Administration of Adipose Derived Stem Cells Decreases Fibrosis Following Chronic Rotator Cuff Tear

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Introduction: Rotator cuff tears are among the most common upper extremity injuries, and are associated with atrophy of muscle fibers, fibrosis, and an accumulation of lipid within and around muscle fibers, commonly referred to as "fatty degeneration." Surgical treatment of torn rotator cuff muscles does not reduce fatty degeneration, and persistent weakness and atrophy is a major drawback to successfully treat these injuries. Adipose Derived Stem Cells (ADSCs) are a class of multipotent, mesenchymal stem cells that have shown promise in increasing tendon to bone healing after rotator cuff tear, and decreasing fibrosis in other disease models (1,2). However, studies assessing the effect of ADSC treatment on muscle fiber contractility and biochemical markers of fatty degeneration in chronically torn rotator cuff have not been performed. To gain greater insight into the effect of ADSC administration on muscle function following chronic rotator cuff tear, we used a well-established chronic experimental technique of full-thickness rotator cuff tear and repair in a NIH nude rats. The nude rat does not produce T-cells, is commonly used for the study of xenografts and develops fatty degeneration in a similar fashion to other rat models of chronic rotator cuff tear (3). We tested the hypothesis that administration of ADSCs into the chronically torn supraspinatus muscles of immunodeficient rats would reduce fibrosis and fat accumulation, and enhance muscle fiber specific force production.

Methods: This study was approved by our IACUC. Adult male T-cell deficient NIH nude (NIH-Foxn1rnu) rats were subjected to a massive full-thickness, bilateral tenectomy of the supraspinatus muscles as described (4). After a period of 28 days, the torn supraspinatus muscles were repaired, and received an injection of either vehicle alone (Lactated Ringers Solution, LRS, 0 ADSCs), 3x10^5 ADSCs, or 3x10^6 ADSCs suspended in LRS. ADSCs were isolated from human adipose tissue and extracted using a fully automated Cell Isolation System (TGI). Rats were allowed to ambulate normally until sacrifice two weeks following the repair procedure. Supraspinatus muscles were isolated, weighed, and prepared for muscle fiber contractility, biochemical measures, and immunohistochemistry. Percent human DNA was assessed by qPCR using primers against human-specific b2-microglobulin, and a standard curve with known ratios of human and rat DNA. For immunohistochemistry, rotator cuff muscles were cryosectioned and labeled with either human Alu probes, which are transposable elements only found in human DNA, or incubated in antibodies against human b2-microglobulin to detect proteins of human origin. Hydroxyproline makes up approximately 14% of the dry mass of fibrillar collagens, and was measured to approximate total collagen content. Total lipid was extracted in (2:2:1.8) chloroform:methanol:aqueous solvent, and spotted on HPTLC plates. Lipid content was assessed with Rhodamine 6G staining and band densitometry. Differences between groups were tested using one-way ANOVAs (a=0.05) followed by Fisher’s LSD post-hoc sorting.
Results: Two weeks following repair of chronically torn muscles, there was 0.2% human DNA in the 3×10^5 ADSC group and 8.7% human DNA in the 3×10^6 ADSC group (Figure 1A). Human β2-microglobulin was present on the plasma membranes of muscle fibers and other mononuclear cells in ADSC treated animals (Figure 1B). Additionally, human Alu DNA probes were also detected in treated samples (Figure 1C). There were no differences in muscle mass (Figure 2A), but there was approximately a 40% reduction in hydroxyproline content in the treated groups compared to controls (Figure 2B). For lipid content, there were no differences between groups for triglyceride species or phospholipid species (Figure 2 C-D). Although no significant differences in muscle fiber morphology or contractility were observed, compared to controls there was a statistical trend in the 3×10^6 group for a 12% decrease in fiber CSA (p=0.07) and a 25% increase in muscle fiber normalized force (sFo) production (p=0.07, Figure 3).

Discussion: The objective of this project was to evaluate the ability of ADSC treatment to enhance muscle regeneration following chronic rotator cuff tear. We were able to successfully incorporate human ADSCs into torn rotator cuff muscles at the time of repair, which persisted for at least two weeks after injection. Following integration, these cells were able to make functional proteins, and reduced collagen accumulation. Additionally, a trend towards an improvement in muscle fiber sFo production was observed in the 3×10^6 ADSC group. Combined with studies that found ADSCs augment tendon to bone healing, ADSC treatment in chronically torn rotator cuff muscles likely will improve muscle quality and lead to a more secure repair. Overall, this study demonstrated that ADSCs could be stably incorporated into injured muscle, and lead to a reduction in fibrosis after repair, a trend towards improved muscle fiber sFo production, and no worsening of fatty degeneration. Combined, these results provide support for a clinical trial in patients with chronic rotator cuff tears.

Significance: As there has been much interest in the use of stem cell-based therapies in musculoskeletal regenerative medicine, the reduction in fibrosis and trend towards an improvement in single fiber contractility, combined with previous studies using ADSCs in torn rotator cuff muscles, suggest that ADSCs may be beneficial to enhance the treatment and recovery of patients with chronic rotator cuff tears.