STUDY ON CARRIERS OF BONE MORPHOGENETIC PROTEIN USED FOR SPINAL FUSION

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Introduction. Osteoinductive growth factors, such as bone morphogenetic proteins (BMPs), are known to stimulate osteoblast differentiation and synthetic activity. The soluble osteoinductive growth factor requires a less soluble carrier for expression of its biologic potency. We have reported the efficacy of the sintered bovine bone named True Bone Ceramics (TBC) coated with type I collagen as a carrier of BMP for lumbar intertransverse process arthrodesis in the animal model. The study has demonstrated that the more effective carrier is the biomaterial which possesses bony or porous structures to allow in-depth bone growth in achieving solid spinal fusion. However, there is some doubt about whether this fusion can be brought about from early stages of recovery, because the strength of TBC itself is fragile in comparison with other artificial hydroxyapatite. The aim of this study was to clarify which carrier was biomechanically more effective for osteoinductive growth factors in spinal fusion. In this study, alphacalcium phosphate (α-TCP) cement was also selected as a carrier, because the material itself has sufficient strength.

Materials and Methods. Twenty-seven adult rabbits, each weighing 3.0 kg, were used. Under sodium pentobarbital anaesthesia, each rabbit underwent a bilateral lumbar intertransverse process arthrodesis at L4-L5 similar to the rabbit model established by Boden and colleagues. Animals were divided into five groups and in each rabbit, and one of the following materials per fusion side was implanted. They were 1) Autologous cortocancellous bone graft (autologous bone group: n=7), 2) α-TCP cement (TCP group: n=5), 3) α-TCP cement containing 100 μg of recombinant human bone morphogenetic protein-2 (rhBMP-2) (BMP-TCP group: n=5), 4) TBC coated with type I collagen infiltrated with 100 μg of rhBMP-2 solution (BMP-TBC group: n=5), 5) collagen sheet containing 100 μg of rhBMP-2 (BMP-collagen group: n=5). Spinal fusions were evaluated by radiographic analysis, manual palpation, biomechanical testing and histologic examination. Radiographic fusion at the operative areas of all rabbits was evaluated 3 and 6 weeks after surgery. The rabbits of each group were sacrificed 6 weeks after surgery and the lumbar spines were removed. At the time of harvest, the lumbar spines were manually palpated at the level of the fused motion segment. Ultimate tensile strength was read directly as the peak load to failure. Stiffness was calculated from the load-displacement curve. The specimens were fixed in 10% neutral buffered formalin, undecalified and embedded in methylmethacrylate. Using hematoxylin and eosin, or Goldner’s Trichrome stains, the new bone formation was observed. All parameters were analyzed by analysis of variance (ANOVA) and Student’s t-test for statistical analysis, and p<0.05 as a minimum level of significance was chosen. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the author’s institute.

Results. Radiographic Analysis. Six weeks after surgery, the fusion rates were 4/7 in the autologous bone group, 1/5 in the TCP group, 4/5 in the BMP-TCP group, 4/5 in the BMP-TBC group and 4/5 in the collagen and BMP group. Radiographs of rabbits which were evaluated as spinal fusion revealed continuous trabecular patterns within the intertransverse fusion mass. Manual Palpation. The fusion rates by manual palpation in each group were 3/7 in the autologous bone group, 1/5 in the TCP group, 5/5 in BMP-TCP group, 5/5 in the BMP-TBC group and 4/5 in the BMP-collagen group. Radiographs of rabbits which were evaluated as spinal fusion revealed continuous trabecular patterns within the intertransverse fusion mass. Mechanical Testing. Both the BMP-TCP and BMP-TBC fusions showed significantly higher tensile strength (p<0.05) and stiffer fusion (p<0.05) than the autologous bone group. Both the BMP-TCP and BMP-TBC fusions tended to show higher tensile strength and stiffer fusion than the BMP-collagen fusions. All the BMP-TCP fusions failed at the interface points where the implanted masses attached to the transverse process. Some fusions in both the BMP-TCP and BMP-collagen groups failed at the middle points of the grafted fragments. (Figure 1) Histologic Examination. The histologic findings in each BMP group demonstrated a cortical bone rim around the edge of the fusion mass. In the BMP-TCP group, there was no sign of intervening soft tissue between the new bone and the implant, and a layer of the new bone was observed covering the surface of the grafted fragment. In the BMP-TBC group, all pores of grafted TBC fragments were filled up with the new bone formation. While, in the collagen BMP group, less mature bone formation were present in the grafted fragments and the new bones were not mutually connected.

Discussion. TBC is the biomaterial possessing a natural trabecular structure and is an organized crystal of bone mineral made by sintering bovine bone at high temperatures. The crystallized element of TBC is similar to artificial hydroxyapatite and the compressive strength of itself is 3 MPa. The crystallized element of α-TCP cement consists of mainly calcium and phosphorous. Although the appearance of the α-TCP cement is paste at the time of implantation, the cement itself attains the compressive strength of 60 MPa within 72 hours after implantation. In the present study, the BMP fusions resulted in higher biomechanical strength and greater stiffness than the autograft. Concerning the carriers of the rhBMP-2, there were no significant differences among groups in fusion rates and biomechanical evaluation. However, both the BMP-TCP and BMP-TBC fusions tended to show higher tensile strength and stiffer fusion than the BMP-collagen fusions. Histologic findings on the collagen sheet used as a carrier demonstrated that the grafted area was occupied with immature bones. The effective carrier for osteoinductive growth factors in achieving the earliest solid spinal fusion is the one that in itself has sufficient biomechanical strength, or is the one that possesses bony or porous structures. Although it may be necessary to carry out detailed experimental study on the characteristics of BMP between species, these observations warrant further clinical investigation concerning the carrier effectiveness of osteoinductive growth factors.

Conclusions. To achieve the earliest solid spinal fusion, we conclude that the effective carrier is the material that in itself has sufficient biomechanical strength, or bony or porous structures, such as α-TCP or TBC.

References.

Figure 1. Biomechanical testing. Bar graphs of relative stiffness show differences among the fusions. * Significantly different from BMP-TCP group. ** Significantly different from BMP-TBC group. (ANOVA, Student’s t-Test, P<0.05)