

EFFECT OF SUBACROMIAL HYALURONIC ACID INJECTION ON THE NORMAL ROTATOR CUFF TENDON: AN EXPERIMENTAL STUDY IN RAT

SUBAKROMİYAL HYALURONİK ASİD ENJEKSİYONUNUN NORMAL ROTATOR MANŞET TENDON ÜZERİNE ETKİSİ: SIÇANLARDA DENEYSEL BİR ÇALIŞMA

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SUMMARY

Introduction: Clinically hyaluronic acid injection has been used in treating shoulder pain and was found to be effective for patients with rotator cuff tears. The aim of the present experimental study was to evaluate cellular changes in the rotator cuff after hyaluronic acid injection into subacromial area, compared to subacromial injections with normal saline.

Material and Method: Ten male Wistar rats were used in the study. Both the right (study n=10) and left (control n=10) shoulders were used for subacromial injection in each rat. The hyaluronic acid doses were given 0.1ml of hyaluronic acid (HYALGAN®) were injected subacromially into the right shoulder (study group) and 0.1ml of saline solution were given into the subacromial space of the left shoulder (control group) once weekly for consecutive 3 weeks. One week after the last injection, the rats were sacrificed with a lethal dose of Xylazine HCl and Ketamine HCl. Next, the right and left shoulder joints including the supraspinatus and infraspinatus tendons were carefully dissected and slices were subjected to routine hematoxylin and eosin (H&E) staining procedure, and then were examined under a light microscope. Differences between groups, regarding animals' weight, were evaluated using Student's t-test for independent variables. The level of statistical significance was set at p<0.05.

Results: Macroscopically: normal tendon structures were observed in both groups. Microscopically: no statistically significant pathologic changes including necrosis, inflammation and giant cell infiltration were observed on microscopic examination of both the control and study shoulders (p= .5921).

Conclusion: Hyaluronic acid injection into the subacromial area has no adverse histopathologic effects on the healthy rotator cuff.

ÖZ

Giriş: Klinik olarak hyaluronat enjeksiyonu omuz ağrı tedavisinde kullanılır ve rotator manşet yırtıklı hastalarda efektif olduğu bulunmuştur. Deneysel çalışmamızın amacı subakromial bölgeye hyaluronat enjeksiyonu sonrası rotator cufftaki hücresel değişiklikleri değerlendirmek ve subakromial alana normal salin enjeksiyonu ile karşılaştırmaktır.

Gereç ve Yöntem: Çalışmada 10 erkek Wistar rat kullanıldı. Her ratın hem sağ (çalışma) hem sol (kontrol) omuzları enjeksiyon için kullanıldı. Hyaluronik asid dozu sağ omuza (çalışma grubu) 0,1 ml sol omuza (kontrol grubu) 0,1 ml salin enjekte edildi ve üç hafta tekrarlandı. Son enjeksiyondan bir hafta sonra ratlar letal dozda Xylazine HCl and Ketamine HCl ile sakrifiye edildi. Daha sonra, supraspinatus ve infraspinatus tendonları dahil olmak üzere sağ ve sol omuz eklemleri dikkatlice diseke edildi ve rutin hematoxilen ve eozin (H&E) boyama prosedürüne tabi tutuldu, ışık mikroskopunda incelendi. İstatistiksel olarak $p < 0.05$ anlamlı kabul edildi.

Bulgular: Makroskopik olarak her iki grupta da normal tendon yapıları gözlemlendi. Hem kontrol hem de çalışma omuzlarının mikroskopik incelemesinde nekroz, inflamasyon ve dev hücre infiltrasyonu gibi istatistiksel olarak anlamlı patolojik değişiklikler gözlenmedi ($p = 5921$).

Sonuç: Subakromiyal alana hyaluronik asit enjeksiyonunun sağlıklı rotator manşet üzerinde olumsuz histopatolojik etkileri yoktur.

INTRODUCTION

Hyaluronic acid is an important component of synovial fluid and articular cartilage (1). The biophysical properties of the synovial fluid, including the viscosity and elasticity, are due to its hyaluronic acid content. The synovial fluid is unique in that it functions differently under different physical conditions (2).

Hyaluronic acid has been shown to influence the levels of prostaglandins and cyclic AMP in synovial fluid. It has anti-inflammatory, analgesic properties and affects the leukocyte functions. The high molecular weight cross-linked derivatives of hyaluronic acid provides a protective effect on chondrocytes exposed to inflammatory stress mediators (3). A possible autocrine stimulation of hyaluronic acid synthesis in synoviocytes has been proposed previously (3). Basically when injected into the joint it serves as a lubricant inside the joint (4), protecting the articular cartilage (5), suppressing the activities of pro-inflammatory mediators and thus the inflammatory process in a joint (6,7).

