MESENCHYMAL STEM CELL THERAPY IN GROWTH PLATE CARTILAGE INJURY

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Introduction: Injury to the growth plate in a growing child can lead to varying degrees of limb length discrepancy and angular deformity as a result of growth arrest [1]. In the older child, correction of deformity can be achieved by stapling, epiphysiodesis or osteotomy. However, the treatment is more challenging in the younger child. Angular deformity in younger child may be corrected by excision of the bony bridge and insertion of fat, silastic, and bone cement as interposition material. However, these methods yield varying degrees of success. Recently, experimental studies have transplanted mesenchymal stem cells (MSCs) into the articular cartilage defect. It showed that MSCs were differentiated into chondrocytes as early as two weeks after transplantation [2]. The objective in the present study was that established growth arrest could be corrected with the transplantation of MSCs, which may provide the appropriate cartilage presence necessary to inhibit the initial formation of bony bridges across the epiphysis and so maintain the growth potential.

Materials and Method: Eighteen immature New Zealand White rabbits (5-7 weeks old) were used as experimental animals because these animals would take 4-6 months before the growth plate was spontaneously fused. The left tibia was used as the experimental leg. The proximal tibia was exposed through an anteromedial incision. The medial half of the proximal epiphyseal plate was excised with a scalpel blade and a small curette. This led to growth arrest [3]. Two groups of rabbits without MSC transplantation left untreated partial defect and treated with gelform served as a control. The three groups of rabbits were implanted with culture-expanded MSC constructs (MSCs+5% gelatin, MSCs+10% gelatin+gelform, and MSCs+gelform+TGF? $_3$) to 6-week test periods. Bone marrow was aspirated from the proximal anterior aspect of the tibia by a sterile surgical procedure. Cells were cultured in low glucose -DMEM with antibiotics and 10% fetal bovine serum according to the method of Lennon et al. [3]. Primary cultures were seeded at 15-20 million nucleated cells per 56 cm^2 and were maintained for 10-14 days. Nonadherent cells were removed from the cultures by the second or third change of medium. Confluent colonies were detached from the plate with trypsin (0.25%) in EDTA (1 mM). The cells were then collected, rinsed, centrifuged twice with medium, counted, and replated to the first-passage stage. When first-passage MSCs were nearly confluent, they were again detached, counted, and pelleted for construction of the implant. The mesenchymal stem cell-matrix implants has been constructed with several different types of matrices such as 5% gelatin, 10% gelatin/gelfoam, and gelform in chondrogenic medium supplemented with TGFβ_3 10 ng/ml. Radiographs were taken from both limbs. Tibiofemoral angle and lengths of both legs were measured. Healing of the defect was investigated histologically by hematoxylin-eosin and Safranin 0/fast green stain. One way ANOVA test and Scheffe's test were employed to find differences between the angular deformity of each group.

Results: As shown in Figure 2, the mean values of angular deformity for all groups were found to be statistically different ($p < 0.0001$). In control, excision of the medial half of proximal tibial growth plate of the rabbit showed to severe varus angulation of the tibia and retardation of growth (30°±4°). Although partial defect was treated with gelform without MSCs, degree of varus deformity (28°±4°) was still shown similar to untreated group. The varus angulation can be seen as early as 2-3 weeks after excision of the physis and progressively worsens with time. In group I (MSCs + 5% gelatin), large amounts of cells were leaked out with the medium. Angular deformity showed 23°±3°. The overall length of experimental leg was also shorter than that of unoperated leg. In group II (MSCs+10% gelatin + gelform), the final amount of deformity (14°±2°) was significantly less than that in group I although there was a trend toward varus angulation. In group III (MSCs + TGFβ_3 + gelfoam), growth arrest with angular deformation was prevented (9°±1°).

Discussion: The present study showed that the implanted MSC constructs with TGF? $_3$ were able to prevent formation of a bony bridge although the present experimental model was used large physeal defects (50% of the physis) and a relatively short period of 6 weeks. MSC with low concentration of gelatin tended to leak out from the physeal defect and the results was poor. Although high concentration of gelatin could prevent leakage of cells from the defect, the use of gelatin alone was not sufficient to prevent formation of a bony bridge. The addition of TGF? $_3$ would be necessary to induce the more differentiation of bone marrow stem cell, and stabilize the phenotype of prehypertrophic epiphyseal chondrocyte [2]. Histological section showed some areas of columnation interspersed with chondrocytes irregularly arranged in the matrix. These findings suggested that the repair of growth plate defects could be enhanced by the implantation of cultured MSCs. Although the transplanted MSC constructs would prevent angular deformity, there was still deficiency in the correction of angular deformity and restoration of full longitudinal growth of tibia. Full correction of angular deformity and length of the tibia was not achieved yet. More work must be done in this area to achieve full correction.

References: [1] Lee et al., J Ped Orthop 18, 155-160, 1998. [2] Wakitani et al., JBJS 76A, 579-590, 1994. [3] Lennon et al., In Vitro Cell Deve Biolo Animal 32: 602-611, 1996.

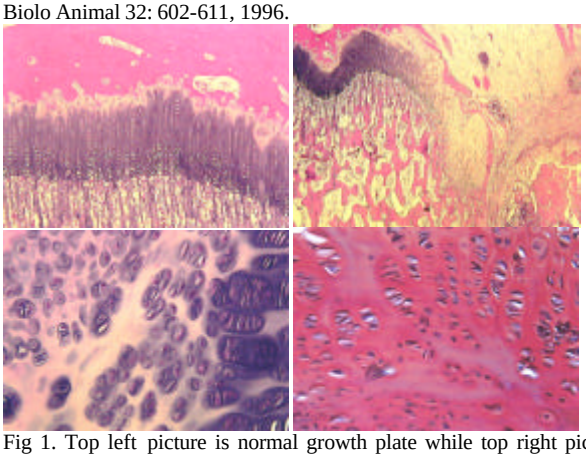

Fig 1. Top left picture is normal growth plate while top right picture shows bone bridge formation of growth plate (x40). Bottom left picture is H&E stain of regenerated chondrocytes of growth plate while bottom right picture shows safranin-o stain of the regenerated growth plate (x400).

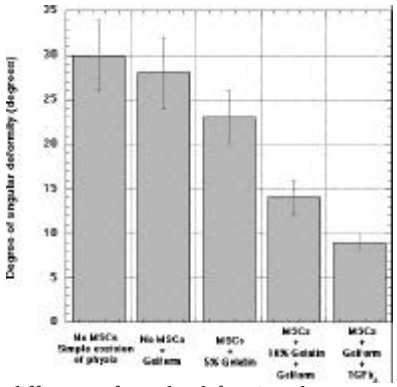

Figure 2. The difference of angular deformity when compared with right and left knee joints in each experimental group

Acknowledgement: This study has been supported by the VA merit grant.

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