

Gene Expression of Procollagen $\alpha 1$ (I) and $\alpha 1$ (III) in Partial-thickness Tears of the Deep Pectoral Tendon in Chickens

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The purpose of this study was to evaluate the healing process of partial-thickness tears of the deep pectoral tendon in chickens which is considered to be equivalent to the human rotator cuff. The superior side (bursal-side) and the inferior side (joint-side) layers of the deep pectoral tendon of 80 adult chickens were lacerated in the same manner. The mRNA expression levels of $\alpha 1$ (I) and $\alpha 1$ (III) procollagen was at a level 2 weeks after laceration. The mRNA expression level of procollagen $\alpha 1$ (I) was significantly higher than that of procollagen $\alpha 1$ (III) in all experimental periods ($p < 0.05$). The expression levels of procollagen-mRNAs were constitutively higher in bursal-side tears than in joint-side tears throughout the experimental periods ($p < 0.05$), even 52nd weeks after laceration. These observations indicate that the bursal-side layer possesses higher healing ability than the joint-side layer in the chicken's deep pectoral tendon where subacromial impingement is absent, and that the remodeling of partial-thickness tears in chicken models needs more than one year.

Key words : Procollagen $\alpha 1$ (I), Procollagen $\alpha 1$ (III), Partial-thickness tear of tendon

INTRODUCTION

Partial-thickness tears of the rotator cuff frequently occur in the middle-aged and the elderly, diagnosed by MRI and/or ultrasound [2]. They are often treated conservatively in clinical practice by use of anti-inflammatory medications, steroid injections, physical therapy etc. Whether the torn cuff heals spontaneously or not in this condition has great clinical implication, but this has not yet been clarified. Yamanaka and Matsumoto reported that 10% of joint-side tears decreased in size and 10% disappeared, but the remaining 80% enlarged in size or progressed to complete-thickness tears as demonstrated arthrographically in a long-term follow-up [16].

As a model for tendon healing, the healing process in the flexor tendons of the fingers has been investigated, and two forms of healing modalities have been proposed (intrinsic and extrinsic healing) [7-9].

Gelberman et al. emphasized that endotenon cells primarily produce repair collagens [3]. Hamada et al. demonstrated, in their in situ hybridization study of $\alpha 1$ (I) procollagen mRNA, that the torn supraspinatus tendon also has intrinsic healing capability [4].

The major structural component of the tendon is type I collagen. In the early phase of tendon healing, type III collagen also increases [14]. Since collagen has an extremely long half-life, mRNA of procollagen (precursor of collagen) may serve as a real-time marker of newly synthesized collagen [5].

In this study, we examined the healing modality of rotator cuff tear with animal models, especially in two forms of partial-thickness tear. The mRNAs expression levels of $\alpha 1$ (I) and $\alpha 1$ (III) procollagen were semiquantified to evaluate the healing process of partial-thickness tear of the deep pectoral tendon in chicken, which was considered to be equivalent to human rotator

cuff, using reverse transcriptase polymerase chain reaction.

MATERIALS AND METHODS

Procedure for making partial-thickness tears in chicken deep pectoral tendon

Eighty white leghorn chickens, aged 8 months and weighing 2.5–3.0 kg, were anesthetized with pentobarbital (0.8 mg/kg body weight) injected intramuscularly. The superior and inferior side layers of the deep pectoral tendon in 80 adult chickens were lacerated in the following manner; the laceration starting from the line 1.0 cm proximal to the humeral insertion, either on the superior or inferior surface, spanning the whole width, vertically incising half the depth of the tendon, and then, turning at a right angle cranially to proceed 1.0 cm to complete a “L” shaped flap (Fig. 1). The chickens were sacrificed on the 1st, 2nd, 3rd, 4th, 6th, 8th, 24th, and 52nd weeks. Lacerated tendons were separated from the surrounding tissues. Sham operation was performed at the opposite side of each operated site in the same manner and the sham-operated site was used as a control.

Measurement of procollagen-mRNAs expression level by reverse transcriptase polymerase chain reaction

First strand cDNA synthesis (reverse transcription) was performed at 23 °C for 10 min

and 42 °C for 60 min in 20 μ l of 50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl₂, 0.1 M DTT, 1 mM deoxynucleotide triphosphate mixture, human placental ribonuclease inhibitor (Takara, Shiga, Japan), 100 pmol oligonucleotide random primer (Takara, Shiga, Japan), and 20 units of reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA). After denaturation, the procollagen-specific cDNA fragments were amplified with 2.5 units of Taq DNA polymerase by hot-start-procedures (TOYOBO, Osaka, Japan; denaturation at 94 °C for 1 min, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 min, 20 cycles) with primers specific for procollagen (α 1)I (sense: 5-TGGCGACATCAGCCGGGTTG-GAGC-3, anti-sense: 5-TCATGCTTCCTGCCT-CAACCGCCC-3), procollagen (α 1)III (sense: 5-GGAACCAACCTTCGTAGACTCTCC-3, anti-sense: 5-GTCCATGTTGTGGTGGTGGCTG-TA-3). RNA preparations from 21-week-fertilized ovum asues were used as a positive control for procollagen. Cycle number (20 cycles) was determined prior to this experiment in detail, so that polymerase chain reaction amplification was linear under these conditions (data not shown).

