

Research Article

Dose-independent adverse effects of corticosteroid injections on rotator cuff healing in a rat model

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ABSTRACT

Objective: The aim of this experimental study in a rat model was to investigate the biomechanical and histological effects of subacromial corticosteroid injections on the healing of the tendon-bone junction after repair in the rotator cuff tear model.**Methods:** A total of 48 rats were divided into 3 groups: the control group, the single-dose steroid group, and the 4-dose steroid group. The supraspinatus tendon was completely dissected from the footprint attachment site and repaired. Saline was injected into the subacromial area in the control group, while 0.6 mL-4.8 mg/kg of single-dose methylprednisolone was applied to the single-dose group, and the same dose was applied 4 times at 1-week intervals to the 4-dose steroid group. All animals were allowed unrestricted movement during this period, and all animals were sacrificed by cervical dislocation 4 weeks after the last dose in the 4-dose steroid group. Cell shape and the number of apoptotic cells were assessed histopathologically, and the fracture load, maximum stress, and energy absorption were assessed biomechanically.**Results:** There were no significant differences in terms of cell shape between the groups. There was a significant difference in the mean number of apoptotic cells in the steroid groups compared to the control group. In the biomechanical evaluation, no differences were found between the groups in terms of maximum tension or breaking load (n.s.). A significant difference was found when the 3 groups were compared in terms of energy absorption ($P = .001$). There was a significant difference in energy absorption between the control group and the steroid-treated groups, but there was also a significant difference between the single-dose group and the 4-dose group ($P = .038$).**Conclusion:** The administration of corticosteroids was found to have a negative effect on healing at the tendon-bone junction, but this effect did not vary with the number of steroid injections. From a biomechanical perspective, it was observed that the energy absorption at the surgical repair site was lower with corticosteroid administration, and this negative effect increased with the increasing number of injections.

Introduction

With an increasing aging population, there is a parallel increase in musculoskeletal degenerative diseases, which create a burden on society. One such disease is rotator cuff (RC) disease,¹ for which the etiology and precise diagnostic criteria remain unclear.² There is increasing evidence that shoulder injuries involving the RC cause severe pain and a deterioration in quality of life and sleep.³ Several non-surgical treatment modalities have been developed for rotator cuff injuries, including corticosteroid (CS) injections, but their relative effectiveness has not yet been well established.⁴

In addition to non-steroidal anti-inflammatory drugs and pain control protocols, CS injections are also widely used for postoperative pain control.^{5,6} Several shoulder surgeons have reported pain reduction and significant improvements in joint range of motion following postoperative CS injections in patients with severe stiffness associated with pain and sleep disturbance.^{6,7} In contrast, some surgeons are skeptical about subacromial CS injections after RC repair due to

concerns about complications associated with the use of CS, such as infection,^{7,8} tendon rupture,⁹ and short-term postoperative adverse effects on the healing process of the repaired tendon.^{10,11} A study examining the costs of preoperative treatments showed that steroid injections were commonly used despite the negative effects on postoperative outcomes and the high costs.¹² Another study compared treatment methods for RC-related shoulder pain for the periods between 2000 and 2004 and between 2012 and 2016, reporting that while the rate of CS injections increased from 9.8% to 19.7%, the rate of nonsteroidal anti-inflammatory drug (NSAID) prescriptions decreased from 33.6% to 18.4%.¹³ All these studies have shown that the frequency of CS injections, preferred as a medical treatment for RC tears, has increased in recent years.

The aim of this study was to demonstrate the histopathological and biomechanical effects of repeated subacromial CS injections on the RC and tendon-bone attachment of full-thickness RC tears created in a rat model. To the best of the authors' knowledge, there are no previous studies in the literature that have evaluated the in-vivo effect of CS injection following

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RC repair on tendon-bone healing. This study is the first of its kind in terms of both these aspects and the comparison of repeated CS injections. The hypothesis of the study is that the use of subacromial CS injection after repair in the RC tear model will adversely affect the healing of the tendon-bone junction biomechanically and histologically.