Clinically hyaluronic acid injection has been used in treating shoulder pain (8-10). Hyaluronic acid was found to be effective for patients with rotator cuff tears (11). Blaine et al found that hyaluronic acid is effective and well tolerated for the treatment of osteoarthritis and persistent shoulder pain that is refractory to other standard non-operative interventions (11).

Clinically, Meloni et al found that the use of ultrasonographic guidance permits accurate injection of the hyaluronic acid at the subacromial level where it serves not only as an anti-inflammatory, but also has a lubricating mechanical effect which loosens the adhesion on the sliding surface of the tendon (12).

Experimentally, damage to the rotator cuff of rats has been observed after repeated local subacromial injections of the steroid (13,14). The side effects of local hyaluronic acid injection into the subacromial region of the shoulder are unknown and to our knowledge no experimental data are available to show the toxic and/or safety of extra-articular subacromial hyaluronic acid injections. We hypothesized that the hyaluronic acid have not negative effect on rotator cuff.

The aim of the present study was to evaluate cellular changes in the rotator cuff after hyaluronic acid injection into subacromial area, compared to subacromial injections with normal saline.

MATERIAL AND METHODS

All experiments were approved by the Ethical Committee of the School of Medicine. Ten male Wistar Rats, weighting 300-350g, were used in the study. All experimental animals housed at a controlled temperature of 22 ± 1 °C and animals had free access to food and water. Animals were anaesthetized with Xylazine HCl (21 mg/kg) of

body weight and Ketamine HCl (45 mg/kg). Both the right (study n=10) and left (control n =10) shoulders were used for subacromial injection in each rat. The hyaluronic acid doses selected were equivalent to those administered to humans. The hyaluronic acid doses were given 0.1ml of hyaluronic acid (HYALGAN®*) were injected subacromially into the right shoulder (study group) and 0.1ml of saline solution were given into the subacromial space of the left shoulder (control group) once weekly for consecutive 3 weeks (15). All injections were performed under sterile condition by the same surgeon to lateral side of shoulder.

One week after the last injection, the animals were sacrificed with a lethal dose of Xylazine HCl and Ketamine HCl. Next, the right and left shoulder joints including the supraspinatus and infraspinatus tendons were carefully dissected by Deltoid split approach and were placed in 10% formalin solution for 24 hours for fixation and 20% diluted formic acid solution for 48 hours for decalcification, respectively. The tissue sections containing joint capsule and humerus head through the shoulder joint were cut.

Macroscopically, no significant finding was observed in both groups of shoulder joints from the rats. The specimens were placed in 10% formalin solution for 24 hours for fixation and 20% diluted formic acid solution for 48 hours for decalcification, respectively. The tissue sections containing joint capsule and humerus head through the shoulder joint were cut. After then, the sections were subjected to routine tissue processing and embedded in paraffin. Paraffin blocks were sliced in 5-µm thickness with microtome and the slices were subjected to routine hematoxylin and eosin (H&E) staining procedure, then were examined under a light microscope (Olympus BX50) by a expert pathologist. In microscopic examination, the following histopathological features were evaluated: oedema/vascular congestion, inflammatory cell infiltration, presence of multinuclear giant cells, and collagen band necrosis.

A semiquantitative histopathological scoring system was used to score the features as follows: oedema/vascular congestion,

inflammatory cell infiltration, presence of multinuclear giant cells, and collagen band necrosis. Accordingly, 0: no histopathological changes, 1: mild, 2: moderate, 3: severe (13,14).

Data were analyzed using SPSS statistical package 21.0 (SPSS Inc., Chicago, IL, USA). Differences between groups, regarding animals' weight, were evaluated using Student's t-test for independent variables. The level of statistical significance was set at $p < 0.05$.

RESULTS

There was no statistically significant difference between the two groups regarding animals' weight ($p > 0.05$).

Macroscopically: The tendons were observed for any change in consistency and color. Normal tendon structures were observed in both the control and study groups. Microscopically; Edema, congestion, focal fragmentation of the collagen bundles and infiltration by inflammatory cells, predominantly lymphocytes and giant cell infiltration cells were the focus of microscopic examination. Only in one specimen of each group local infiltration by lymphocyte and giant cells were observed (Figure 1).

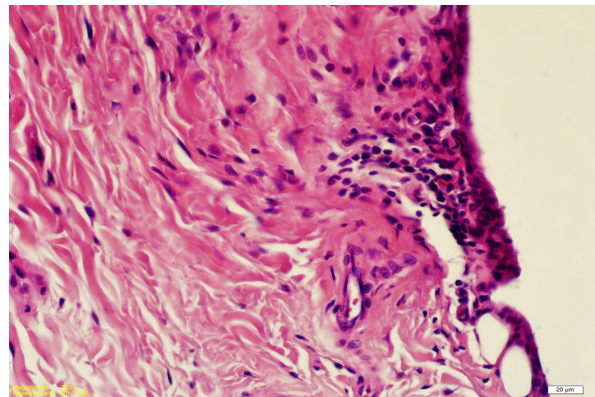


Figure 1. Only in one study specimen of each group local infiltration by lymphocyte and giant cells were observed.

