• Distribution and homing of stem cells after intra-articular injection to normal and arthritic joints

[1]Watts, AE; +[1]Nixon, AJ
[1]Comparative Orthopaedics Laboratory, Cornell University, Ithaca, NY
+ajn1@cornell.edu

INTRODUCTION

Cell based therapies for the repair of articular cartilage injuries are being pursued due to poor self-repair and limited efficacy of pharmacologic therapies. Adult, bone-marrow derived mesenchymal stem cells (MSCs) are a stem cell source for autologous cell transplantation to musculoskeletal tissues with good proliferation and chondrogenic differentiation potential. Despite the early promise of cartilage repair with naïve MSC implantation, evaluation in large animal models does not support clinical use.

Homing of stem cells in response to local chemokine production and capillary fragility has been shown for bone injury, but is not nearly as clear in cartilage damage or progression of arthritis. Anecdotal evidence and clinical experience suggests that intra-articular injection of autologous bone marrow derived mesenchymal stem cells (MSCs) is an effective treatment for joint injury where more routine treatment protocols have failed.(1) Clinical efficacy may be due to focal localization of injected MSCs into articular cartilage defects, or potentially remote formation of synovial niches that enhance joint repair by synovial trophic factor elaboration or anti-inflammatory effects at the capsular and synovial level.

We hypothesized that autologous bone marrow derived mesenchymal stem cells (MSCs) would engraft to abnormal cartilage in OA joints but not in normal joints following intra-articular injection.

METHODS

Twenty-nine joints from 10 skeletally mature (median age, 4.5 years; range, 2-10 years) Thoroughbred or Thoroughbred cross horses were characterized as normal (noOA) or abnormal (OA) through clinical, lameness and radiographic examination. Bone marrow MSCs were isolated through tissue culture plastic adherence. Second passage autologous MSCs (3x10e6 for fetlocks and 5x10e6 for femoropatellar joints) were labeled with fluorescent nanoparticles (Quantum® dots; Qdot®) or left unlabeled (7 joints), and then injected to 17 noOA and 12 OA joints. Three ml of modified Eagles' medium (MEM) and 5 ml MEM were used as a carrier solution for metacarpophalangeal and femoropatellar injection, respectively. Joint tissues (cartilage, synovium, osteochondral) were collected 1 week after MSC injection for frozen and formalin fixed histologic preparations and evaluated for the presence of fluorescently labeled cells and tissue architecture. Synovial fluid analysis included fluorescent evaluation of cytospins for the presence of florescent labeled cells and complete synovial analysis.

Twelve joints from Thoroughbreds (n=6; median age, 5 years; range, 3-7 years) with a similar distribution of OA and noOA fetlocks and stifles were injected with the same volume of carrier solution (MEM) without cells. Synovial fluid assay was performed one week after MEM injection. Joint tissues were not harvested.

Descriptive statistics were generated. Comparisons between groups for proportion of positive tissue sections was made through Fisher's exact test. Comparisons between groups for synovial fluid values were made with Wilcoxon's rank sum test. Two-tailed p-values ≤ 0.05 were considered significant.

RESULTS

Clinical findings following MSC injection included increased lameness (n=2; 4/5 AAEP grading scheme), and severe (11), moderate (3), and slight effusion (4). Synovial fluid abnormalities included elevated nucleated cell counts (median 2,800/ul; interquartile range 1,750-4,450/ul), consisting of large mononuclear cells and small lymphocytes (Table 1). There were no statistically significant differences in synovial fluid parameters between Qdot® labeled MSC and unlabeled MSC injected joints. Of the MSC injected joints, only total nucleated cell count (TNCC) was different between OA (median, 2,300 cells/ul; interquartile range, 1,420-2,875 cells/ul) and noOA joints (3,400 cells/ul; 2,400-5,150; 2-tailed p=0.04). Total nucleated cell count, percent macrophages and total protein were significantly different (lower) in MEM injected joints versus MSC injected joints (Table 1). Qdot® labeled MSCs were identified in synovial fluid from all Qdot® injected joints 1 week after injection.