Materials and methods

This study was approved by the Bursa Uludağ University Ethics Committee for Animal Experiments (Approval No: 2019-01/06 Date: 09.01.2019). All surgical procedures were performed by the first 2 authors. The animals were cared for, fed, and housed under the supervision of the responsible veterinarian of the same centre. The shoulders of Wistar albino rats were chosen for the study because of the similarity of the shoulder anatomy to that of humans with a coracoacromial arch, as well as the low cost and ease of care of the animals. The study material consisted of the left shoulders of 48 female Wistar albino rats aged 4 months. No restrictions were placed on the animals' activity. It was planned to exclude any rats that showed decreased food and water intake, weight loss, or decreased responsiveness to stimuli. No such conditions were observed in any of the animals.

The rats were divided into 3 groups: the control group, the single-dose steroid group, and the 4-dose steroid group. Within each group, the animals were then divided into 2 subgroups for biomechanical and histopathological examination.

Surgical technique

After the administration of anesthesia, a 15-mm incision was made starting from the lateral border of the acromion. The RC was then accessed by splitting the deltoid muscle. After dissection, the supraspinatus muscle was identified and a full-thickness dissection was performed at 2 mm from the humerus insertion point. A tunnel was created in the humerus near the tuberculum majus, using the tip of a 0.5-mm syringe. Using the modified Mason-Allen suture technique,¹⁴ tendon repair was performed with 5/0 Prolene sutures passing from the tendon and through the tunnel opened in the humerus. The deltoid muscle and fascia were repaired, and then the subcutaneous tissue and skin were sutured. The surgical technique is outlined in Figures 1 and 2.

Corticosteroid injection

The first injection was given immediately after the repair, before the wound was closed. All other injections were performed in the same way, without the use of ultrasound. The posterolateral corner of the acromion was identified using a 25-gauge needle by rubbing the undersurface of the acromion and aiming the needle anterolaterally into the subacromial space. The plunger was gently pushed until no resistance was felt during injection. Group 1 rats were injected with saline into the subacromial space, and groups 2 and

3 were injected with methylprednisolone succinate (Prednol-L 0.6 mL-4.8 mg/kg lyophilized injectable ampoule). Corticosteroid injections were repeated once a week for a total of 4 doses using the same technique in the 4-dose group. All animals were allowed unrestricted movement during this period, and all the animals were sacrificed by cervical dislocation 4 weeks after the last dose was administered to the 4-dose steroid group. All the rats were monitored 3 times per week throughout the experimental period. The humerus, scapula, and supraspinatus muscles were completely removed from each rat.

Histopathological examination

Tissue samples were taken from the tendon-bone interface for histopathological analysis. The samples were fixed in 10% formaldehyde, and after 24-48 hours of decalcification, the fixation and processing procedures were carried out. Sections of 5 microns in thickness were cut from the tissues embedded in paraffin blocks and placed on microscope slides. Samples were stained with haematoxylin and eosin for routine histopathological evaluation. Microscopic images were scored by 2 observers for cell shape and the number of apoptotic cells. A score of 1.0 to 4.0 points was assigned, and the average of the 2 observers scores was used for analysis. Higher scores were given if the cell shape changed from round to spindle, and higher scores were given if the number of cells decreased. Better healing was demonstrated by a change in cell shape from round to spindle and a decrease in the number of apoptotic cells. In order to avoid inter-observer variability, a scale expressing both histological data and numerical values was created, and the average of the scores of the 2 pathologists was taken into account. The numerical scale was created to ensure comparability with those used in previous studies on this subject.¹⁵

Biomechanical examination

The soft tissues were cleaned of the material removed from the rats, and then the ends of the humerus and scapula were wrapped in non-slip sandpaper and clamped in the 2 jaws of a universal gripping device (Shimadzu AG-X-HS, Japan) (Figure 3). A constant rate of 100 mm/min was applied until the tendon ruptured from the repaired area. For each specimen, the device measured the energy absorbed, the maximum tension, and the load applied until the tendon ruptured. Results were recorded electronically as decimals. Correlation values were calculated for each parameter using the parallel forms method. The correlation value for each parameter was greater than 0.7.

