Poor Healing Capacity of Chronic Torn Supraspinatus Tendon

Chun-Yi Lee, Chao-Tien Hsu[2], Weng-Cheng Chang[2], Horng-Chaung Hsu[1]

Department of Orthopaedic Surgery, China Medical College Hospital, Beikang; [1]Department of Orthopaedic Surgery, China Medical College Hospital; [2]Institute of Medical Science, China Medical College, Taichung, Taiwan, R.O.C.

Background. Tear of rotator cuff typically begins in the supraspinatus tendon, however the healing capacity of torn supraspinatus tendon is unclear. The aims of this study were to measure the content of α1 type-I and α1 type-III procollagen mRNA from the torn edge of supraspinatus tendon and to estimate its healing capacity.

Methods. Twenty torn supraspinatus tendon specimens were obtained from 20 complete-thickness tear patients. The control group consisted of six normal supraspinatus tendons obtained from other shoulder operation patients. The content of α1 type-I and Type-III procollagen mRNA was measured semiquantitatively by RT-PCR.

Results. mRNA levels of collagen I and III were lower in the torn supraspinatus group than in the normal group ($p < 0.001$). There were no statistically significant differences between size of tear and duration of pain in mRNA levels of collagen I and III.

Conclusions. This study revealed lower amounts of α1 type-I and type-III procollagen mRNA in torn rotator cuff which suggests that chronic torn supraspinatus tendons have a poor healing capacity. **(Mid Taiwan J Med 2002;7:1-6)**

Key words

healing capacity, RT-PCR, supraspinatus, α1 type-I procollagen mRNA, α1 type-III procollagen mRNA

INTRODUCTION

Flexor tendon of fingers, as a model for tendon healing, have intrinsic and extrinsic healing capacities [1-4]. Torn supraspinatus tendons also have intrinsic and extrinsic capacities. Uhthoff and Sarka examined torn rotator cuffs using immunohistologic techniques and concluded that extrinsic healing superseded intrinsic healing in the overall process of repair of torn rotator cuffs; routine bursectomy was not suggested [5]. Hamada K observed the expression of α1 (I) procollagen mRNA in torn supraspinatus tendons by in situ hybridization and noted that a high level of α1 (I) procollagen mRNA expression was detected in the tenocytes and undifferentiated mesenchymal cells near the torn portion in tendons with complete-thickness tear and observed little expression of α1 (I) procollagen mRNA in the epitenon cells and fibroblasts of the subacromial bursa. He concluded that the intrinsic healing process superseded the extrinsic healing process [6].

The major structural component of the tendon is type-I collagen. In the early phase of tendon healing, however, type III collagen increases [7]. This collagen is gradually

Received : October 15, 2001. Revised: December 10, 2001.
Accepted : January 8, 2001.
Address reprint requests to : Horng-Chang Hsu, Department of Orthopaedic Surgery, China Medical College Hospital, No 2, Yuh-Der Road, Taichung 404, Taiwan, R.O.C.

Table 1. Primer sequences and PCR reaction conditions

mRNA species	5'primer	3'primer
Collagen I	5'-GCA AAG AAG GCG GCA AAG GTC-3'	5'-CCC CTC ACG TCC AGA TTC ACC -3'
Collagen III	5'-CCCAGAACATCACATATCAC-3'	5'-GCGAGAAGATGACCCAGATCATGTT-3'
β-Actin	5'-CAAGAGGAACACATATGGAG-3'	5'-GCTTCTCCTTAATGTCACGCACGAT-3'

PCR = polymerase chain reaction; D = denaturing; A = annealing, E = extension; C = cycles; S = sizes (base pair). Primer sequences are derived from Genbank.

replaced by type I collagen as the scar tissue matures. This process is essential for the maintenance of structure and function of the tendon [8].

