

Influence on Functional Recovery of Mononuclear Cell Transplantation in Rotator Cuff Repair: A Preliminary Report

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Abstract

Rotator cuff tears occur mainly due to overload of the tendons, which leads to a progressive degenerative process and to rotator cuff tears. Surgical treatment is indicated in more advanced stages of the lesion. Retear affects approximately 35% of patients. The goal of the present study was to investigate the effect of mononuclear cell transplantation in rotator cuff repairs in relation to patient functional recovery. Thirty patients with rotator cuff tear were divided into two groups and submitted to rotator cuff repair. In addition to the surgical procedure, the experimental group was submitted to transplantation of mononuclear bone marrow cells. One to two years post-op, both groups were submitted to evaluation of pain, amplitude of movement, retears, and isokinetic evaluation of flexion-extension movements, external and internal rotations, and abduction and adduction of the shoulder. The operated shoulder was compared to the healthy shoulder. Mann-Whitney test was used to evaluate the percentage improvement of the operated shoulder in relation to the healthy shoulder. SPSS (13.0) was used for statistical analyses; the level of significance was $p < 0.05$. The control and the experimental groups presented near-normal values for the variables analyzed in the comparison between shoulders. However, the experimental group showed better results than the control group. Functional recovery of patients submitted to repair with mononuclear cell transplantation was different than that of controls: the incidence of retear was lower in the experimental group; also, the muscle torque and balance values were closer to the healthy shoulder values for the experimental group than the controls.

Keywords: Rotator cuff; Mononuclear stem cells; Functional evaluation

Introduction

Rotator cuff disease compromises the health status and functional independence of the elderly population [1]. More advanced stages of the disease are treated surgically with rotator cuff repair [2]; the goal of the procedure is to recover shoulder function [3].

Rotator Cuff (RC) disease is one of the common causes of shoulder pain and loss of muscle strength [4]. Rotator cuff tears occur due to degenerative factors and micro traumas that damage the tendons at the point of insertion onto the humeral head [4]. Massive RC tears occur in association with tendon lesions; in these lesions there is muscle atrophy and deposition of fatty tissue [5]. The lesion begins with an inflammatory process; after, with repetitive stress, tendinosis and rotator cuff tears may follow [6]. Because it is a degenerative disease, the prevalence increases with age [1].

Surgical treatment is indicated in cases of advanced lesions with rotator cuff tear [1]. Surgery is also indicated in cases of constant pain, muscle weakness, and especially progressive muscle weakness [4]. Rotator cuff repair is the most common procedure [2].

Most patients submitted to surgery already present with advanced degeneration of the tendons, which are also atrophied. Due to the atrophy, there is re-tearing of tendons even after successful surgical procedure. Several studies have shown, by means of magnetic resonance imaging or ultrasound that the degeneration of the tendons remains after surgical treatment. The quality of the tendons at the moment of surgical repair is one of the main factors that determine the success of the treatment. However, up until the present moment it is also one of the factors that cannot be changed [2,7-9].

The balance between skeletal muscle structures is one of the factors that need to be considered in shoulder lesions. In RC lesions the biggest

imbalance is in the synergy between outer and inner rotations. The outer rotator muscles act eccentrically during internal rotation to avoid excessive translation of the humeral head in the glenoid cavity [10].

The stability of the shoulder depends on adequate alignment of the articulation, and on muscle balance between muscle activation and muscle strength applied to the bones. The timing of the temporal recruitment pattern and the level of muscle activation during movement are important factors for movement coordination [11-14].

Studies suggest that proper dynamic function of shoulder stabilization muscles, especially the rotator cuff, can be a determinant factor in the prevention and/or severity of the lesions to skeletal muscle tissue [15]. In the past few years there has been an increase in the use of stem cells in the treatment of degenerative diseases. There have been studies in the areas of cardiology, neurology, and dentistry for the treatment of different types of disease [16]. However, there are few publications about the use of stem cells in orthopedics [16]. We identified a single study that showed good results in the application of mononuclear bone marrow stem cells to rotator cuff repair [17].

Based on the replication and differentiation of stem cells, and based on the positive results of the study, it is possible that the use of stem cells on conjunctive tissue such as the tendon will stimulate its repair,

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as is the case of other tissues. Stem cell transplantation into damaged tendons may provide additional stimuli for tendon repair. It may help create healthy and resistant tissue with enhanced regeneration and structural recovery abilities. It may also stimulate the recovery of viscoelastic properties and of muscle strength.

