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Investigation of the biomechanical and histopathological effects of autologous conditioned serum on healing of Achilles tendon

Erdinç Genç [a, *], Ozan Beytemur [b], Serdar Yuksel [b], Yılmaz Eren [c], Aysel Çağlar [d], Bedri Onur Küçükyıldırım [e], Mehmet Akif Güleç [b]

[a] Mengucek Gazi Training and Research Hospital, Department of Orthopedics and Traumatology, Erzincan, Turkey
[b] Bağcılar Training and Research Hospital, Department of Orthopedics and Traumatology, İstanbul, Turkey
[c] Şişli Etfal Training and Research Hospital, Department of Orthopedics and Traumatology, İstanbul, Turkey
[d] Bağcılar Training and Research Hospital, Department of Pathology, İstanbul, Turkey
[e] Yıldız Technical University, Department of Mechanical Engineering, İstanbul, Turkey

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ABSTRACT

Objective: The aim of this study to evaluate the effects of autologous conditioned serum (ACS) on the healing of transected rat Achilles tendons via the assessment of biomechanical and histological parameters.
Methods: The study was conducted on 45 male Sprague–Dawley rats. Five rats were used as donors for ACS preparation. Animals were randomly assigned to the experimental or control group. In both groups, the Achilles tendon was cut transversally and then sutured. In the placebo control and ACS-treated groups, saline or ACS, respectively, was injected into the repair zone three times after surgery. Ten rats from each group (ACS group, $n = 20$; control group, $n = 20$) were euthanized at days 15 and 30 after surgery for histopathological ($n = 5$) and biomechanical ($n = 5$) testing. The histopathological findings were interpreted using the Bonar and Movin scales. Tendon remodelling was evaluated via the immunohistochemical staining of collagen type 3. Biomechanical effects were assessed by tensile testing.
Results: The Bonar and Movin scale scores were significantly better in the ACS-treated group on both day 15 ($p = 0.003$ and $p = 0.003$, respectively) and day 30 ($p = 0.005$ and $p = 0.004$, respectively). The immunohistochemical density of collagen type 3 was significantly lower in the ACS-treated group on day 30 ($p = 0.018$). The type 1/3 collagen ratios of the groups were similar on days 15 and 30, as determined by Sirius Red staining ($p = 0.910$ and $p = 0.133$, respectively). In the biomechanical assessment results, the ACS-treated group's maximum load to failure values were significantly higher on day 15 ($p = 0.049$).
Conclusion: Injection of ACS had a positive effect on the histopathological healing of rat Achilles tendons on days 15 and 30 and on biomechanical healing on day 15. ACS treatment contributed to lowering the collagen type 3 density by day 30. According to our study, ACS may be favourable for the treatment of human Achilles tendon injuries and tendinopathies.

Introduction

The Achilles tendon is the strongest, largest tendon in the body.[1] However, it commonly ruptures in middle-aged men who exercise.[2] The incidence of tendon rupture is estimated as 18/10 000.[3,4]

Along with surgical and conservative methods for Achilles tendon rupture treatment, novel treatment methods have been developed due to the establishment of new biological approaches. Numerous articles have shown that individual growth factors are useful for tendon healing in animal models.[4] Such growth factors include vascular endothelial growth factor (VEGF), transforming

* Corresponding author. Başbağlar Mh. Mengücek Gazi Eğitim ve Araştırma Hastanesi Giriş Kat Ortopedi Polikliniği, 24000, Erzincan, Turkey
E-mail addresses: erdincgenc@hotmail.com (E. Genç), beytemur@yahoo.com (O. Beytemur), Serdar84yuksel@gmail.com (S. Yuksel), Yilmazeren55@gmail.com (Y. Eren), ayselkaracaglar@gmail.com (A. Çağlar), kucukyil@yildiz.edu.tr (B.O. Küçükyıldırım), akifgulec@yahoo.com (M.A. Güleç).
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growth factor (TGF) β −1, platelet-derived growth factor, insulin-like growth factor-1, basic fibroblast growth factor (FGF-2) and bone morphogenic proteins (BMP-12 and BMP-13).[5–11]

Due to mechanical stress in tendons, interleukin 1 (IL-1) expression upregulates and stimulates the release of cytokines, which play a role in inflammation. This pathway is a potential contributor to existing inflammation in tendinopathy, and the inhibition of such a pathway may be useful for tendinopathy treatment.[12] IL-1RA is a natural competitive inhibitor of IL-1; by inhibiting the signal pathway, it prevents the inflammatory cascade.

Autologous conditioned serum (ACS), which is used for the treatment of osteoarthritis and similar inflammatory diseases, is an injectable agent rich in endogenous IL-1RA. Meijer et al reported that contact between the blood and small glass spheres allowed a rapid, strong increase in the synthesis of many anti-inflammatory cytokines, including IL-1RA.[13] ACS is also rich in anti-inflammatory cytokines, such as IL-4, IL-10 and IL-13; furthermore, its tumour necrosis factor (TNF)- α, FGF-2, VEGF and hepatocyte growth factor (HGF) values are high.[14,15]

The aim of the present experimental study is to examine whether local ACS treatment implemented after tendon surgery would be useful for healing over a 4-week period. Our hypothesis is that ACS administration will have positive immunohistochemical, histopathological and biomechanical effects on the healing of Achilles tendons.

