**ABSTRACT INTRODUCTION:**

Determination of whether the edge of the ruptured rotator cuff tendon has healing potential is of great importance for both arthroscopic and open rotator cuff repairs. Proper examination of the molecules in the ruptured rotator cuff tendon may provide help in answering this question. Type I and type III collagen have been measured in previous studies, because these are the primary fibrillar collagens of both normal and ruptured rotator cuff tendons, playing important roles in the healing of ruptured tendon. In this study, we used real-time reverse transcription-polymerase chain reaction (RT-PCR) to examine expression of mRNAs of procollagen types I and III at the edges of ruptured rotator cuff tendons. We also evaluated the integrity of the repaired cuffs at least 1 year after surgery, and we examined the relationships between procollagen-mRNA expression and postoperative cuff integrity.

**METHODS:**

Specimens were obtained during surgery on 12 patients with full-thickness rotator cuff tears. The average patient age was 58 years (range, 40 to 69 years). There were 14 male and 5 female patients, and the injuries were in 12 dominant and 7 non-dominant shoulders. The cross-sectional rupture size (longitudinal × transverse) ranged from 1 cm² to 20 cm², (average, 5.9 cm²). The average period from symptom onset was 8 months (range, 1 month to 3 years). A margin 5 to 7 mm wide was removed from each ruptured rotator cuff tendon during surgery.

Five cadaveric rotator cuff tendons without apparent rupture were obtained as controls within 6 h after death. There were 4 male and 1 female donors, none of whom had shoulder pain before death. The average age was 63 years (range, 55 to 72 years). There was no significant difference in age between patients with ruptured rotator cuff tendons and controls. The rotator cuff tendon was harvested as close to the critical portion as possible.

The tissue specimens were snap frozen and stored at -80°C for total cellular RNA and protein isolation.

The conditions of real-time PCR were first treatment at 50°C for 2 min, then incubation at 95°C for 15 sec, and then annealing at 62°C for 1 min without extension. We repeated this process (denaturing and annealing) for 40 cycles. The amounts of mRNAs for procollagens mRNA and β-actin mRNA were measured linearly. Expression of procollagen mRNA was then normalized against that of β-actin as an internal standard by the delta-delta-CT method. The relative ratios of the PCR products of the procollagens in each sample (ruptured and control tendons) compared with those in MG cells were calculated (i.e., MG cells were used as positive controls for procollagens).

Tissue specimens were solubilized in buffer [0.25 M sucrose, 3mM Tris-HCl (pH7.5), 0.1mM EDTA] after the insoluble materials had been removed by centrifugation for 60 min at 40000 x g. They were then electrophoresed on 15% SDS-PAGE gels, and transferred onto PVDF membranes. After inhibition of non-specific binding, the protein blots were incubated with antibodies for 1 h, at 37°C, and then reacted with anti-mouse-IgG antibody labeled with horseradish peroxidase. Immune complex was detected by chemiluminescence.

Postoperative cuff integrity was examined at least 1 year postoperatively (mean 14.1 months, range 12-15 months) by using Sugaya’s classification (1). Cuff integrity was classified into 5 categories from three views of T2-weighted images: type I, repaired cuff with sufficient thickness and homogeneously low intensity on each image; type II, sufficient thickness with partial high intensity area; type III, insufficient thickness but without discontinuity, suggesting partial thickness delaminated tear; type IV, presence of minor discontinuity in only one or two slices on both coronal and sagittal images, suggesting a small full-thickness tear; and type V, presence of a major discontinuity in more than two slices on both coronal and sagittal images, suggesting medium or large full-thickness tear.

The Mann-Whitney U-test was used for comparison among the different parameters recorded. The Wilcoxon rank test was used to determine the significance levels of the scores between preoperative and postoperative status. Spearman’s correlation rank test was used to analyze possible relationships between the procollagen-mRNAs and clinical parameters. Results with a P-value less than 0.05 were considered significant.

**RESULTS SECTION:**

Expression of the mRNAs of both procollagens at the edges of the ruptured tendons was significantly greater than that in the controls. As with the results of the real-time RT-PCR analysis, at the edges of the ruptured rotator cuff tendons there was greater production of both procollagen proteins, compared with the controls.

Postoperative cuff integrity was evaluated by Sugaya’s classification system. There were 6 type I, 3 type II, 2 type III, and 1 type IV. When graded from 5 point for type I to 1 point for type 5, postoperative cuff integrity was significantly correlated with the expression of both procollagen-mRNAs at the edges of the ruptured tendons (procollagen type I: r = 0.63; P < 0.04, procollagen type III: r = 0.60; P < 0.03)

We found significant inverse correlation between period from symptom onset and the levels of expression of both procollagen-mRNAs (type I: r = -0.75; P < 0.0005, and type III: r = -0.52; P = 0.0331).

**DISCUSSION:**

Procollagen production at the edge of the rotator cuff tendon has been examined before (2,3). To our knowledge, our study is the first to relate it to clinical outcome. Our results revealed that mRNA expression of both procollagen types I and III at the edge of the ruptured rotator cuff tendon was closely correlated with the postoperative cuff integrity.

Lo et al., using RT-PCR, found that type I and III collagen mRNA expression at the edges of ruptured rotator cuff tendons was significantly greater than that in control rotator cuff tendon obtained from fresh cadavers. They concluded that tendon at the edge of ruptured rotator cuff may potentially contribute to the healing process after repair (2). The results of our RT-PCR analysis of procollagens type I and III thus concurred with those of Lo et al. Additionally, our Western blot analysis demonstrated increased production of both procollagen proteins at the edge of the ruptured rotator cuff tendon. These results therefore suggested that the tissue at the edge of the ruptured rotator cuff tendon in our series was active in healing.

Previous studies have examined the correlation between period from symptom onset and the expression of procollagen type I and type III (2,3). Lo et al. reported the inverse correlation between period from symptom onset and collagen type I mRNA expression (2). In situ hybridization studies have demonstrated that, at edge of ruptured rotator cuff tendon, procollagen type I mRNA expression in the tenocytes is correlated with period from symptom onset, but procollagen type III mRNA expression in these cells is not (3). We found a significant and inverse correlation between period from symptom onset and the expression of both procollagen-mRNAs at the edge of the ruptured rotator cuff tendon.

Limitations of this study include the fact that the small number of samples analyzed could obviously have influenced our results with regard to the association with postoperative findings. Despite these limitations, we believe that our data sufficiently correlated procollagen-mRNA expression at the edge of the ruptured rotator cuff tendon with postoperative cuff integrity.

In conclusion, procollagen type I and III mRNA expression at the edge of the ruptured rotator cuff tendon was significantly correlated with postoperative cuff integrity. Expression of both procollagen-mRNAs was significantly associated with period from symptom onset.

**REFERENCES:**