The biomechanical evaluation recorded data on energy absorption capability, maximum tension, and breaking load obtained in the axial tensile tests. Energy absorption capability is the ability of a material to store energy. The ability to absorb load or an impact on the material indicates whether or not it is easily deformed. The unit is joule (J). The breaking load is the value of the force at the moment of rupture of the material, expressed in Newtons (N). Tension is described as the force acting on a unit area. Maximum tension is the greatest tension value reached at the moment of rupture of the sample. The units are N/tex.

Statistical analysis

Power analysis was used to determine the minimum number of animals required to achieve statistical significance, thus avoiding unnecessary use of experimental animals. The results of the power analysis indicated that to predict a statistically significant difference in effect size ($f=0.40$) for an 80% confidence interval with a 5% margin of error, a subgroup of 8 rats was required. As there were 6 subgroups, the total study population was set at 48 rats. The data obtained in the

HIGHLIGHTS

- Corticosteroids had a negative effect on the healing of repaired acute rotator cuff tears in a rat model.
- Corticosteroids reduce the number of apoptotic cells in repaired acute rotator cuff tears.
- Corticosteroids decreased tendon energy absorption with the number of injections
- The negative effect of corticosteroids was dose-independent.

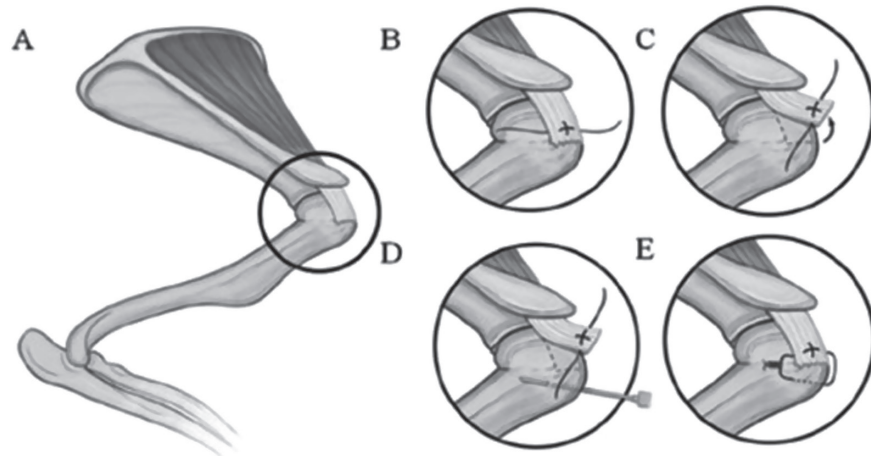


Figure 1. Schematic representation of the modified Mason Allen suture technique: A. Identification of the supraspinatus tendon under the acromion. B. Modified Mason Allen suture placed in the intact supraspinatus tendon. C. Detachment of the supraspinatus tendon from its attachment. D. Passing a 0.5 mm syringe tip through the proximal humerus. E. Knotting the suture through the bone tunnel.

study were statistically analyzed using SPSS vn.22.0 (IBM SPSS Corp.; Armonk, NY, USA) software. Continuous variables were expressed as mean \pm SD values. The values obtained in the histopathological

and biomechanical studies were statistically examined using the Mann-Whitney U test for the comparisons of 2 independent groups of non-parametric data. For 3 groups, the Kruskal-Wallis test was used. A value of $P < .05$ was considered statistically significant.

Results

The minimum, maximum, mean and SD values of the parameters used in the histopathological evaluation, and the comparisons between the control, single-dose, and multiple-dose steroid groups are shown in Table 1. There were no significant differences in cell shape between the 3 groups (n.s.). Differences were found between the 3 groups in terms of the number of apoptotic cells ($P = .028$). There was a 36% reduction in the mean number of apoptotic cells in the steroid groups compared to the control group. The observers' subjective assessments reported that the cells were rounder in shape and that there was a greater intensity of apoptotic cells in the steroid-treated groups than in the control group.

When all specimens were examined prior to biomechanical testing, there were no macroscopic findings such as defects in the repair area. During the biomechanical testing, all tendons tore at the site of surgical repair. The minimum, maximum, mean and SD values of the parameters used in the biomechanical evaluation, and the comparisons between the control, single-dose steroid, and multiple dose steroid groups are shown in Table 2. No significant differences were found between the 3 groups for breaking load (n.s.) and maximum tension (n.s.). A significant difference was found when the 3 groups were compared in terms of energy absorption ($P = .001$). There was a significant difference in energy absorption between the control group and the steroid-treated groups, but there was also a significant difference between the single-dose group and the 4-dose group ($P = .038$). These results showed that steroid administration had a negative effect on tendon strength and that this negative effect increased with the increasing number of injections.

