Effects of Alendronate on Bone Formation Induced by Recombinant Human Bone Morphogenetic Protein-2

Introduction:
Bone morphogenetic protein-2 (BMP-2) possesses both anabolic and catabolic actions to bone. Recombinant human BMP-2 (rhBMP-2) is widely used for spinal fusion surgery. However, vertebral osteolysis after posterior interbody lumbar fusion using rhBMP-2 was recently reported.

Bisphosphonates, such as alendronate (ALN), are widely used drugs for diseases associated with bone resorption. ALN was originally used for the treatment of osteoporosis, but more recently it has also been used in many orthopedic fields. ALN is also known to be effective in blocking bone resorption when applied locally.

The aim of this study was to determine whether local ALN administration could inhibit beta-TCP resorption and/or bone formation induced by rhBMP-2.

Materials and Methods:
New Zealand White rabbits, weighing 3.1 to 3.3 kg, were used for this study. Under general anesthesia, a 15mm long segmental bone defect was created in the diaphysis of the rabbit ulna. Rabbits were separated into 2 groups and were treated as follows: Group A - defects were filled with a complex of beta-TCP granules (75% porosity) and 6.5% collagen gel with 25µg of rhBMP-2; Group B - beta-TCP granules were immersed in a 10⁻³ M ALN solution for 30 minutes, excess solution was removed with sterilized filter paper and the pre-treated TCP granules were then added to the same amount of collagen and rhBMP-2. Defects were then filled in the same manner as Group A. After surgery, all animals were allowed to move freely in their cages without casting.

Bone regeneration and beta-TCP resorption were assessed by X-rays, micro-CT scans and histology. X-ray radiographic findings of each rabbit were examined at 2, 4, 6, and 8 weeks postoperatively. Animals were sacrificed at 2 and 8 weeks and the treated ulnas were removed. After non-destructive examination using micro-CT, non-decalcified specimens were stained with TRAP.

Results:
X-Ray findings
Group A - Two weeks after surgery, beta-TCP granules were already disappearing, especially in the center of the defect. New bone formation was observed, suggesting that replacement of beta-TCP by bone had occurred. At 4 weeks, almost all beta-TCP granules had disappeared. At 6 and 8 weeks, the defect was completely repaired with newly formed bone (Fig. 1). Group B - Two weeks after surgery, most of the complex still remained in the defect. At 4 weeks, beta-TCP granules were still visible and partial bone formation was observed. At 6 weeks, most of the TCP granules were replaced by bone. At 8 weeks, the defect was completely repaired with newly formed bone (Fig. 2).

Micro-CT evaluation of 2 and 8-week bone replacement
At 2 weeks in group A, micro-CT images showed partial beta-TCP resorption as approximately 50% of beta-TCP granules were resorbed and new bone formation was found. In group B, most of the beta-TCP granules still remained in the defect (Fig. 3). At 8 weeks, the defect was completely repaired with cortical bone in both groups, but there was marked bone marrow cavity formation in group A (Fig. 4). TRAP staining shows that new bone formation from radial site was occurred in group A, but not in group B. TRAP-positive cell are present on the surface of TCP in both groups. However, the number of TRAP-positive cells were greater in group A (Fig. 5).

Discussion
In this study, we used an injectable complex of beta-TCP granules and collagen as a carrier for BMP-2. One advantage of this complex is that beta-TCP is not only a carrier for BMP-2, but also acts as a monitor for osteoclastic resorption.

Recently, adverse effects of rhBMP-2 in spinal fusion surgery, such as ectopic bone formation and local bone resorption, were reported. BMP-2 is well known to induce bone formation and it also stimulates bone resorption through both direct and indirect stimulation of osteoclast formation and activation of mature osteoclasts. We studied the effects of ALN on bone formation and TCP resorption induced by BMP-2 in rabbits and investigated the possibility that ALN administration may resolve osteolysis caused by rhBMP-2 in a clinical setting. In the

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