The goal of the present study was to evaluate the effect of mononuclear bone marrow cell transplantation on the recovery of shoulder functionality after rotator cuff tear repair. We evaluated muscle torque, muscle imbalance, pain, and movement amplitude.

Materials and Methods

The study included 30 patients with rotator cuff tear submitted to rotator cuff repair. Participants were randomly selected by the orthopedic team at the Hospital de Clinicas de Porto Alegre [17]. Patients were divided into two groups with participants of both sexes and older than 50 years. Each group had 15 patients. Exclusion criteria were full irreparable tears for both groups. In both groups the surgical procedure was performed through a mini open incision and an arthroscopic approach was used to avoid confusion on true postoperative MR. All sutures were done through the bone with the aid of a reinforced steel needle. The control group included patients submitted to rotator cuff repair without cell transplantation; the experimental group was submitted to repair with mononuclear bone marrow cell transplantation.

Prior orthopedic procedure, bone marrow mononuclear cells were obtained. In short, after general anesthesia, the patient was positioned in lateral recumbent position and 100 ml of bone marrow was obtained from the posterior iliac crest. Bone marrow aspirate was then transferred to the Culture and Characterization of Hematopoietic Cells Laboratory at the Experimental Research Center. The bone marrow mononuclear fraction was obtained - in aseptic conditions, according to the good manufacturing practices - by Ficoll Hypaque density gradient, and then resuspended in saline solution enriched with 10% autologous serum to a final volume of 10 ml. Final cell viability, as determined by trypan blue, was above 90%. An aliquot of the final suspension was submitted to flow cytometry analysis for the presence and counting of CD34, CD45 and CD38 cells in a 3-color FACS Calibur BD Flow Cytometer (Becton Dickinson, San Jose, CA). The average and the median concentration of CD 34+ injected in the patients was respectively 5.65×10^6 and 3.81×10^6 of mononuclear cells [17].

During the preparation of the mononuclear cell suspension, the patient was placed in the beach-chair position and the surgery was initiated. Incision was performed to the transdeltoid lateral approach. All patients underwent acromioplasty for an increase in the subacromial space. The tendon-tendon and tendon-bone sutures were performed with Ethibond 5/0. After closure of the lesion, 10 ml of the cell concentrate were injected in the tendon and bone in participating in the "footprint". After plan closure, the patient was immobilized in a sling for 4 weeks. Physical therapy was initiated after the fourth week in a conventional rehabilitation protocol [17].

After one year post-op, patients were contacted for functional evaluation. The evaluation included a questionnaire for identification of the patient, movement amplitude, pain, and isokinetic test (muscle torque and imbalance). In the final study, the control group included nine subjects and the experimental group included 12 subjects.

The isokinetic test was carried out using the following criteria for exclusion: [1] history of more than one surgery on shoulder articulation; [2] neurological, skeletal muscle, metabolic and heart

alterations that would not allow for the participation in maximal voluntary contraction tests; [3] contraindication to participate in stress tests; [4] arterial pressure higher than 240/120 mmHg; [5] angina and electrocardiogram alterations that indicate ischemia; and [6] presence of neurological, skeletal muscle, inflammatory, metabolic or active neoplasia. We also observed the Humac™ Norm System User's Guide (Humac™/Norm Testing & Rehabilitation System - User's Guide - model 770. Computer Sports Medicine, 2005) [18].

The present study was approved by the ethics committee of Universidade Federal do Rio Grande do Sul. All participants signed an informed consent form that assured patients of their rights according to resolution number 196/96 of the National Committee of Health; the form informed patients of all the procedures that would be carried out.

Isokinetic tests were carried out at the exercise lab (LAPEX) of the School of Physical Education [18]. Patients were positioned in the dynamometer in a sitting position; they maintained shoulder abduction and full extension of the elbow to execute movements of extension, flexion, abduction, and adduction. Patients remained in the sitting position to perform internal and external rotation; however, in this case the elbow was maintained at a 90-degree angle flexion. Next, a warm-up and familiarization session was carried out. Patients performed 10 submaximal repetitions (50% of maximal voluntary contraction) for the following movements: flexion and extension, abduction and adduction, and external and internal rotation of the shoulder. The warm-up was carried out at an angle speed of 120°/s. After a two-minute rest, we carried out isokinetic and isometric muscle torque evaluations.

