RESEARCH ARTICLE

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Effects of Systemic Enalapril Treatment on Skin and Peritendinous Adhesion: An Experimental Study

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Objective: One of the most important clinical complications after tendon repair surgery is fibrosis and adhesion formation, and some studies have shown that angiotensin II is responsible for pathological scarring and fibrosis. Enalapril, an angiotensin-converting enzyme inhibitor, inhibits excessive fibrosis and hypertrophic scarring while reducing conversion to angiotensin II. However, the effects of enalapril on skin fibrosis and peritendinous adhesion on tendon injuries remain unknown. We aimed to investigate the effects of enalapril on peritendinous adhesion and skin fibrosis during the extrinsic healing period of the tendon.

Materials and Methods: Twenty-one rats were randomly divided into three groups: sham (Group S), control (Group C) and enalapril (Group E). Both hindlimbs were operated. In Group S, the Achilles tendon was disassembled so that only the tendon sheath was opened. In groups C and E, the Achilles tendon was transversally and completely cut and then repaired. Postoperatively, only Group E was provided enalapril treatment for 28 d via oral gavage. Full-thickness Achilles tendon biopsies with skin were taken from healing regions to evaluate peritendinous adhesion and skin fibrosis.

Results: The formation of skin fibrosis, but not of peritendinous adhesion, was significanly lower in the enalapril group.

Conclusion: Enalapril reduces fibrosis in skin or subcutaneous tissues but has no significant effect on epitendineum and tendon structure.

Key Words: *Angiotensin-converting enzyme inhibitor, fibrosis, adhesion formation*

Sistemik Enalaprilin Cilt- Peritendinoz Adezyon ve Tendon İyileşmesi Üzerine olan Etkileri: Deneysel Çalışma

Amaç: Tendon onarımları sonrası görülen en önemli klinik problemlerden birisi fibrozis ve adezyon formasyonudur. Anjiotensin II patolojik skar ve fibrozisten sorumlu tutulmaktadır. Enalapril, Anjiotensin I'in Anjiotensin II'ye dönüşümünü azaltarak patolojik fibrozis ve hipertrofik skarı önlediği gösterilmiştir ancak tendon yaralanmalarında ciltteki fibrozis ve peritendinöz adezyon üzerine olan etkileri araştırılmamıştır. Bu çalışmada Enalaprilin, tendonun ekstrinsik iyileşme döneminde, peritendinöz adezyon ve cilt üzerine olan etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmada kullanılan 21 deney hayvanı; Sham grubu (Grup S), kontrol grubu (Grup K) ve enalapril grubu (Grup E) olmak üzere üç gruba ayrıldı. Grup S'de sadece tendon kılıfı açılacak şekilde aşil tendonu disseke edildi. Grup K ve grup E'de ise aşil tendonu transvers olarak tam kat kesilip yeniden onarıldı. Postoperatif dönemde Grup S ve Grup K'ye herhangi bir tedavi verilmezken Grup E'ye 28 gün boyunca oral enalapril verildi. Çalışmanın sonunda aşil tendonu üzerindeki cilt ile bağlantıları korunacak şekilde biyopsileri alınarak peritendinöz adezyon ve ciltteki fibrozis açısından değerlendirildi.

Bulgular: Ciltdeki fibrozis skoru ortalaması enalapril grubunda en az iken peritendinöz adezyon üzerine etkisi olmadığı tespit edildi.

Sonuç: Enalaprilin cilt-ciltaltı dokularda fibrozisi gerilettiğini fakat epitenon ve tendon üzerinde etkili olmadığını gözlemledik.

Anahtar Kelimeler: *Aniotensin dönüştürücü enzim inhibitör, fibrozis, yapışıklık*

Introduction

Tendon adhesion is the most important complication after tendon repair surgery and is characterized by the formation of fibrosis between the tendon and surrounding tissues (1). Tendon adhesion occurs in 20% of patients despite developments in surgical techniques, materials and rehabilitation (2). The most effective method for preventing tendon adhesion formation is through an active rehabilitation program during the postoperative period (3), but this is not achievable with nerve, vascular and bone injuries, and the risk of tendon adhesion increases. Although several agents have been experimentally and clinically tried to prevent tendon adhesion formation, these candidate drugs have not yet been accepted for routine clinical application (4).