No statistically significant pathologic changes including necrosis, inflammation and giant cell infiltration were observed on microscopic examination of both the control and study shoulders ($p = .5921$). (Table 1).

Table 1. A semiquantitative histopathological scoring system was used to score the features as follows: oedema/vascular congestion, inflammatory cell infiltration, presence of multinuclear giant cells, and collagen band necrosis. Accordingly, 0: no histopathological changes, 1: mild, 2: moderate, 3: severe

K1-9			Control group
C1-9			Study group
	oedema/vascular congestion	collagen band necrosis	inflammatory cell infiltration
K1	0	0	0
K2	0	0	0
K3	0	0	1 multinuclear giant cells, and focal focus of inflammation (lymphocyte)
K4	0	0	0
K5	0	0	0
K6	0	0	0
K7	0	0	0
K8	0	0	0
K9	0	0	0
K10	0	0	0
C1	0	0	0
C2	0	0	0
C3	0	0	1 focal lymphocyte infiltration
C4	0	0	0
C5	0	0	0
C6	0	0	0
C7	0	0	0
C8	0	0	0
C9	0	0	0
C10	0	0	0

DISCUSSION

Clinically, intra-articular injection of hyaluronic acid has proved to be efficient in relieving pain in patients with osteoarthritis of the knee (16-18) and in peri arthritis of the shoulder (8-10). Previous studies have suggested that the injection of hyaluronic acid into the glenohumeral joints, and perhaps the bursa, may be safe and effective in treating shoulder pain that has arisen from several etiologies. (4,11, 12, 19-23).

In this experimental study we found that following subacromial hyaluronic acid injection, no statistically significant pathologic changes were observed in the rotator cuff tendons which may suggests toxic effects of hyaluronic acid comparing to control group. Therefore, hyaluronic acid didn't seem to induce changes in rotator cuff

tendons composition which may results in complications secondary to subacromial injection.

Alterations of cuff tissue, expressed as cellular damage, should be anticipated after local injections. Previous histopathologic studies have confirmed isolated damage in cuff tissue after steroid injections into the subacromial space of the rat shoulder (13,14). Despite the emerging use of hyaluronic acid injections instead of steroid injections, either for intra-articular osteoarthritis or for cuff pathologies, the significance of its local toxicity or the exact cellular interaction is still debated. While some consider it as a safe medication without adverse effect, no real histopathologic studies were conducted to show this claim in the subacromial area.

To our knowledge no previous experimental study have proven the safety of hyaluronic acid injection into the subacromial area. Analysis of composition of supraspinatus tendon, after steroid injection, has revealed edema, congestion, focal fragmentation of the collagen bundles and infiltration by inflammatory cells, predominantly lymphocytes and plasma cells (13,14). Therefore, in our study we specifically searched for these parameters to reveal any harmful effects of hyaluronic acid injections.

Rat shoulder is comparable to that of the human shoulder in term of anatomy and has been used as an experimental model to evaluate injections in the periarticular area of shoulder joint (24-26). Currently, the recommended injection schedules are 1 injection weekly for 3 weeks for the cross-linked higher molecular weight injection. In our study we have used a similar protocol to mimic that in the daily practice.

Temporary local reaction after hyaluronic acid injection into the knee joint has been reported to be approximately 1% to 4%. Among these reactions, local pain, swelling, and redness are the most frequent (17). Another reported complication after hyaluronic acid injection into the knee joint is pseudosepsis, or a severe acute inflammatory reaction (27).

There exact data which shows the rate of local complications after hyaluronic acid injection into the subacromial area is much fewer than that available for the knee joint. In previous clinical studies, no significant adverse reaction or complication were observed after hyaluronic acid injection into the subacromial area(19, 28, 29).The present study contributes to the histopathologic details of this safety that could hopefully lead to the wider use of hyaluronic acid for shoulder pathologies.

However further clinical studies with larger number of the subjects may be necessary to clarify the rate of complications after the hyaluronic acid injection into the subacromial area.

Possible limitations of this study are related to the use of a single commercially available hyaluronic acid preparation. This was chosen based on the clinical daily use of the injection in our clinic. However, further studies are possibly necessary in order to compare the different hyaluronic acid products beyond this.

In conclusion, hyaluronic acid injection into the subacromial area has no adverse histopathologic effects on the healthy rotator cuff and may be considered for the conservative management of subacromial pathologies. We use this Hyalgan for rotator cuff tear and shoulder pain.

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