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Is resection of the tendon edge necessary to enhance the healing process? An evaluation of the homeostasis of apoptotic and inflammatory processes in the distal 1 cm of a torn supraspinatus tendon: part I

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Background: We hypothesize that the expression of proapoptotic and antiapoptotic molecules and cytokines is dependent on the distance from the torn supraspinatus tendon edge and this expression may influence its potential for healing. The aim of this work is to evaluate the expression of proapoptotic Bax molecule and caspases 3, 8, and 9; antiapoptotic Bcl-2 molecule; and proinflammatory tumor necrosis factor (TNF) α and anti-inflammatory interleukin 10 (IL-10) in 3 sections taken from a 1-cm section of the edge of a torn supraspinatus tendon: 3 mm distal and 3 mm proximal, as well as the remaining 4-mm middle section between them.

Methods: Nine patients, with a mean age of 58 years, were included in the study. All fulfilled strict inclusion criteria regarding the morphology of the tear and reconstruction technique. Samples were taken from the ruptured supraspinatus tendon at the time of arthroscopic repair. Quantitative real-time polymerase chain reaction assay was used for analysis.

Results: The expression of caspases 9, 8 and 3; Bax; and TNF-α significantly decreased from the distal to the proximal parts of the tendon edge ($P < .05$). However, a significant increase in Bcl-2 and IL-10 expression was also found in the same direction ($P < .05$).

Conclusions: Tenocytes can reduce the expression of proapoptotic caspases 3, 8, and 9 and Bax, as well as proinflammatory TNF-α, by increasing the expression of Bcl-2 and IL-10 within 1 cm of the supraspinatus edge in a distal to proximal direction. Resection 4 to 7 mm from the edge of the torn supraspinatus tendon may enhance the healing process by reaching a reasonable compromise between molecular homeostasis of apoptotic and inflammatory processes and mechanical aspects of rotator cuff reconstruction.

The study was approved by the local ethics committee.

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Despite decades of experience in tackling the problem, the reconstruction of a damaged rotator cuff often results in the tear recurring.[4,5,16,21,35,42] The recurrence of the tear leads to the progression of degenerative changes in the muscle, decreasing its strength further.[12] It has been shown experimentally that, in the case of second-degree fatty degeneration of the supraspinatus or higher, according to the Goutallier computed tomography classification,[14] considerable morphometric alterations occur.[10,11] Despite this, the muscle is still able to generate 34% of control-value strength.[11] Hence, it is vital for the reconstructed tendon to heal completely.[12]

A review of the current literature indicates that one reason for the healing difficulties seen to affect the repaired tendon may be apoptosis,[2,22,24,25,27,28,40,41] as well as upregulation of the proinflammatory cytokines associated with the homeostasis of its extracellular matrix.[27,40] Although earlier research has shown a greater degree of apoptosis[2,22,24,25,27,28,40,41] and a higher concentration of proinflammatory cytokines to be present in the torn supraspinatus tendon[27] and the synovium,[34] there are no data regarding the defense activity of the tenocytes in response to increased concentrations of antiapoptotic molecules and anti-inflammatory cytokines. A greater understanding of the homeostatic basis of the apoptotic and inflammatory processes should enable a more comprehensive estimation of the healing potential of the torn tendon edge and better establish the degree of resection needed to increase the chance of healing.

The hypothesis of the study is that the expression of proapoptotic and antiapoptotic molecules, as well as proinflammatory and anti-inflammatory cytokines, depends on the distance from the edge of the torn supraspinatus tendon and that this trend may determine the potential for the rotator cuff to heal at any one point. The aim of this work is to evaluate the expression of proapoptotic factors (Bax proteins and caspases 3, 8, and 9) antiapoptotic factors (Bcl-2 proteins), as well as proinflammatory cytokine tumor necrosis factor (TNF) α and anti-inflammatory interleukin (IL) 10, in 3 sections taken from a 1-cm section of the resected margin of a torn supraspinatus tendon: a 3-mm section from the distal end, a 3-mm section from the proximal end, and the remaining 4-mm middle section.

Materials and methods

Informed written consent was obtained from all patients. The study included 9 patients: 7 men and 2 women. The mean age of the patients was 58 years (range, 51-66.1 years; SD, 5.01 years). Samples were taken from the superior part of the ruptured supraspinatus tendon at the time of arthroscopic repair of a U-shaped rotator cuff tear.[9] The inclusion criteria were 3-fold: (1) the tear dimension was between 2 cm and 3 cm from lateral to medial and between 2 cm and 2.5 cm from anterior to posterior; (2) the tear extended through the full thickness of the supraspinatus and the anterior part of the infraspinatus,[29] with an intact subscapularis tendon; and (3) repair using the margin-convergence technique was successful,[6] with acceptable tension after a 1-cm-long and 1-cm-deep resection of the superior edge of the supraspinatus tendon (cuff mobility was evaluated through stitches after prior mobilization and selective capsulotomy, cutting the coracohumeral ligament from the coracoid process).

The criteria for exclusion were as follows: the absence of concomitant disorders such as biceps pathology requiring tenodesis or tenotomy, fractures, rheumatoid arthritis, osteonecrosis, glenohumeral arthritis, or labral pathology; the patient was receiving steroid injections; the patient was not in good general condition; and the patient was a smoker.

All samples were placed in Trizol reagent (Ambion, Foster City, CA, USA). Before examination, the edges of the samples were cut off and the specimens cut into 1 × 1-cm squares. Next, 3 mm of material was cut from the tear-margin end of the sample (distal), 3 mm was cut from the proximal end, and the remaining 4 mm formed a middle section. Each section was divided into 3 sections to give 3 measurements from each part, which were then combined to give an overall mean value for the section. The expression of caspases 9, 8, and 3 and the proapoptotic Bax and antiapoptotic Bcl-2 molecules, as well as TNF-α and IL-10 cytokines, was assessed according to Gach et al.[13]

Quantitative real-time polymerase chain reaction assay

All molecular assays were performed as previously described. The expression of human Bax; Bcl-1; TNF-α; IL-10; glyceraldehyde 3-phosphate dehydrogenase (GAPDH); and caspases 3, 8, and 9 was quantified by real-time polymerase chain reaction (PCR) using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Total cellular RNA (1 μg) from the studied material was extracted by use of Trizol reagent (Invitrogen [Thermo Fisher Scientific], Carlsbad, CA, USA) using a single-step purification protocol.[1] RNA pellets were dissolved in ribonuclease-free water, and their concentrations and purity were determined by spectrophotometer readings at 260 and 280 nm. RNA that had undergone polyadenylation was isolated by use of an Oligotex kit (Qiagen, Chatsworth, CA, USA): 50 ng of poly(A) RNA was used for the first-strand complementary deoxyribonucleic acid (cDNA) synthesis with the SuperScript II ribonuclease Transcriptase System (Invitrogen), using $Oligo(dT)_{12\text{-}18}$ Primers (Invitrogen) as described per the manufacturer's instructions. We amplified cDNA