Since type-I and III collagens have an extremely long half-life, it is difficult to distinguish newly synthesized type-I and -III collagens from older preexisting collagen. Conversely, the mRNA of α1 (I) and α1 (III) procollagens, which is one of the precursors of type-I and III collagens, has a half-life of about 8-9 hours and may serve as a real-time marker for newly synthesized type-I and III collagens [9].

The present study was undertaken to evaluate the healing capacity of in vivo torn supraspinatus tendon by semiquantitatively measuring the level of expression of mRNA of α1 (I) procollagen and α1 (III) procollagen.

MATERIALS AND METHODS

Patients and Tissue Samples

Twenty supraspinatus tendon specimens were obtained from 20 patients with complete-thickness tears. The age of the patients at the time of surgery ranged from 43 to 77 years (mean: 66 years). Three normal supraspinatus tendon specimens were obtained from avulsion facture of greater tubercle of humerus from rotator interval tear patients who received surgery within 6 hours. The other 3 normal supraspinatus tendon specimens were obtained from anterior instability of shoulder patients. The age of the patients at the time of surgery ranged from 32 to 69 years (mean: 51 years).

Samples of supraspinatus tendons were rinsed in sterile phosphate buffered saline, frozen immediately in liquid nitrogen, and stored at −80 ℃ prior to processing. All samples were obtained with consent of the patients.

RNA Extraction

Total RNA was extracted from samples by the TRI spin method as previously described [10,11]. Briefly, tissues snap-frozen in liquid nitrogen were powdered by a Braun Dismembrator (B. Braun Biotech, Allentown, Pennsylvania, USA). TRIzol reagent (Life Technologies, Gaithersburg, Maryland, USA) was added to the powder (1 mL TRIzol reagent/100 mg tissue), and the sample was allowed to thaw before being transferred to 1.5 mL Eppendorf tubes. Chloroform was added (0.2 mL/mL TRIzol), the tubes were vortexed, and then centrifuged at 12,000 ×g for 15 minutes to separate the aqueous and organic phases. RNA was precipitated from the aqueous phase by mixing with isopropyl alcohol. The RNA precipitate formed a gel-like pellet on the side and bottom of the tube after centrifugation. After removing the supernate, the pellet was washed once with 75% alcohol. The sample was then vortexed and centrifuged at 7500 ×g for 5 minutes at 4 ℃.

Semiquantitative Reveres Transcription Polymerase Chain Reaction

Two micrograms of total RNA from each tissue sample was reversely transcribed with a 1st strand cDNA Synthesis Kit for RT-PCR (Boehringer Mannheim, Foster city, California, USA), as described by the manufacturer, to convert mRNA to the corresponding cDNA. Three-microliter aliquots of cDNA were then amplified by polymerase chain reaction in a final volume of 100 μL containing 20 mM Tris-

Table 1. Continued

D	A	E	C	S
91	61.5	72	35	344
91	55	72	35	366
91	55	72	35	300

HCl, 50 mM KCI, 1.5 mM $MgCl_2$, 0.5 μgM of each polymerase chain reaction primer, and 2.5U Taq DNA polymerase (Life Technologies). Amplification was performed with a Cetus 9600 thermocycler (Perkin-Elmer). The polymerase chain reaction cycling parameters are shown in Table 1. The primer sets used for polymerase chain reaction were oligonucleotides 20-25 base pairs in length whose synthesis was based on the most conserved sequences in Genbank.