Discussion

The main finding of this study was that subacromial CS injection reduced the biomechanical strength of the tendon-bone junction and impaired histological healing. The histopathological evaluation of this study showed that although there were no statistically significant

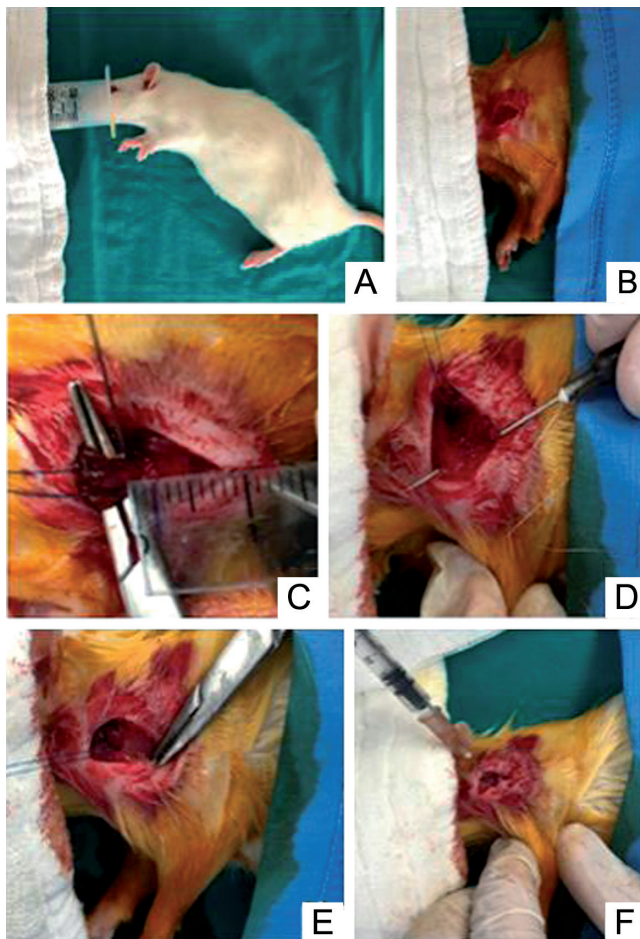


Figure 2. Surgical Procedures: A. Continuation of anesthesia via inhaler after anesthesia and positioning of the subject. B. Skin incision of approximately 15 mm starting from the lateral border of the acromion. C. Finding the supraspinatus muscle after separating the deltoid split and determining the level to be cut with a ruler. D. Tunnelling to the humerus after the supraspinatus tendon is separated. E. Repair of the tendon using the modified Mason Allen suture technique with the suture passed through the tunnel. F. Subacromial injection after the deltoid muscle is also repaired.