Transforming growth factor-β (TGF-β) is a cytokine with numerous biological activities in wound healing in mammals. There are three isotypes of TGF-β: TGF-β1, TGF-β2 and TGF-β3. TGF-β1 is known to be effective in wound healing and scar tissue formation and plays a key role in tendon healing and adhesion formation (5).

Clinical and experimental studies have reported that angiotensin-converting enzyme inhibitors (ACEI) inhibit TGF-β1 activity and prevent hypertrophic scar formation (6, 7). However, the effects of ACEI on skin and tendon adhesion remain unknown. In this study, we aimed to investigate the effects of systemic enalapril (an ACEI) administration on peritendinous adhesions and skin fibrosis after primary tendon repair surgery in rats.

Materials and Methods

Research and Publication Ethics: All experimental procedures and animal care were approved by Firat University Animal Experiments Ethics Committee on 10/15/2014 (protocol number: 2014/106).

Twenty-one adult (7 rats/group), male, Wistar albino rats, weighing 300±20 g (mean±standard devation), were used in the study. All surgical procedures were performed under anesthetic and aseptic conditions. A combination of ketamine hydrochloride (50 mg/kg, Ketalar; Pfizer, Istanbul) and xylazine (10 mg/kg, Rompun; Bayer, Istanbul) was subcutaneously administered as an anesthetic before the surgical procedure.

Surgical Procedures: Subjects were randomly assigned to sham (Group S), control (Group C) and enalapril treatment (Group E) groups. Both posterior legs of the animals were cleaned using an antiseptic solution (Batticon; ADEKA, Istanbul), the skin was horizontally incised and the Achilles tendon was reached with dissection. In Group S, only the tendon sheath of the Achilles was dissected from proximal to distal and integrity of the tendon was intact (Figure 1). In groups C and E, the Achilles tendon was transversally and completely incised and then repaired with 5/0 round polypropylene suture (Prolene; Ethicon Inc., Somerville, NJ) according to the modified Kessler method (Figure 2) (8). The same protocol was bilaterally performed, and a total of 42 achilles tendons were operated. The animals' movements were not restricted during the healing process. Groups S and C were not given any medication postoperatively. Enalapril treatment was applied to Group E with oral gavage for 28 consecutive days at 10 mg/kg/day after surgery. The study was terminated at postoperative day 28 to histologically evaluate skin fibrosis and peritendinous adhesion. The rats underwent a second operation under anesthesia. Scar tissues related to the surgery were resected from 5-mm proximal to 5-mm distal to include skin fibrosis and peritendinous adhesion. Skin was also resected for the evaluation of the bilateral Achilles tendon. Following biopsy, the rats were euthanized by carbon monoxide inhalation.

Microscopic Evaluation: Tendon biopsy materials (n=42) were fixed in 10% formaldehyde and embedded in paraffin. Five-micrometer-thick sections were taken, and samples were stained with H&E and Masson trichrome. The sections were evaluated for peritendinous adhesion and skin fibrosis. In each group, skin fibrosis and peritendinous adhesion were evaluated using the scoring method previously described (9) (Table 1).

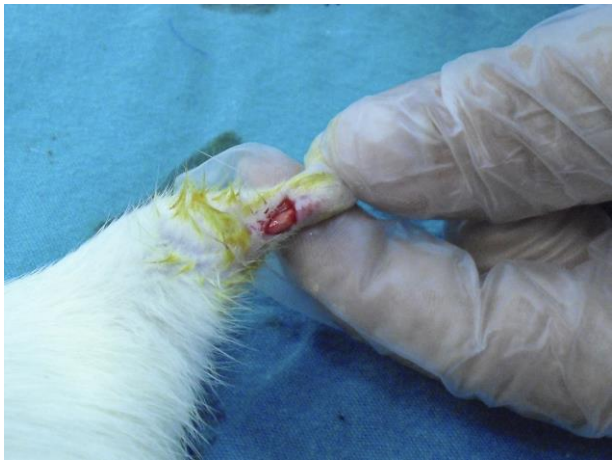

Figure 1. In Group S, tendon sheath of Achilles tendon was dissected from proximal to distal