Total RNA from the torn edge of rorator cuff tendon was analyzed by semiquantitative reverse transcription-polymerase chain reaction to determine relative levels of the content of type-I and type-III collagens. β–actin was used as the internal standard. For all reported experiments, conditions were determined to be in the linear range for both the polymerase chain reaction and the image analysis system. Briefly, for each group of samples, all of the samples were subjected to reverse transcription at the same time, and subsequently all samples of cDNA were amplified by polymerase chain reaction at the same time to avoid any potential experiment - to-experiment variation in efficiency. Three microliter aliquots of cDNA were amplified by polymerase chain reaction as previously described. Ten-microliter aliquots of the polymerase chain reaction products were electrophoresed on 2% agarose gels with Tris-acetate-EDTA buffer, stained with ethidium bromide, and destained in distilled water. All gels were photographed with Polaroid no. 55 film. The photographs were then analyzed with a CCD (charge-coupled device) camera and appropriate software (Quality one, PDI, Bioscience, La Jolla, California, USA). Integrated

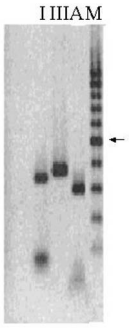

Fig. 1 Polymerase chain reaction analysis of mRNA from torn human supraspinatus tendon. Lane designations: I = Collagen I (334bp), Relative OD (Optical density) to β-actin = 0.734; III = collagen III (366bp), Relative OD = 1.036; A = β-actin (300bp), relative OD = 1.000 and M = Marker DNA. The arrow indicates 500 base pairs.

density values for the genes were normalized to their corresponding β-actin values to yield semiquantitative assessment (Fig. 1).

Statistical Analysis

Descriptive statistics determined the group means and standard deviations. Data were analyzed in a 2-factor repeated-measures multivariate analysis of variance (MANOVA). The 2 factors were the copy number normalized for β-actin. Subsequently, non-paired t test was performed between tear and normal groups with Bonferroni correction. Significance levels were set at 0.05 and 0.01 for the multivariate analysis. All statistical analyses were performed using personal computer-based statistical software (SPSS/PC+ version 8.0; SPSS, Chicago, Illinois).

RESULTS

Expression of β-actin mRNA was noted in all rotator cuff specimens. There were no statistically significant differences between the control and tear groups (p = 0.39).

The level of expression of mRNA of procollagen I and III did not differ statistically with respect to age and gender in torn samples. There were also no statistically significant differences in terms of tear size and duration of pain in torn samples.

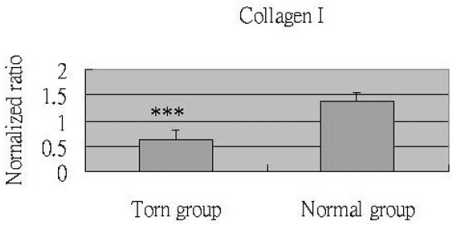

Fig. 2 Semiquantitative reverse transcription-polymerase chain reaction analysis of mRNA levels of procollagen I. Polymerase chain reaction products were analyzed as described in the Materials and Methods section. Values presented represent the mean ± SEM for each group. Normal group: 1.38 ± 0.17; Torn group: 0.63 ± 0.18. Statistically significant differences between groups (p< 0.001, analysis of variance) are indicated with three stars.

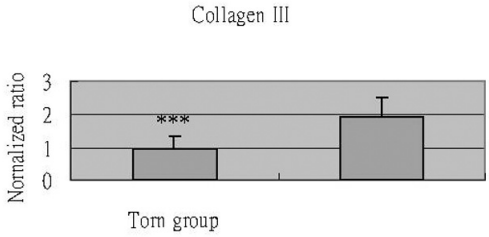

Fig. 3 Semiquantitative reverse transcription-polymerase chain reaction analysis of mRNA levels of procollagen III. Polymerase chain reaction products were analyzed as described in the Materials and Methods section. Values presented represent the mean ± SEM for each group. Normal group: 1.9 ± 0.61; Torn group: 0.95 ± 0.39. Statistically significant differences between groups (p<0.001, analysis of variance) are indicated with three stars.

The level of expression of collagen I and III was statistically lower in torn supraspinatus samples than in control samples (p < 0.001) (Figs. 2 and 3).

DISCUSSION

Rotator cuff tendons have not been studied as extensively and are not as well characterized as flexor tendons. Investigators have demonstrated several unique aspects of the structure and biology of rotator cuff tendons. These characteristics undoubtedly reflect the rotator cuff's unique function of stabilizing and assisting in motion of the most mobile and unstable joint in the body [12].