Polymerase chain reaction amplification products separated through a 3% agarose gel were blotted onto a membrane. The blots were hybridized with ³²P-labeled oligonucleotide probes for procollagen (α 1)

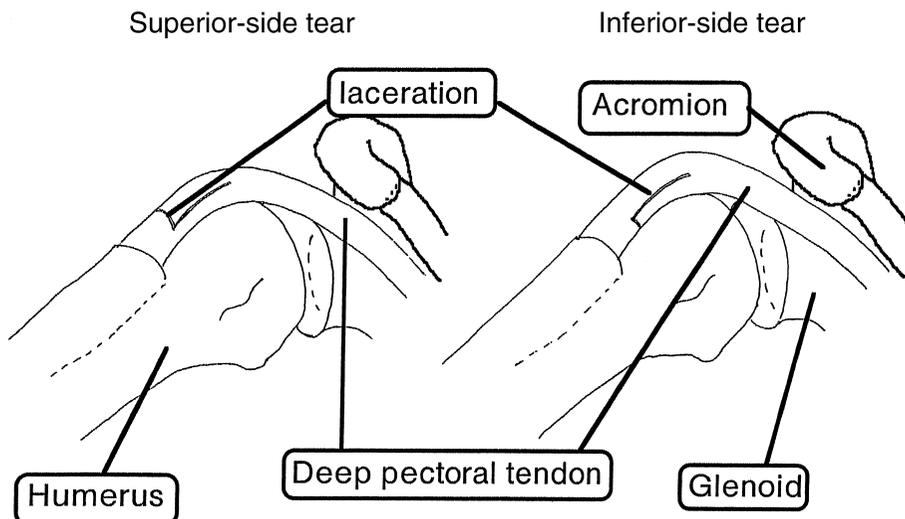


Fig. 1 Scheme of two types of partial-thickness tears in deep pectoral tendon in chicken

I (5-ATGCTCAGCTTTGTGGATACGCGGA-3) and procollagen ($\alpha 1$) III (5-TGGCAGGGT-TAGCACT-3) at 55 °C for 16 hours. The blots were exposed to a Kodak RP film at 80 °C with double intensifying screens. Signal intensity was quantified with the Interactive Build Analysis System (Carl Zeiss, Germany). The relative number of units of polymerase chain reaction amplification products in each sample compared to β -actin was calculated by Interactive Build Analysis System (Carl Zeiss, Germany).

Statistical analysis

The Mann-Whitney U test was used for comparisons between controls and the 2 categories of partial-thickness tears. P values less than 0.05 were considered statistically significant.

RESULTS

The mRNA expression levels of $\alpha 1$ (I) and $\alpha 1$ (III) procollagen in two types of partial-thickness tear were measured at the 1st, 2nd, 3rd, 4th, 6th, 8th, 24th, and 52nd weeks. The mRNA expression levels of $\alpha 1$ (I) and $\alpha 1$ (III) procollagen showed their maximum levels at 2 weeks after laceration. The mRNA expression level of procollagen $\alpha 1$ (I) was significantly higher than that of procollagen $\alpha 1$ (III) in all experimental periods ($p < 0.05$, Fig. 2). The expression levels

of procollagen-mRNAs were constitutively higher in the superior-side tears than in the inferior-side tears throughout the all periods ($p < 0.05$, Fig. 3A and 3B), even at the 52nd week from laceration.

DISCUSSION

As an animal model of human rotator cuff tear, various animals have been used so far. Recently, Soslowky et al. have reported that the shoulder of the rat is an appropriate model for investigation of rotator cuff diseases [12]. In the present study, we used the deep pectoral tendon of the chicken as an animal model of human rotator cuff. Although this model does not furnish subacromial impingement, the following advantages were provided as compared with the previous models: 1) the shoulder joint of the chicken is non-weight bearing, 2) its main motion is similar to abduction of the human shoulder, and 3) the size of the model enables us to perform easy operative procedure. In this study, we were able to produce two different types of tear of deep pectoral tendon in chicken, equivalent to those of the human partial-thickness rotator cuff tear (bursal-side and joint-side tears).