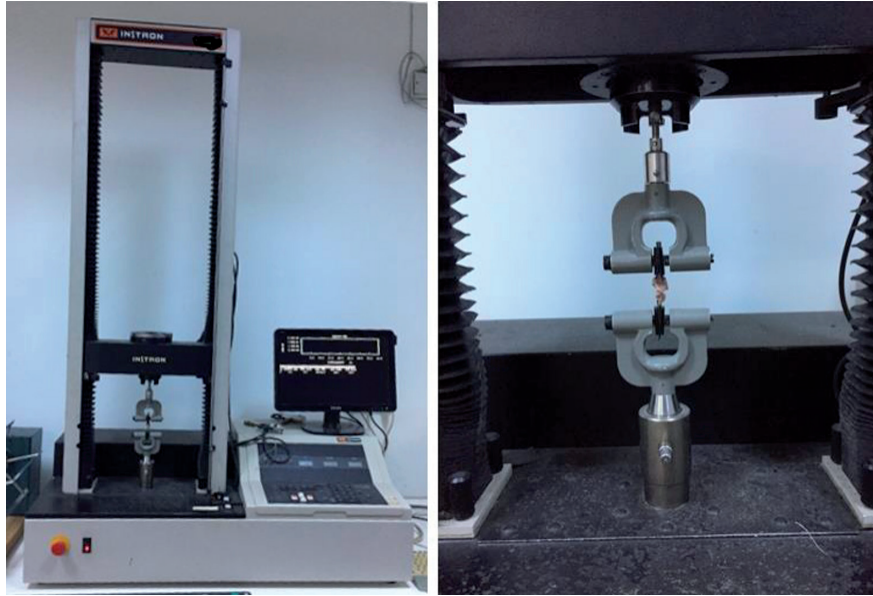


Figure 3. Universal tension device (Instron, Shimadzu AG-X-HS, Japan).

differences between the control group and the steroid groups in terms of cell shape, the differences in the number of apoptotic cells between the groups were statistically significant, indicating that steroids, independent of dose, had a negative effect on the healing of the tendon and tendon-bone junction. In the biomechanical evaluation, there were significant differences in energy absorption between the control and steroid groups. This result showed that steroids had a negative effect on the repaired RC tendons and that this negative effect was exacerbated by a higher number of doses.

In a previous study investigating the causes of RC tears, the risk was found to be 7.44 times higher in patients who received a CS injection.¹⁶ In contrast, there are studies that show positive effects of CS injection in conservative management. Hajivandi et al¹⁷ concluded that steroid injection enhanced the effect of physiotherapy in patients with full-thickness RC tears, followed by conservative treatment. Although there are publications supporting CS injections in the course of conservative treatment, the number of studies showing the effect of the same after surgical treatment of RC tears is very limited. One systematic review reported that CS injection was

beneficial on clinical scores and pain in the first 3 months after the repair of RC tears, but these effects were not maintained at 6 months and beyond.⁷ In contrast, Baverel et al¹⁰ reported that preoperative CS injection posed no risk for clinical scores and re-tear, although postoperative CS injection posed a greater risk for re-tear and lower scores. The same study also reported that these results might depend on the degree of fatty infiltration other than CS injection. As it is very difficult to investigate the histological effects of CS injections with human studies, the current animal study on this topic can be considered valuable. Maman et al¹¹ investigated the effects of CS injection on a rat RC model and showed that a single or multiple-dose of CS after RC tear repair resulted in a significant 26.1% reduction in the maximum load on the RC. In contrast to this study, histological evaluation was also performed in the current study and there were no differences between the groups in terms of maximum load. Although the maximum load value in the current study resulted in a reduction of approximately 23% in the single-dose CS group and approximately 17% in the multiple-dose group, this was not statistically significant.

Table 1. Comparison of control group, single dose group and 4-dose group in terms of cell shape score and apoptotic cell amount score

Group	Cell Shape Score	Apoptotic Cell Amount Score
Control group n:8	2.64 ± 0.58	3.21 ± 0.90
Mean ± SD (min-max)	(1.50-3.00)	(1.50-4.00)
Single dose group n:8	2.13 ± 0.49	2.06 ± 0.68
Mean ± SD (min-max)	(1.50-3.00)	(1.25-3.50)
4-dose group n:8	2.16 ± 0.48	2.06 ± 0.90
Mean ± SD (min-max)	(1.50-3.00)	(1.00-3.50)
P		
Control group/ single dose group/ 4-dose group	n.s*	.028*
Control group/ single dose group		.015 [†]
Control group/ 4-dose group		.028 [‡]
Single dose group/ 4-dose group		n.s [‡]

*Kruskal-Wallis test.

[†]Mann-Whitney U test.