The major type of collagen in normal rotator cuff tendon is type I, with trace amounts of type III. Riley and co-workers found that the collagen contents of the supraspinatus tendon does not vary with age, gender, or distal versus proximal location [13].

Using in situ hybridization with a probe specific to human procollagen β-1 type III mRNA, Tomoganaga and co-workers found high levels of it as late as 18 months following apparent trauma in the tendon substance and synovial tissues in complete-thickness tear [14]. Using in situ hybridization with a probe specific to human procollagen α1 type I mRNA, Hamada and co-workers found that the level decreased as early as 4 months after apparent trauma [5]. In our study, the level of expression of mRNA of procollagen I and III did not differ statistically with respect to age and gender in torn samples. There were also no statistically significant differences in tear size and duration of pain in torn samples. Acute macrotrauma with tear at mid-substance was not found in our series. It was hard to determine the time of apparent trauma and duration of shoulder pain. All tears were located at the critical zone. Repetitive microtrauma is thought to be a more important factor in rotator cuff degeneration than in acute trauma. In our study, the torn edge of supraspinatus tendon was found grossly to be white and thin in all tears. The size of tear and duration of shoulder pain were not apparently related to these gross findings. All of our patients had chronic degeneration of rotator cuff.

Uhthoff and Sarkar used an immunohistochemical study to examine the torn edge of human supraspinatus and demonstrated type III collagen production at its bursal side. Much of the initial healing response may, in fact, be derived from bursal tissue, suggesting that routine bursectomy at the time of tendon repair may be ill advised [6]. Kumagai et al, using in situ hybridization, found the presence of procollagen III mRNA at the subacromial bursa and in the tendinous tissue. Type III collagen is a component of the normal synovium and its presence in the bursal side of supraspinatus may not be

evidence of extrinsic healing [15,16]. In our study, procollagen III mRNA levels were significantly lower at the torn edges of human supraspinatus which suggested no active healing process there.

Hamada K and co-workers, using in situ hybridization, detected a high level of α(1) type I procollagen mRNA expression in the tenocyte and undifferentiated mesenchymal cells near the torn portions of tendons with complete-thickness tears and little expression of α(1) type I procollagen mRNA in the epitenon cells and the fibroblasts of the subacromial bursa. They suggested that there is much higher synthetic activity of type-I collagen in the tendon than in the synovial tissue of the acromial bursa in the middle or late stages of torn supraspinatus tendon healing. In our study, procollagen I mRNA levels decreased significantly at the torn edges of human supraspinatus which indicates that no active healing process occurred.

In our study, lower levels of procollagen I and III mRNA expression were found in chronic torn samples which suggests that the torn supraspinatus tendons were at the atrophy stage and had little healing capacity.

ACKNOWLEDGMENTS

The authors would like to thank associated professor Win-Min Liang for her assistance in biostatistical analysis. This study was supported by a grant from China Medical College Hospital (DMR-88-039).