Material and methods

Local ethics committee approval for animal experimentation was obtained on 08.06.2015 (no. 2015/26). Forty-five 12-month-old adult male Sprague–Dawley rats with a mean body weight of 400–450 g, including five rats as ACS donors, were used in the study. The animals were kept five rats to a cage at a temperature of 22 °C under a 12-h: 12-h light–dark cycle. They were fed ad libitum with standard rat feed and had free access to water.

Forty rats were divided into two groups, with group 1 as the control group ($n = 20$) and group 2 as the ACS group ($n = 20$). Prophylactic gentamycin (8 mg/kg) was administered to the rats 30 min prior to the surgical procedure. The surgery was initiated with administration of inhaled anaesthesia, which started with 4% isoflurane (Forane) as an induction dose and continued with 2% as a maintenance dose. The posterior side of the right cruris was shaved, iodine was applied under aseptic surgical conditions and the area was covered using a sterile green cover; a standard posterior longitudinal incision of 2 cm was applied, and the Achilles tendon was revealed (Fig. 1). A complete transverse incision was performed using a no. 11 scalpel (Plusmed, Turkey) at 4–5 mm on the proximal side of the Achilles tendon–calcaneus junction. The end of the Achilles tendon was non-traumatically resutured using the modified Kessler method PDO II 4/0 (BOZ, Turkey). The incision site was sutured with four 3/0 propylene (Dogsan, Turkey) sutures placed at equal distances by under sterile conditions, and dressing was applied with povidone iodine (BatticonR, Adeka, Turkey; Fig. 2). No immobilisation method was applied to the rats during the postoperative period.

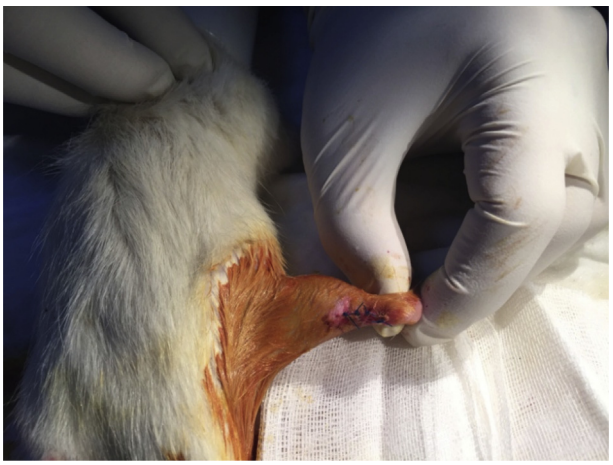

Fig. 2. Postoperative Achilles tendon with sutures at equal distances.

The five rats included in the donor group were decapitated after the collection of 5–6 cc of blood under anaesthesia. The blood samples collected from the donor group were transferred into special Orthokine (Orthogen AG, Düsseldorf, Germany) injectors[2] containing glass spheres, with a surface area of 21 mm, under a temperature of 37 °C. The samples were centrifuged using a centrifugation device (Megafuge, Kendro, Germany) at a rate of 3500 rpm for 10 min; following this, concentrated serum was collected in 0.2-mL quantities and kept at −20 °C. The samples were

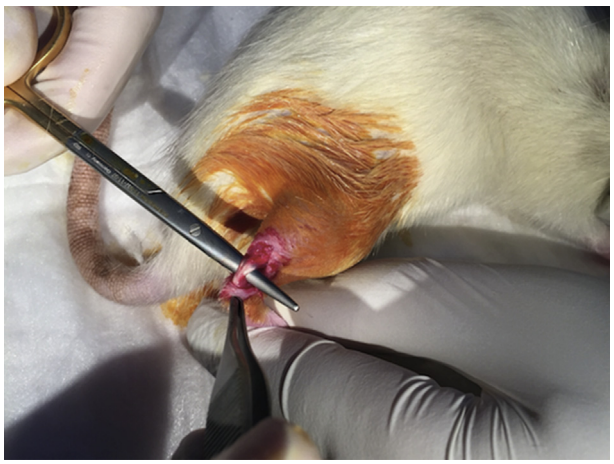

Fig. 1. Exploration of the Achilles tendon.

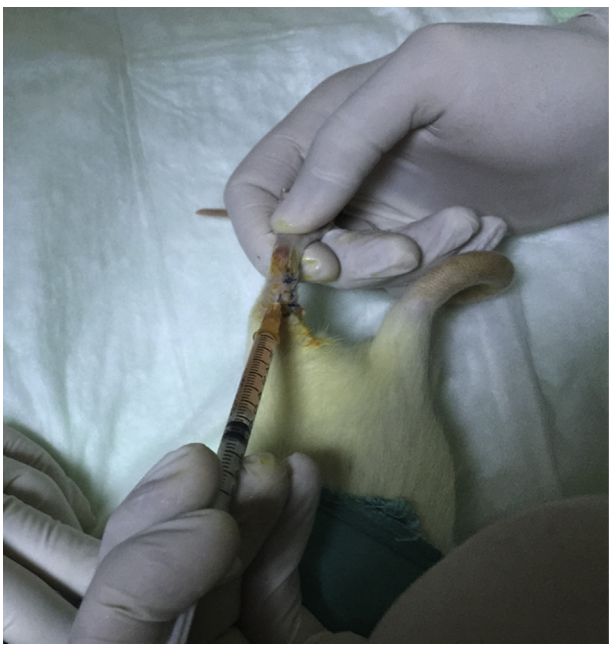

Fig. 3. Autologous conditioned serum (ACS) administration after surgery.

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