The mRNA expression levels of procollagen in the superior-side (bursal-side) tear were constitutively higher than those in the inferior-side(joint-side) tear throughout

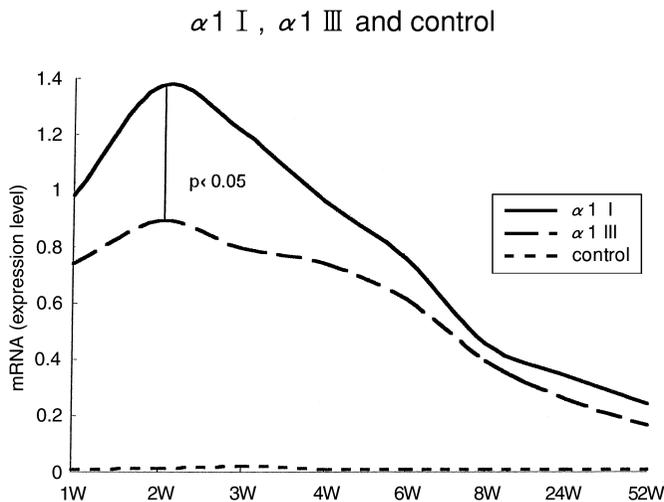


Fig. 2 The mRNA expression levels of $\alpha 1$ (I), $\alpha 1$ (III) and control in experimental periods. Statistical significance among three parameters was constitutively noted throughout experimental periods.

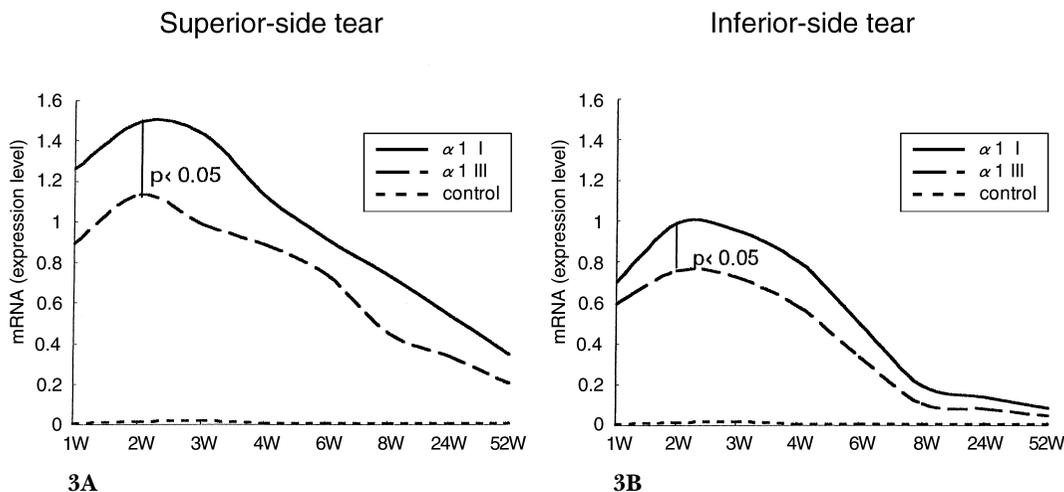


Fig. 3 The expression levels of procollagen-mRNAs in superior-side tear (3A) and inferior-side tear (3B) in experimental periods. Statistical significance among three parameters was constitutively noted throughout experimental periods.

experimental periods. Hamada *et al.* performed *in situ* hybridization studies of procollagen $\alpha 1$ (I) on the human rotator cuff [4]. In their study, although there was no statistical significance, the mean value of signal positive cells was higher in the bursal-side tear than in the joint-side tear. Thus, our results may support that in the human rotator cuff tear, the bursal-side layer possesses more active healing capability than joint-side layer. The reason why the expression of $\alpha 1$ (I) and $\alpha 1$ (III) was higher in the superior-side tear than in the inferior-side tear may be considered due to 1) more abundant vascularity in the bursal-side than in the joint-side layer [15] and 2) the differences of the epitenon cell reaction against trauma between the two layers.

In the human and pig skins, type III collagen expressed in the early stage is replaced by type I collagen 2–3 weeks after laceration [1, 11]. In contrast, our results revealed that mRNA expression level of $\alpha 1$ (I) procollagen was constitutively higher than that of $\alpha 1$ (III) procollagen. The previous report on chicken flexor tendon is in accordance with our results [6]. From these observations, it is suggested that healing modality of the rotator cuff tendon and flexor tendon differs from that of skin.

The present study demonstrated that in chicken deep pectoral tendon where sub-

acromial impingement is absent, the superior-side (bursal-side) layer could have higher healing capability than the inferior-side (joint-side) layer. Thus, this study provides a new insight for understanding the healing modality in two forms of partial-thickness tear (bursal- and joint-side tears) in the human rotator cuff.

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