**Effect of Corticosteroid on Collagen Gene Expression in Injured Rotator Cuff Tendon**

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**Introduction:** Subacromial corticosteroid injections are commonly utilized in the conservative management of rotator cuff disease. The beneficial anti-inflammatory and analgesic properties of corticosteroid are tempered by potentially significant side effects on connective tissue. Animal studies, performed primarily on Achilles and patellar tendons, have associated corticosteroid exposure with tendon atrophy and decreased biomechanical properties [1,2]. These studies, however, have yielded conflicting results that fail to clarify corticosteroid effects.

The specific impact of corticosteroid on rotator cuff tendons is not well understood. It is difficult to apply conclusions from other tendon studies to the rotator cuff due to its unique anatomic location between an overlying bony arch and an underlying joint space. No study to date has examined the effects of corticosteroid on injured rotator cuff tendons. Our aim was to characterize the injury response of rotator cuff tendons through analysis of the type III to type I collagen expression ratio, a tendon injury marker, and examine the effects of corticosteroid on this response.

**Methods:** Sixty-six Sprague-Dawley rats with a mean body weight of 570 gm (422 – 700 gm) were used in this IACUC approved study. The rats were randomly assigned to four groups: control, steroid treated, tendon injury, and tendon injury plus steroid treatment. Six rats served as sham surgery control. Subcutaneous injections of antibodies (gentamicin, 8mg/kg) were given pre-operatively. A 1 cm transverse skin incision was made along the lateral border of the acromion. A portion of the deltoid origin was released from the acromion by sharp detachment. The acromion was then gently retracted, exposing the infraspinatus tendon. Unilateral tendon injuries were created with full thickness defects across 50% of total infraspinatus tendon width, 1 mm from humeral insertion. Steroid treatments of a single, human equivalent methylprednisolone dose (0.6mg/kg) were injected into the subacromial space near direct visualization. Steroid treatment directly followed the creation of injury in the injury plus steroid treatment group. For closure, the fascia of the detached deltoid muscle was sutured to that of the trapezius muscle. The skin was then closed with staples.

At 1, 3, and 5 weeks post-injury, the infraspinatus tendon was harvested and snap frozen in liquid nitrogen. Total RNA was extracted from tendons using the RiboPure isolation system (Ambion, Austin, TX). Real time RT-PCR was performed on total RNA extracts using the Assays-on-Demand Gene Expression System (Applied Biosystems, Foster City, CA). Primer/probe sets specific for type I (alpha 2(I) chain) and type III (alpha 1(III) chain) collagen were used. The experimental mRNA levels were individually normalized to ribosomal 18s levels. The collagen III to collagen I ratio was computed for each sample. The data was analyzed using one-way ANOVA and Tukey’s post-hoc procedure. Statistical significance was set at p<0.05.

**Results:** Tendon expression of normalized type III and type I collagen were separately characterized prior to calculation of the type III / I ratio. In the tendon injury group, a significant 5.0 fold (p<0.05) increase in type III collagen expression was already present by week 1 post-injury. Type I collagen expression did not exhibit any significant increase above control levels until 5 weeks post-injury. Similar trends were also seen in the steroid treated and tendon injury plus steroid groups.

The collagen type III / I expression ratio remained at baseline at all time points in the control and sham groups. At 1 week, the collagen type III / I ratio increased more than 4.0 fold (p<0.05) above control in the tendon injury and tendon injury plus steroid groups (Figure 1). The ratio remained greater than 2.0 fold (p<0.05) above control at 3 weeks in both groups and returned to baseline at 5 weeks. Interestingly at 1 week, the steroid treated group showed a significant increase in type III / I ratio, more than 4.5 fold (p<0.01). This level decreased to baseline by 3 weeks.

**Discussion:** The objectives of this study were to: 1) characterize the role of type I and type III collagen in the injury response of rotator cuff tendons and 2) determine possible modulating effects of corticosteroid on this response. Type I collagen is the main collagen found in normal tendons, making up greater than 90% of total collagen content. When tendons are injured, an increased proportion of type III collagen is produced during the early injury/reparative response. Collagen protein typing of injured tendons have shown type III collagen content can acutely increase from <5% of total collagen content to >15% post-injury [3]. As injured tendons remodel and mature, baseline type III to type I collagen ratios are restored. This temporal change in collagen composition is reflected in the data from our injury group. As expected, a significant increase in the expression of type III collagen was evident by one week post-injury. Type I collagen expression, however, remained at baseline levels until a significant increase was observed at five weeks. This variation in expression levels is consistent with an active tendon injury response that progresses from acute phase towards maturation.

The type III to I collagen ratio between the injury and injury plus steroid groups suggest a single, human equivalent dose of corticosteroid does not significantly alter the acute phase response of an injured rotator cuff tendon. However, exposure of uninjured tendons to the same steroid dose initiated an injury response that was equivalent to that of structural injury. This significant steroid response in normal tendon was short-lived and the type III to I collagen ratio returned to control levels by three weeks. These findings suggest that a single dose of steroid has no long-term effect on collagen expression in either injured or uninjured rotator cuff tendons. At the same time, the significant effect seen in the steroid group suggests that even a single steroid dose might not be entirely benign in the short-term. The dramatic increase in type III to type I collagen expression can temporarily change rotator cuff tendon properties if the same proportions are translated to the protein level. It also raises concern of a potential cumulative steroid effect if consecutive doses are given before type III to I collagen ratios can return to baseline.

**Figure 1. Collagen III to Collagen I Gene Expression Ratio vs. Time**

* compared to control group p<.05
** compared to control group p<.01

**References:**