These tests consisted of three repetitions at a speed of 60°/s for isokinetic evaluation, and three repetitions at the angle of maximal strength for isometric evaluation. The procedure was carried out for each articular movement evaluated. Between each series there was a two minute rest to minimize the effects of fatigue. During the isokinetic tests we gave patients the same verbal stimulation to achieve maximal effort [19].

Peak torque for each group was used to evaluate relative improvement of torque. Muscle imbalance was calculated for each movement by the antagonist/agonist ratio for abduction/adduction, flexion/extension, and external/internal rotation. The torque and/or muscle imbalance values for the non-operated shoulder were used as the healthy control values (100%). Based on these values we calculated the relative improvement of the operated shoulder compared with the non-operated (healthy) shoulder. After the evaluation, patients stretched and ice was applied to the shoulder articulation for 20 minutes. Ice was applied to prevent possible muscle discomfort due to non-habitual maximal effort. Amplitude of shoulder articulation was obtained using a goniometer (Advanced Rehab™ (Systems, Inc.). We measured external and internal rotation, abduction, adduction, flexion and shoulder extension movements [20]. Patients were asked about pre- and post-op shoulder pain during early evaluation. Patients were given a ruler showing a 0 to 10 scale of pain; they were asked to report the pain levels they felt at that specific moment [21-24]. Tendon healing and number of retears were controlled by clinical evaluation and using magnetic resonance imaging.

Statistical Analysis

Quantitative and population variables are presented as average (plus or minus standard deviation); t tests for independent samples were

used for comparison between groups. Other variables are presented as median and minimum and maximum values. Preliminary statistical analysis showed that the data were non-parametric. Therefore, we adopted the Mann-Whitney test to evaluate the percentage improvement for the operated shoulder in relation to the healthy shoulder in the comparison between groups. The Chi - Square test was used to compare the number of re-ruptures between the groups. Statistical analyses were carried out using SPSS (Statistical Package for the Social Sciences) version 13.0. The level of significance adopted was $p < 0.05$.

Results

Table 1 shows the population results. Groups were homogenous; there were no significant differences in body mass, height and age. Analyses of pre- and post-op shoulder pain reports showed that both groups had similar results. Both the control and experimental groups reported improvement of pain after surgery. The experimental group reported pre-op pain levels of 8.75 ± 1.42 , and post-op levels of 0.67 ± 2.0 ; the control group reported pre-op levels of 8.2 ± 1.9 and post-op levels of 1.4 ± 2.9 . Therefore, both groups showed similar improvement in pain. In general, the movement amplitude, muscle torque, and muscle imbalance increased for both groups when the operated shoulder values were compared to the healthy shoulder values (Table 2). However, the values of movement amplitude of flexion, abduction muscle torque, and abduction/adduction muscle imbalance showed a greater improvement for the experimental group than the control group (Table 2). Five patients in the control group presented with re-tear after one year of surgery; one patient in the experimental group showed re-tear during the same period, no significant difference.

Discussion

Evaluation of retears after rotator cuff repair showed that 33.3% of patients submitted to surgery but no transplantation of stem cell presented with retears. Other studies have shown high failure rates in tendon repair of approximately 27.8% [25]. The same authors carried out another study [26] that showed positive results for 11 of the 12 patients who were submitted to rotator cuff repair. However, in that study not all patients initially presented with total tear of the rotator cuff.

Retear in the experimental group (stem cell transplantation) was 6.6%. The incidence of re-tear was thus almost five times less in the experimental group than the control group. We also observed that the patients with the lowest indicators of improvement in the comparison with the healthy shoulder also showed the highest rates of retears. Similarly, Marcondes et al. [27] evaluated the ratio between muscle strength and functionality in patients with shoulder impingement syndrome. The authors found a greater deficit of shoulder flexion and rotation strength associated with functional deficit.

Yet another study reported that suture failure after surgery

Group	N	♂	♀	Age (years)	Body mass (kg)	Height (cm)
Experimental	12	8	4	58.5 ± 7.3	72.1 ±12.1	160.5 ± 8.3
Control	9	6	3	53.8 ± 7.9	67.4 ±10.6	147.8 ± 7.8
P	-	-	-	0.825	0.902	0.225

♂=males; ♀=females; p=p value

Table 1: Characteristics of the population: sex, age, body mass and height (average \pm SD).