QDot® labeled MSCs were found predominantly in the synovial membrane compared to cartilage (p<0.0001; Figure 1). Adherence of labeled MSCs to cartilage was minimal and found in 17 of 97 cartilage sections. The proportion of positive sections from synovium and cartilage was not different between OA and normal joints (P=0.79).

	MSC injected joints		MEM only injected joints		2-tailed p value
Total nucleated cell count cells/microl	2,800	1,750-4,450	850	300-1,700	<0.001*
Lymphocytes %	32	19-47	34	29-53	0.5
Macrophages %	64	48-76	42	27-60	0.05*
Neutrophils %	5	0-1.5	7	2-36	0.12
Total protein g/dl	<2.5	<2.5-3.15	<2.5	NA	0.01*

Table 1. *Synovial fluid cytology (median; interquartile range) one week after bmMSC in modified Eagles' medium (MEM) injection or MEM alone. Values that were significantly different (P≤0.05) are marked by an asterisk.*

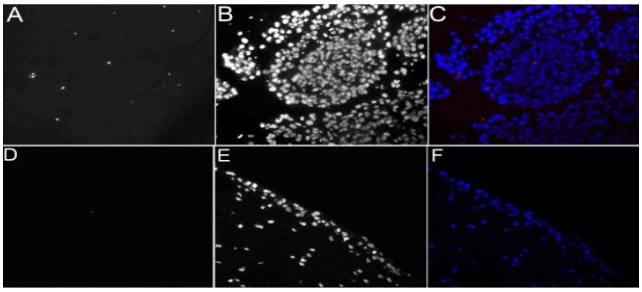

Figure 1. *Synovium (A, B, C) and cartilage (D, E, F) from an OA fetlock (400x), displaying A & D) Qdot® labeled MSCs, B & E) Hoechst stained nuclei and C & F) merge.*

DISCUSSION

Contrary to our hypothesis, MSCs did not home to cartilage injury in normal or OA joints. This suggests that intra-articular injection of MSCs would not be a useful technique for cartilage re-surfacing. However, the large presence of labeled MSCs within the synovial tissue indicates that MSCs may engraft to synovial tissue. Mild, joint flares were common after intra-articular MSC injection. This was surprising given the relatively low dose of MSCs used. In a caprine stifle model of OA, a much larger cell dose (10x10e6 cells) was used without evidence of joint reaction.(2) These cells were suspended in hyaluronan rather than MEM. It seems unlikely that the addition of HA, rather than MEM, would reduce the joint reaction, given that clinical flares seem relatively common when HA is injected without steroid combination. We elected to use MEM rather than HA, in an effort to reduce the likelihood of post injection joint flares. Additionally, compared to joint flares in clinical patients, flares were mild with transient lameness and effusion that were easily distinguished from synovial sepsis. Although the flares were mild and self-limiting, this joint reaction is concerning, and additional experiments for a dose response test as well as characterization of the flare is warranted.

There were no differences in synovial cytology parameters for joints injected with unlabeled MSCs and Qdot® labeled MSCs, suggesting that Qdot® labeling is safe for intra-articular cell tracking. When comparing synovial cytology between OA and noOA injected joints (both labeled and unlabeled cells) there was a significant reduction of TNCC for OA joints compared to noOA joints. This may be a result of the immune modulating ability of MSCs when placed within an inflamed environment.(3)

SIGNIFICANCE

If intra-articular injection of MSCs is effective in reducing joint disease, it may be through modulation of synovial fluid constituents, inflammation, or cytokine profile, rather than direct cartilage repair.

References:
1. Ferris DJ, et al. Proc Annu Conven AAEP, 2009;Las Vegas; p59-60.
2. Murphy JM, et al. Arthritis Rheum. 2003 Dec;48(12):3464-74.
3. Yagi H, et al. Cell Transplant. 2010;19(6):667-79.

Acknowledgements: Study funded by private gifts to the laboratory.