Effects of alendronate on bone formation and osteoclastic resorption of beta-tricalcium phosphate

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Introduction: Bisphosphonates such as alendronate (ALN) are widely used drugs for diseases associated with bone resorption. They act directly on osteoclasts, inhibit the isoprenoid biosynthesis pathway, and interfere with protein prenylation. ALN is also effective in blocking bone resorption when applied locally. The mechanism of beta-tricalcium phosphate (TCP) resorption involves solution-mediated disintegration and cell-mediated disintegration. We previously reported that osteoclasts played a major role in the bioresorption of beta-TCP. Thus, resorption of beta-TCP is important for bone formation. The aim of this study was to determine whether local ALN administration would inhibit osteoclastic resorption of beta-TCP and bone formation.

Materials and Methods: Experiment 1. Cylindrical beta-TCP blocks (4 mm in diameter and 10 mm in height) with a mean pore size of 200 μm and a porosity of 75% were immersed in ALN solutions at 10^-2 to 10^-8 M for 2 days. Excess ALN was then removed with sterilized filter paper. New Zealand White rabbits weighing 3.1 to 3.3 kg were used. Under general anesthesia, bilateral cylindrical bone defects (4.1 mm x 10 mm) were created by drilling. Bone cavities were filled with the ALN-treated beta-TCP blocks. Rabbits were sacrificed at 2 weeks postoperatively, femoral specimens were harvested, and decalcified sections were obtained for hematoxylin-eosin (HE) and tartrate-resistant acid phosphatase (TRAP) staining.

Experiment 2. Cylindrical beta-TCP blocks (4 mm in diameter and 5 mm in height) were treated with ALN in the same manner as experiment 1. Bone marrow cells were obtained from bilateral femora of a 6-week-old Fisher rat. The ALN-treated beta-TCP blocks were soaked with the bone marrow cells and were implanted into 12-week-old Fisher rats subcutaneously. Implanted beta-TCP blocks were harvested at 6 weeks postoperatively. Decalcified sections were used for HE and TRAP staining and non-decalcified sections were stained with toluidine blue.

Results: Beta-TCP blocks with allogenic rat bone marrow cells enabled marked bone formation 6 weeks after implantation subcutaneously (Fig.1A). TRAP-positive cells were also present on the surface of the beta-TCP blocks. In contrast, no bone formation was detected in the beta-TCP blocks without implantation of marrow cells. In the ALN-treated beta-TCP blocks, new bone formation was inhibited in a dose-dependent manner (Fig.1B). This inhibitory effect was seen at a concentration of 10^-6 M, but ALN at 10^-8 M did not inhibit. The number of TRAP-positive cells was also reduced by local administration of ALN. In the rabbit cancellous bone defect model, similar inhibitory effects of ALN on bone formation and TRAP-positive cells were observed (Fig.2).

Discussion: Beta-TCP resorption is thought to involve both solution- and cell-mediated disintegration. In our previous study, the mechanism of beta-TCP resorption was considered to be cell-mediated disintegration by numerous TRAP-positive giant cells. In addition, the time lag between the peak value of TRAP-positive cell population and that of new bone formation rate suggested that a coupling-like phenomenon could be occurring in the beta-TCP filled bone defects. Thus, osteoclast-mediated resorption of beta-TCP may be important for enabling bone formation. In this study, we used two different experimental models, and the results showed that local application of ALN at a concentration of 10^-2 to 10^-6 M reduced the number of osteoclasts on the surface of beta-TCP blocks. This phenomenon was consistent with the previous study of the effects of ALN on pit formation by mouse osteoclasts. Inhibition of osteoclast formation resulted in reducing beta-TCP resorption and enabled bone formation. Thus, these results suggest that osteoclast-mediated resorption plays an important role in beta-TCP resorption and bone formation. In addition, this experimental model may be a useful alternative to the pit formation assay to examine the effect of bisphosphonate on bone resorption. It was also reported that treatment with ALN(2mg/kg/day) before or during fracture healing, or both, resulted in no adverse effects on the union, strength, or mineralization of bone in mature beagle dogs. However, our study suggests that higher doses of ALN used for treatment of metastasis may inhibit fracture healing.