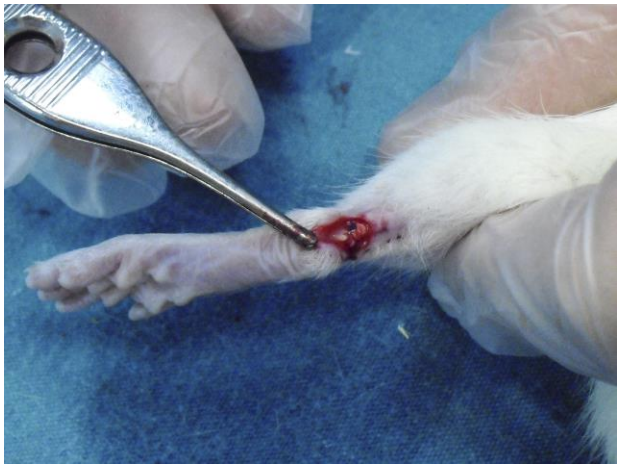

Figure 2. In groups C and E, the Achilles tendon was transversally and completely incised and then repaired

Table 1. Grading criteria of adhesions in histologic evaluation

Points	Features of Adhesions
	Quantity
0	No apparent adhesions
1	A number of scattered filaments
2	A large number of filaments
3	Countless filaments
	Quality
0	No apparent adhesions
1	Regular, elongated, fine, filamentous
2	Irregular, mixed, shortened, filamentous
3	Dense, not filamentous
	Grading of Adhesions
0	No adhesions
1–2	Slight adhesions
3–4	Moderate adhesions
5–6	Severe adhesions

Statistical Analysis: Data analysis was performed by using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). The differences in measurements among the groups were statistically significant was evaluated by the Kruskal–Wallis test. When the P values from the Kruskal–Wallis test statistics were statistically significant, Conover's multiple comparison test was used to learn which group differed from which others. A P values less than 0.05 was considered statistically significant. Data are shown as their median (min–max) values.

Results

None of the animals died, and no postoperative complications related to wound healing or infection were observed. Skin fibrosis score was 2 (0–4) in group S, 2.5 (0–6) in Group C and 2 (0–4) in Group E. Skin fibrosis scores in group C were significantly higher than those in Group E (P<0.05) (Figure 3). Peritendinous adhesion score was 0 (0–2) in Group S, 3 (0–6) in Group C and 3.5 (2–6) in Group E. Scores in groups C and E were significantly higher than those in Group S (P<0.001). There was no significant difference between groups C and E (P=0.648). Diffusion of adhesion formation in groups C and E had similar characteristics (Figure 4), and the amount of adhesions in these groups was significantly higher than that in Group S (P<0.001). Grade of peritendinous adhesions is summarized in Table 2.

