THE EFFECTS OF BONE MORPHOGENETIC PROTEIN AND BASIC FIBROBLAST GROWTH FACTOR ON CULTURED MESENCHYMAL STEM CELLS FOR SPINE FUSION

INTRODUCTION. Recently, mesenchymal stem cells (MSCs) residing in bone marrow have been noticed and studied in various fields because of its capability to differentiate into various cells. It is known that MSCs can differentiate into an osteogenic lineage under the proper conditions. The MSCs would help to heal bone defects and to induce bone formation in a similar fashion to BMPs. We have investigated the feasibility of using MSCs to minimize some problems (e.g. requirement of a high dose to achieve consistent effects, and high manufacturing cost) due to use of BMPs in spinal fusion. It has been reported that the cultured MSCs act as a substitute for autograft and BMP fusions. Moreover, our previous study hypothesized that it would be important to differentiate into osteogenic cells and to implant a large number of cells so that the solid spinal fusion has been achieved. In this study, we selected two factors, which are BMP and basic fibroblast growth factor (b-FGF) being implicated the osteogenic regulatory process by virtue of their proliferation, mitogenic and differentiation activities. The purpose of this study was to determine the efficacy of BMP and b-FGF for cultured MSCs in lumbar arthrodesis.

MATERIAL AND METHODS. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the authors’ institute. Thirty-seven adult rabbits, each weighing 4.0 to 4.5 kg, were used. The rabbits were anesthetized with ketamine (10 mg/kg, intramuscularly) and sodium pentobarbital (25 mg/kg, intravenously). Each underwent single-level bilateral posterolateral intertransverse process fusions at L4-L5. The animals were divided into four groups and in each group, one of the following materials was implanted with Type I collagen sheet (1x2x5 cm) on each side: 1) autologous bone, 2.5 g corticocancellous bone harvested from the posterior iliac crest (autologous bone group, n=9); 2) cultured bone marrow cells (MSC; n=7), 3) cultured bone marrow cells with recombinant human bone morphogenetic protein-2 (rhBMP-2) (Marrow-BMP; n=7), 4) cultured bone marrow cells with b-FGF (Marrow-BFG; n=7), 5) cultured bone marrow cells with rhBMP-2 and b-FGF (Marrow-BMP-FGF; n=7). Fresh bone marrow cells from iliac crest of each animal were cultured in a standard medium for 2 weeks. For an additional 1 week, the marrow cells (1x10⁶ cells/ml) were cultured in 10⁻⁴ M dexamethasone, Type I collagen gel and 2.5G porous hydroxyapatite (HA) particles with or without rhBMP-2 (2µg/1gHA) and b-FGF (5µg/1gHA). The animals were sacrificed 6 weeks after surgery. Spinal fusions were evaluated by radiograph, manual palpation, and histology. All parameters were analyzed by analysis of variance (ANOVA). Fusion rates among the groups were compared using Fisher’s exact test. A probability level less than 0.05 was considered significant.

RESULTS. The mortality rate after surgery was two rabbits in the autologous bone group. The fusion rates were 5/7 rabbits in the autologous bone group, 0/7 rabbits in the Marrow group, 2/7 rabbits in the Marrow-BMP group, 3/7 rabbits in the Marrow-BFG group, and 6/7 rabbits in the Marrow-BMP-FGF group. The histology in some cases of the Marrow-BMP and Marrow-BFG groups demonstrated that fibrous tissues and cartilages remained in the grafted areas. The less mature bone formation was present in the grafted fragments and the new bone was not connected mutually. In the Marrow-BMP-FGF group, new bone formation was growing from the transverse process, and that ingrowth of new bone continued between the recipient bone and the matrix of HA particles. The pores of fragments were filled up with bone matrix.

DISCUSSION. The activities of MSCs have been studied in vitro and in vivo and have the capability to differentiate to various cells, including osteoblasts, and cartilage cells. Few studies have addressed the use of cultured bone marrow cells in a spinal fusion model. Lindholm et al transplanted fresh bone marrow in lumbar spinal arthrodesis. The results indicated that bone marrow in combination with autologous bone graft appeared to enhance the rate of fusion compared with either bone marrow or autologous bone graft alone. Some reports have not revealed sufficient efficacy of marrow cells when freshly aspirated cells were implanted. These authors hypothesized that the number of implanted cells might be too low to achieve spinal fusion, and that it might be necessary to differentiate the MSCs into osteoblasts with osteoinductive factors prior to implantation. In our previous study, the implantation of the cultured MSCs in porous HA particles and that treated dexamethasone resulted in good bone formation for lumbar spine fusion in animal model. The porous HA particles and dexamethasone would enhance the differentiation of the MSCs into osteogenic cells. It hypothesized that the more differentiation into osteogenic cells and the more proliferation of cells before implantation might bring the success of the spinal fusion. In this study, the cultured MSCs with BMP and b-FGF produced a favorable degree of fusion in the spinal fusion model. The differentiation and proliferation of MSCs had been further accelerated by the use of BMP and b-FGF.

REFERENCES.


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