent increase, but no significant differences in bone volume were found between groups induced by different doses of bisphosphonate at concentrations of 10^{-4} M and 10^{-6} M (Fig. 3). At 8 and 9 weeks after surgery, the decreased area of the bone structure was significantly less in all the 10^{-4} M injected alendronate-immersed groups than in the 10^{-4} M non-injected alendronate-immersed (Fig. 3).

The number of TRAP-positive osteoclasts rapidly increased until 2 weeks after bone transplant the in control group, and then decreased rapidly, due to the loss of bone volume (Figs. 3, 4 and 6). Osteoclasts in the experimental groups were significantly less than those in the controls until 4 weeks after surgery (Figs. 4 and 6). At 7 and 9 weeks after surgery, the number of TRAP-positive osteoclasts were significantly less in the 10^{-4} M injected alendronate-treated groups than in the 10^{-4} M non-injected alendronate-treated groups (Figs. 5 and 6). Although these effects seen at all concentrations may be enough in clinical application, we will have to change the alendronate concentration taking the quantity of tissue fluid into consideration.

Previous studies clearly demonstrated the induction of osteoclast apoptosis by bisphosphonates [2, 5, 9, 11, 12]. Furthermore, osteoclast apoptosis may occur as a result of treatment with any of the bisphosphonate fam-
alendronate to the bone resorption mechanisms may depend on the dose of drug and schedule of administration [1, 6]. The present study suggests that the bone resorption in the early stages of bone transplantation is controlled by local action of alendronate.

In conclusion, the present results suggest that
alendronate at a concentration of $10^{-4}$ and $10^{-6}$ M inhibits resorption of ectopic bone graft.

**References**