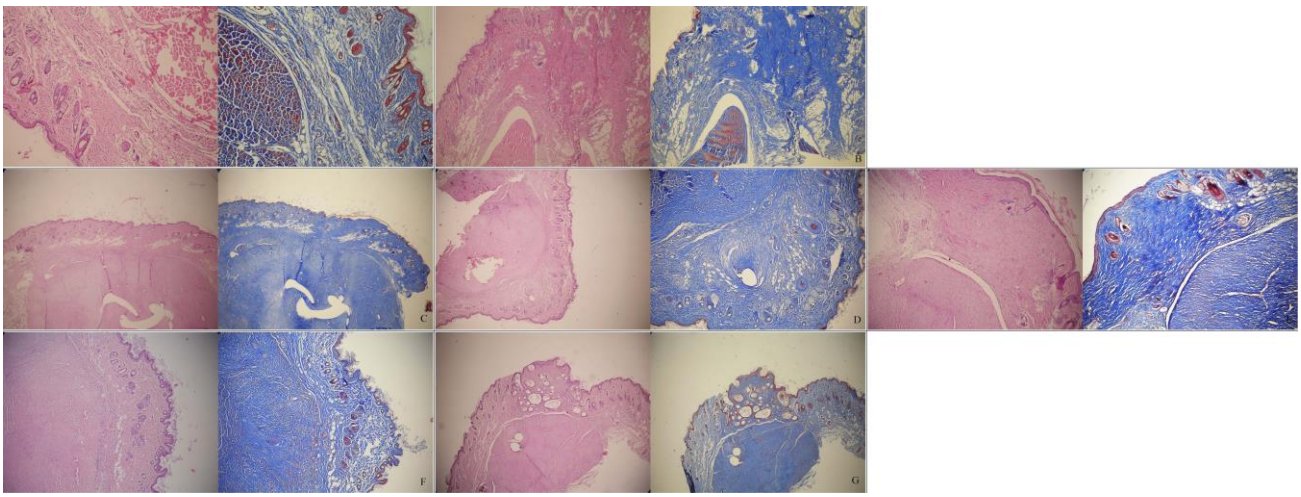

Figure 3. A, B) 1+, 2+ dermal fibrosis of the sham group, respectively. C, D, E) 1+, 2+, 3+ dermal fibrosis of the control group, respectively. E, F) 1+, 2+ dermal fibrosis of the enalapril group, respectively. (H&E and Masson trichrome, 200×)

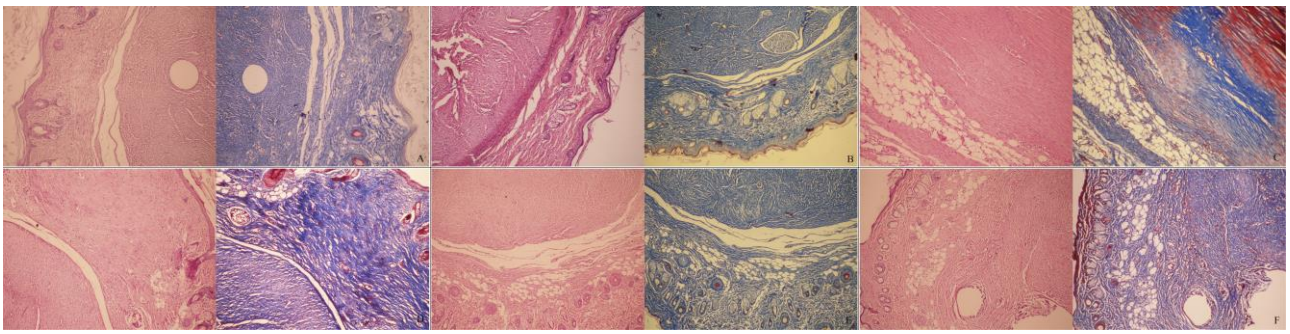

Figure 4. A, B, C) 1+, 2+, 3+ peritendinous adhesion of the control group, respectively. D, E, F) 1+, 2+, 3+ peritendinous adhesion of the enalapril group, respectively. (H&E and Masson trichrome, 200×)

Table 2. Peritendinous adhesion grades of the study groups

	N	Non n (%)	Slight n (%)	Moderate n (%)	Severe n (%)
Group S	14	12 (85.7)	2 (14.3)	0	0
Group C	14	1 (7.1)	4 (28.6)	7 (50.0)	2 (14.3)
Group E	14	0	6 (42.8)	6 (42.8)	2 (14.3)

Discussion

Extrinsic and intrinsic mechanisms are responsible for tendon healing, and a pathway is mediated via the vincular blood system and tenocytes (10). The extrinsic pathway is initiated by inflammatory cells and fibroblasts that migrate from the soft tissue around the injured tissue (11). This pathway is important in restoring tendon persistency and strength, but fibrosis and scarring may cause adhesion formation by disrupting the normal shear function of the tendon within the synovial sheath (12). After an appropriate tendon repair surgery, 20% of patients still have clinically limiting adhesions, and 10% of them will undergo a second surgical procedure (13, 14).