REFERRENCES

1. Lindsay WK, Thomson HG. Digital flexor tendons: an experimental study. I. The significance of each component of the flexor mechanism in tendon healing. *Br J Plast Surg* 1960;12:289-316.
2. Potenza AD. Tendon healing within the flexor digital sheath in the dog: an experimental study. *J Bone Joint Surg [Am]* 1962;44:49-64.
3. Tokita Y, Yamaya A, Yabe Y. An experimental study on the repair and restoration of gliding function after digital flexor tendon injury. *Nippon Sekeigeka Gakkai Zasshi (J Japan Orthop Assoc)* 1974;48:107-27.
4. Gelberman RH, Amiel D, Harwood F. Genetic expression for type I procollagen in the early stages of flexor tendon healing. *J Hand Surg [Am]* 1992;17:551-8.
5. Hamada K, Tomonaga A, Gotoh M, et al. Intrinsic healing capacity and tearing process of torn supraspinatus tendons: in situ hybridization study of alpha 1 (I) procollagen mRNA. *J Orthop Res* 1997;15:24-32.
6. Uhthoff HK, Sarkar K. Surgical repair of rotator cuff ruptures: the imporance of the subaromial bursa. *J Bone Joint Surg [Br]* 1991;73:339-401.
7. von der Mark K. Localization of collagen types in tissues. [Review] *Int Rev Connect Tissue Res* 1981; 9:265-324.
8. Williams IF, McCullagh KG, Silver IA. The distribution of types I and III collagen and fibronectin in the healing equine tendon. *Connect Tissue Res* 1984;12:211-27.
9. Hamalainen L, Oikarinen J, Kivirkko KI. Synthesis and degradation of type I procollagen mRNAs in cultured human skin fibroblasts and the effect of cortisol. *J Biol Chem* 1985;260:720-5.
10. Chomczynski P, Sacchi N. Single-step method of RNA lsolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1997;162:156-9.
11. Reno C, Marchuk L, Sciore P, et al. Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. *Biotechniques* 1997;22:1082-6.
12. Blevins FT, Djurasovic M, Flatow EL, et al. Biology of the rotator cuff tendon. [Review] *Orthop Clin North Am* 1997;28:1-16.
13. Riley GP, Harrall RL, Constant CR, et al. Tendon degeneration and chronic shoulder pain: changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. *Ann Rheum Dis* 1994;53:359-66.
14. Tomonaga A, Hamada K, Gotoh M, et al. Localization of mRNA of procollagen αl type III in torn supraspinatus tendons by in situ hybridization. *The Shoulder Joint* 1995;19:5-9.
15. Kumagai J, Sarkar K, Uhthoff HK. Repair process of surgically produced rotator cuff tear histological and immunohistochemical study using monoclonal antibodies against collagen type I and III. *Trans Orthop Res* 1993;18:315.
16. Kumagai J, Sarkar K, Uhthoff HK. The collagen types in the attachment zone of rotator cuff tendons in the elderly: an immunohistochemical study. *J Rheumatol* 1994;21:2096-100.

破裂的脊上肌腱癒合能力貧乏

李俊億 許朝添[2] 張文正[2] 許弘昌[1]

中國醫藥學院北港附設醫院 骨科 中國醫藥學院附設醫院 骨科部[1] 中國醫藥學院 醫學研究所[2]

背景 典型之旋轉肌肌腱斷始自脊上肌腱，斷裂的脊上肌腱癒合的能力並不清楚，此研究的目的是測量脊上肌腱斷裂的邊緣之第一型及三型膠原纖維的含量，並以決定其癒合之能力。

方法 從20個斷裂的旋轉肌腱獲取20個斷裂的脊上肌腱。6例正常的脊上肌腱也取得自手術。RT-PCR被使用於測量第一型及三型膠原前纖維的mRNA含量，並半定量之。

結果 第一型及三型膠原纖維之mRNA含量，實驗組明顯低於對照組（$p<0.001$）。實驗組中破裂的大小及病患疼痛的時間與第一型及三型膠原纖維內mRNA的含量無關。

結論 此研究顯示實驗組第一型及三型膠原前纖維的mRNA含量低，顯示破裂的脊上肌正處於不用性萎縮之狀態，缺乏癒合能力，所以以手術修補破裂的旋轉肌腱，以達到完全的癒合是必要的。（中台灣醫誌 **2002;7:1-6**）

關鍵詞

癒合能力，RT-PCR ，脊上肌腱，第一型膠原纖維，第三型膠原纖維

聯絡作者：許弘昌

地　　址：404台中市北區育德路2號

中國醫藥學院附設醫院 骨科部

收文日期：10/15/2001　　修改日期：12/10/2001

接受日期：1/8/2002