Relative values	Experimental	Control	P
Abduction amplitude	70.7 (52.8; 119.6)	62.5 (33.3; 225)	0.247
Flexion amplitude	85.2 (66.7; 100)	70.3 (48.9; 260)	0.049*
External rotation amplitude	90.2 (53.8; 216.7)	94.8 (50.8; 288)	0.808
Isometric abduction torque	83.3 (37.6; 242.9)	49.8 (32.7; 93.7)	0.034*
Isokinetic abduction torque	87.4 (28; 163.2)	45.8 (21.4; 133.3)	0.049*
Isometric flexion torque	82.8 (45.7; 188.9)	55.9 (27.3; 383.3)	0.169
Isokinetic flexion torque	89.7 (35.3; 258.8)	64.1 (41.4; 117.6)	0.345
Isometric external rotation torque	88.2 (37.5; 300)	51.9 (40; 150)	0.277
Isokinetic external rotation torque	80.1 (33.3; 128.6)	52.9 (25; 114.3)	0.193
Muscle imbalance abduction/adduction	88.5 (28.1; 180.1)	46.8 (43.3; 59.3)	0.009*
Muscle imbalance flexion/extension	88.89 (30.8; 173.4)	47.9 (20.6; 112.8)	0.058
Muscle imbalance external/internal rotation	85 (47.3; 262.5)	68.7 (38.8; 145.3)	0.169

* $p < 0.05$

Table 2: Percentage values for movement amplitude, muscle torque, and muscle imbalance in the comparison between operated and healthy shoulders. Values are presented as medians (minimum and maximum range).

occurred in 90% of cases. The authors reported that the rate may be reduced to 40% with the application of an extracellular matrix graft to the myotendinous junction; the graft is applied to improve cicatrization of rotator cuff repair, decrease gap formation and improve load to failure. However, the study is still in the experimental stage [7].

There is evidence of improvement in strength and muscle balance after rotator cuff repair [28]. In our study we found an improvement in muscle strength and muscle balance after rotator cuff repair combined with transplantation of stem cells, especially in shoulder abduction and flexion. Randelli et al. [28] showed that application of platelet rich plasma in arthroscopic rotator cuff repair was associated with improvement in muscle strength for these same movements; they also showed improvement in functional scores for the experimental group in comparison to controls [28]. The literature indicates that post-op muscle strength loss is of approximately 30% in the comparison between operated and healthy shoulder [29,30].

Post-op pain was similar for both groups. Our results were similar to studies that showed improvement in pain and discomfort after rotator cuff repair, and that showed improvement in functional scores [3] and pain [31-33]. Another study that also evaluated two groups submitted to rotator cuff repair and used platelet rich plasma reported better pain reduction in the platelet rich group in comparison to the control group [28,34]. Movement amplitude was also associated with better improvement ratings in the stem cell group in comparison to the control group; the improvement, however, was only for the shoulder flexion movement. The improvement in shoulder movement amplitude after rotator cuff repair was approximately 51% [35]; our results showed an improvement of 70% in the control group and 85% in the experimental group. Other studies have shown an improvement of 47% after surgery for the shoulder flexion movement [36].

In spite of the functional improvement that was detected in transplanted mononuclear cells group, there are many questions

without answers to explain that. This study searched only for the clinical and functional evaluation of the patients, and so no biopsies were made. Since no biopsies were made in those patients, it is not possible to affirm that the tissue formation somehow induced by the mononuclear cells was of better fibrous quality than usually seen in conventional repairs. However, it is well accepted that the main reason for rotator cuff lesions is the previous degenerative process that turns the tendon more prone to ruptures. This tissue weakness would be the main responsible for the repairs' short-term failure in spite of how well done was the surgical procedure performed. Maybe the initial healing process was improved by the aid of the mononuclear cells and this improvement goes beyond the time expected for its action, but the fact is that patients treated with it showed a better shoulder after two years follow-up than someone would wait for. Even though this initial finding was promising, further studies were necessary to better explain the influence of mononuclear cells in the tendon healing process.

Conclusions

Functional recovery of patients submitted to rotator cuff repair with transplantation of stem cells was different than the control group with no stem cell transplantation. The stem cell group showed lower retear rates and better ratio of muscle torque and muscle balance values in relation to the healthy shoulder, in comparison to the control group.

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