

ALLOGENEIC STEM CELL THERAPY FOR JOINT DISEASE

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INTRODUCTION:

Mesenchymal stem cells (MSCs) differentiate to mesenchymal tissues such as bone, cartilage, tendon and meniscus (1-3). The delivery of these cells to the arthritic joint may have potential in slowing progression of the disease. In a previous study, intra-articular injection of autologous MSCs and hyaluronan (HA) in goat knees following ACL resection and total medial meniscectomy resulted in some regeneration of meniscus with protection of the articular cartilage (3). In this study, the ability of allogeneic MSCs, delivered as a suspension in HA at various times after injury, to impact meniscal regeneration and retard OA progression was studied.

METHODS:

MSCs, prepared from bone marrow aspirates taken from male donor animals, were retrovirally transduced to express enhanced green fluorescent protein (4). Twenty recipient male goats, unrelated to the donors, were randomized into 4 groups, and unilateral, total medial meniscectomy was performed. After 1 or 6 weeks, the operated joint was treated by injection of a suspension of 10×10^6 MSCs in 5 ml of HA (Hyalartin V, Pharmacia at 4 mg/ml) (n=5 for both groups) or with 5 ml HA (n=5 for both groups). The goats were subjected to a daily exercise regime, beginning 2-weeks post-surgery and which continued until sacrifice at 12 weeks. The harvested knee joints were macroscopically assessed for meniscal regeneration, joint effusion, osteophyte production and cartilage degradation. Tissues were fixed in formalin for microcomputerized tomography (μ CT) and/or histological analysis. Sections of meniscal tissue were stained with Safranin-O and an antibody specific for type II collagen. μ CT scanning of the middle medial condyle was performed by SCANCO Medical AG (Bassersdorf, Switzerland) and the results were analyzed for changes to the subchondral plate and underlying trabecular bone structure. The following parameters were quantified in treated and contralateral control joints: subchondral bone thickness, trabecular bone volume (BV/TV, %), trabecular bone number (DT-Tb.N, mm^{-1}) and spacing () and bone connectivity (ConnDens) (5). Results were analyzed using ANOVA and ANCOVA and included the corresponding measurements in the contralateral femur as covariates. After completion of μ CT the middle medial condyle was processed for histological staining with Toluidine Blue (TB).

RESULTS:

Gross evaluation of joints at sacrifice showed that regenerated neomeniscal tissue was associated with the medial compartment, consistent with earlier studies. The proportion of newly formed tissue was greater in those joints treated with cells 6 weeks after surgery compared to those joints treated with HA alone but this difference was not significant. However, in goats injected with cells 1 week after surgery, the amount of neo-meniscal tissue regenerated was significantly higher when compared to knees treated with HA alone. Immunohistochemical and histological staining of the regenerated neomeniscal tissue indicated a highly cellular, fibrous network rich in type I collagen with isolated zones rich in proteoglycan, but negative for type II collagen. Up to 4 TB-stained sections, spanning the middle medial condyle, were scored for articular cartilage structural damage using a modification of a multiparameter grading system described previously (6). Extent of cartilage loss, extent of fissuring, and the presence of deeper clefts in the articular cartilage were assessed (6). Fig. 1a shows an example of a cell-treated joint assigned a score of 0 (normal) and a HA-treated joint assigned a score of 12. Total loss of cartilage is assigned a score of 24. Damage to the cartilage was less in cell-treated joints, suggesting that the cartilage may be protected from the abnormal mechanical forces associated with the loss of meniscus.

Group	Time (wks)	Score (Mean \pm SD)
HA	1 week	9.4 \pm 2.9
HA + MSCs	1 week	4.4 \pm 5.2
HA	6 weeks	5.0 \pm 2.6
HA + MSCs	6 weeks	2.4 \pm 2.5

Table 1. Articular cartilage damage on the middle medial condyle

However, these results did not achieve significance, given the number of animals used (n=5; Table 1).

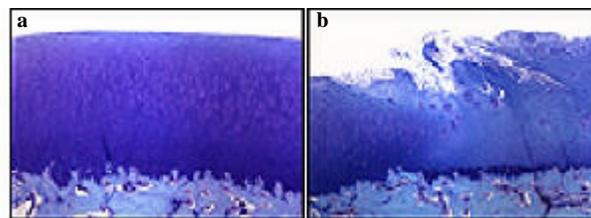


Figure 1. Protection of articular cartilage by MSCs. Appearance of the articular cartilage on the middle medial condyle 6 wks after injection of MSCs + HA (a) and HA alone (b).

μ CT analysis was performed to determine the effect of stem cell therapy on bone changes that occur as a result of instability in this model. Meniscectomy caused thickening of the subchondral plate and underlying trabecular bone in the medial femoral condyle (higher BV/TV). For the ANCOVA, the covariate was significant for subchondral thickness ($p < 0.002$), trabecular ConnDens ($p = 0.013$), DT-Tb.N ($p < 0.005$) and DT-Tb.Sp ($p < 0.003$). The effect of MSC treatment compared to treatment with HA alone (ANOVA) approached statistical significance for DT-Tb.N ($p = 0.066$) and DT-Tb.Sp ($p = 0.07$) (Fig. 2). There was no significant effect on subchondral thickening. DT-Tb.N was lower (overall: -0.21, 1wk: -0.18, 6wk: -0.23) and DT-Tb.Sp was higher (overall: 0.033, 1wk: 0.020, 6wk: 0.045) in MSC treated animals.

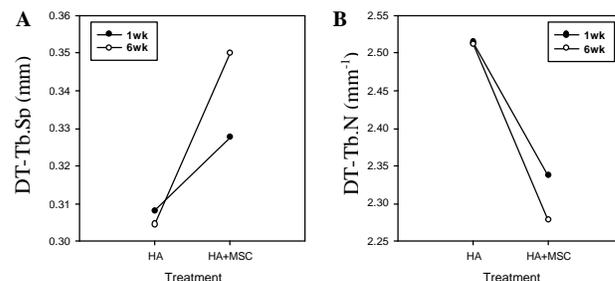


Figure 2. Effect of cell administration on trabecular bone spacing (A) and number (B) on the middle medial femoral condyle.

DISCUSSION:

Injection of allogeneic MSCs into destabilized, osteoarthritic joints resulted in regeneration of meniscus. Early introduction of MSCs resulted in a more favorable repair response. It is possible that delivery of MSCs may impact the granulation phase of wound healing. In parallel with this tissue regeneration, there was a chondroprotective and osteoprotective effect. Early changes to bone as a result of instability were evident by μ CT comparison of treated and contralateral joints. Changes to trabecular volume, number and spacing were positively impacted by treatment with MSCs. These observations suggest the therapeutic benefit of injected MSCs in the OA joint.

Thus, allogeneic MSCs may contribute to the natural repair process in joints after meniscectomy and impact the progression of osteoarthritic changes that occur as a result of destabilization of the joint.

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