Ample studies have focused on the effectiveness of physical barriers made from various biological origin/synthetic materials to prevent tendon adhesion formation (15, 16). These barrier structures limit the formation of peritendinous adhesions by preventing the fibrotic tissue from reaching the tendon. In addition, previous studies have investigated the efficacy of various drugs such as NSAIDs, steroids, TGF-β neutralizers, 5-fluorouracil and plant flavonoids (4).These drugs were expected to prevent adhesion formation by changing cellular and molecular activities in tendon periphery inflammation and tendon healing (17). Although experimental results with these treatment methods have been successful, none of them have been widely accepted and/or used in clinical practice as a treatment (18).

In general, various complications, such as tendon adhesion, tissue trauma, ischemia, foreign body reaction, infection and hemorrhage, are due to an excessive phase of inflammation during the extrinsic healing period as well as due to the accumulation of excessive inflammatory cells in the subsynovial tissue around the tendon. These inflammatory cells secrete a large amount of cytokines and growth factors such as TGF-β, which have been shown to promote the synthesis of type 1 collagen and other extracellular matrix proteins by stimulating dermal fibroblasts with autocrine and paracrine signaling (7). TGF-β1 is involved in the generation of multiple connective tissue pathologies such as keloid and hypertrophic scars and Dupuytren contracture (19) and is the primary responsible cytokine for adhesion formation in mesothelial tissues, as tendon, synovium and peritoneum (20). Adhesion formation reduces with the inhibition of TGF-β with neutralizing antibody at the early stage of wound healing (10). Because TGF-β1 is a key factor in the formation of skin fibrosis and tendon adhesion, treatment approaches through TGF-β1 are thought to reduce tendon adhesion formation (20).

ACEIs inhibits the conversion of angiotensin I to its active form, angiotensin II [7]. Angiotensin II plays an important role in wound healing by stimulating collagen production and fibrosis (21). Overactivity of angiotensin II has been associated with pathological fibrosis in organs such as heart, aorta, kidney and lung (7). Tang et al. reported that angiotensin II increases TGF-β expression. Thus, angiotensin II-related collagen synthesis could be blocked by inhibiting the conversion of angiotensin I to angiotensin II using ACEI (22). Additionally, it has been shown that enapril reduces hypertrophic scar formation by reducing TGF-β and angiotensin II levels (7); hence, it was used as a candidate drug to inhibate TGF-β in this study.

Previous studies have shown that oral treatment of enapril significantly reduced the formation of skin fibrosis around the tendon injury during wound healing in rats; however, no significant effect was observed on tendon adhesion formation.

TGF-β secretion after tendon injuries increases in the first 3 days, reaches maximum on day 3 and decreases to normal levels on day 7 (10). In our study, enapril treatment was initiated after tendon injuries, and it was determined that the first 72 h did not reach the dose that would affect the tendon, and fibrosis was reduced in wound healing but not tendon adhesion formation.

Studies have reported that combination therapy with ibuprofen, an NSAID, and PELA [a di-block copolymer of poly (L-lactic acid)-polyethylene glycol] inhibits tendon adhesion formation with local application after tendon injury, but does not prevent adhesion formation with oral administration 22. Similarly, the systemic administration of TGF-β antibodies, such as TGF-β inhibitors, does not prevent tendon adhesion (23), but local administration has been shown to reduce adhesion formation (10, 24). Overall, the results of our study and previous studies suggest that systemic treatment does not reach the optimal concentration to reduce tendon adhesion formation.

Although the systemic administration of antibodies against TGF-β and NSAIDs does not affect local application, previous studies on reducing tendon adhesions with local applications have revealed that enapril inhibits tendon adhesion formation with a combination of a local barrier that allows for its controlled release. Further studies are needed to test this proposition.

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