Table 2. Comparison of biomechanical parameters of energy absorption capability, maximum tension, and breaking load obtained between the control group, single dose group, and 4-dose group

Group	Energy Absorption Capability (J)	Maximum Tension (N/tex)	Breaking Load Obtained (N)
Control group n:8	0.6 ± 0.2	1.9 ± 0.6	95.5 ± 29.8
Mean ± SD (min-max)	(0.3-0.8)	(1.1-2.9)	(54.0-144.0)
Single dose group n:8	0.3 ± 0.2	1.5 ± 0.7	73.5 ± 34.9
Mean ± SD (min-max)	(0.1-0.6)	(0.4-2.9)	(21.0-146.0)
4-dose group n:8	0.1 ± 0.1	1.6 ± 0.9	79.0 ± 45.5
Mean ± SD (min-max)	(0.0-0.2)	(0.4-3.0)	(22.0-152.0)
P			
CG/SDG/4DG	.001*	n.s*	n.s*
CG/SDG	.021 [‡]		
CG/4DG	<.001 [‡]		
SDG/4DG	.038 [‡]		

4DG, 4-dose group; CG, Control group; SDG, Single dose group.

*Kruskal-Wallis test.

[‡]Mann-Whitney U test.

However, energy absorption was found to be significantly lower in the steroid-administered groups in the current study. In addition, the study by Maman et al¹¹ examined the effect of CS injections on intact and injured RC, not repaired RC tears. However, this study examines CS injections into repaired RC tears. Mikolyzk et al¹⁸ found in their rat experimental animal model studies that steroids had a negative histological and biomechanical effect on the damaged RC in the first week, but this effect was reversed by the third week. In support of the Mikolyzk et al¹⁸ study, Lee et al¹⁹ showed that in their rat model studies, negative effects were observed during the inflammatory period in rats that induced RC tears and received CS injection on the same day, while these effects disappeared after the inflammatory period. However, no repair was performed in these studies and the effects were only assessed in the damaged RC model. In addition, there was no evaluation of repeated doses of steroids.

Maman et al¹¹ in their study examined the greater tuberosity with micro-CT after steroid, they found that osteopenia in the greater tuberosity decreased in the first week and returned to normal by the third week. In another rat study on anchor pull-out strength and Cs injections, greater tuberosity anchors were applied, and anchor pull-out values were examined. Similar to this study, they found that although anchor pull-out values were significantly lower in the first week, they were close to normal by the fourth week without a significant difference.²⁰ Although anchor fixation was not performed in this study, when the subjects were analyzed at week 4 after the last injection, the steroid-treated group had lower breaking load values. This effect may indicate that healing may also be affected by osteopenia.

This study had several limitations. First, this rat model was a model of acute RC defects and may differ from chronic RC defects. However, an acute RC model was appropriate for this study. Second, although the rat model of RC tears was very similar to that of human shoulders, there are different anatomical and histological characteristics. The duration of healing in the tendon-bone region in rats is not fully understood. Although the evaluations in the current study were performed 4 weeks after the last CS injection, there are no definitive data in the literature regarding this period, which could be considered a limitation of this study. Third, factors such as the type of incision made at the tendon-bone junction, the age of the animals, and the healing capacity of the rats may be other limitations. These types of injuries in humans, which are associated with degenerative changes and reduced blood flow, tend to rupture in middle-aged people.

This study showed that post-operative steroid injections in repaired acute full-thickness RC tears impaired healing. This finding is important as there are no other animal studies that have considered both biomechanical and histological aspects. In clinical practice, steroid administration should be avoided in repaired acute tears, especially in the first month. However, this study investigates the effect of CS injections within the first month on healing in an acute tear model. Therefore, studies on the effect of CS injections on healing in chronic repaired tears or 1 month after repair will contribute to the literature.

The histopathological evaluation of this study showed that CS had a negative effect on repaired RC tendon healing, and this effect was dose-independent. From a biomechanical perspective, the decrease in tendon energy absorption increased with the number of injections.

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

Ethics Committee Approval: This study was approved by the Ethics Committee of Bursa Uludağ University Ethics Committee for Animal Experiments (Approval No: 2019-01/06 Date: 09.01.2019).

Informed Consent : N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – H.Ş., Y.A., A.Ö.; Design – H.Ş., Y.A., A.Ö.; Supervision – Y.A., A.Ö.; Resources – ; N/A Materials – H.Ş.; Data Collection and/or Processing – Ö.A., H.S.; Analysis and/or Interpretation – H.Ş., Y.A., N.Ç., Ö.A., H.S.; Literature Search – H.S.; Writing Manuscript – H.Ş., N.Ç., Ö.A.; Critical Review – Y.A., N.Ç., A.Ö.

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