

Comparison of the effects of phonophoresis and ultrasound therapy on recovery of experimental tenorrhaphy in rabbits*

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Summary: Tendon ruptures occur generally at distal portions of extremities and require relatively long recovery period. Traumatic ruptures of Achilles tendon need 6-8 weeks of immobility period including pre and postoperative days. In order to avoid other orthopedical problems by this application and to speed up the recovery period, postoperative local administrations are done. Therapeutic Ultrasound (TUS) is one of these applications. Phonophoresis is the local absorbing procedure of some drugs by means of TUS which is applied to the affected area. Forty male New Zealand rabbits were used in the study which were one year old and mean 3 kg body-weight. The rabbits were divided into 4 groups and experimental Achilles tendon rupture was created in both legs of all rabbits and repaired immediately by tenorrhaphy. The right legs of rabbits of groups 1 and 3 were treated postoperatively with TUS for 10 days. The right legs of rabbits in groups 2 and 4 were treated postoperatively with phonophoresis for 10 days. Left legs of all rabbits served as the control group. All rabbits were euthanized at the end of the 30th day. Samples which were taken from groups 1 and 2 were examined histopathologically and those in groups 3 and 4 were examined biomechanically. In biomechanical investigations, the rupture points of the tendons in N (Newton) were compared. At the end of the study, it was concluded that TUS is a superior for tendon healing when compared to phonophoresis.

Keywords: Achille Tendon, phonophoresis, rabbit, therapeutic ultrasound.

Deneysel olarak tenorafi yapılan tavşanlarda ultrason terapi ile fonoforezin iyileşme üzerine etkilerinin karşılaştırılması

Özet: Tendo kopukları çoğunlukla ekstremitelerin distal kısımlarında meydana gelmekte ve uzun süreli bir iyileşme periyodu gerektirmektedir. Travmatik aşil tendo kopuklarında operasyon öncesi ve sonrası da kapsayan 6-8 haftalık bir bandaj uygulaması ile hareketsizlik sağlanmaktadır. Bu uygulamanın neden olacağı diğer ortopedik problemleri önlemek ve iyileşme periyodunu hızlandırabilmek amacıyla, operasyon sonrası lokal uygulamalar yapılmaktadır. Ultrason terapi (TUS) bunlardan biridir. Fonoforezis ise emilim özelliği olan ilaçların TUS eşliğinde lokal olarak bölgeye emdirilmesidir. Deneysel çalışmamızda 40 adet erkek, 1 yaşında, ortalama 3 kg ağırlığında, Yeni Zelanda tavşanı kullanıldı. Tavşanlar 4 gruba ayrıldı ve tüm tavşanların her iki arka bacağına deneysel Aşil tendo rupturu oluşturuldu ve hemen akabinde tenorafi ile onarıldı. Birinci ve üçüncü gruptaki tavşanların sağ bacaklarına operasyondan sonraki 10 gün boyunca ultrason terapi uygulandı. İkinci ve dördüncü gruptaki tavşanların sağ bacaklarına ise operasyondan sonraki 10 gün boyunca fonoforez uygulandı. Tüm çalışma gruplarında, sol bacaklar kontrol grubunu oluşturdu. Tavşanlar 30. gün sonunda ötenazi edilerek grup 1 ve 2'deki örnekler histopatolojik olarak, grup 3 ve 4'teki örnekler ise biyomekanik olarak incelendi. Biyomekanik inceleme için tendoların kopma noktaları N (Newton) cinsinden karşılaştırıldı. Çalışmanın sonucunda; ultrason terapinin fonoforezle karşılaştırıldığında tendo iyileşmesi için daha üstün bir metod olduğu kanısına varıldı.

Anahtar sözcükler: Aşil Tendo, fonoforez, tavşan, ultrason terapi.

Introduction

To repair Achilles tendon ruptures of dogs and cats completely, immobilization of tarsal joint by bandage after tenorrhaphy is the method generally used. Also, tarsal arthrodesis is preferred for some patients, calcaneal fractures can be seen as a complication. Since healing is

long-lasting period for tendons due to its avascular structure, immobilization is perceived as a solution for many years (2, 3, 19, 20, 23). Recently, researchers try to find new techniques for poor functional recovery, tarsal range of motion loss and myoatrophy complications by shorten the duration of immobilization and to accelerate

* This study is summarized from Phd thesis.

healing. Various graft materials, injectable medicines and therapeutic ultrasound (TUS) are used for this purposes (7, 9, 12, 13, 26).

Therapeutic Ultrasound (TUS) is used for treatment of musculoskeletal disorders, bursitis, inflammation of joint capsule and tendon injuries for more than 50 years (5, 10, 24, 27). Therapeutic Ultrasound delivers the cells and metabolites through blood circulation to the region by the reduce edema, activation of mast cell degranulation and to provide neoangiogenesis in the early periods of tendon healing (6, 14, 17, 18, 22).

In phonophoresis, ultrasound energy is used for increasing the absorption of topically applied medicines through the skin and to provide deep tissue activation. Gel, liquid or cream form of medicines are applied to skin and these forms serves as a medium for the effectiveness of ultrasound. However, transfer of the ultrasound energy from a material to another is depending on the physical properties of these substances (1, 21, 27).

In our study, we aimed to introduce, the TUS and phonophoresis and add to clinical practice after tenorrhaphy of achilles tendon ruptures which are routinely used in most of the university clinics and rehabilitation centres abroad.

Materials and Methods

Animals and Experimental Design: We used 40 adult, male, 1 year old New Zealand rabbits, weighing 2.8-3.2 kg. We fed all animals ad libitum with standard diet of rabbit chow and water and kept them in a temperature-controlled environment. Animal procedures were approved by University of Istanbul, Animal Experiments Local Ethic Committe (assize number: 2009/143).

Rabbits were divided in 4 groups (10 rabbits in each of the groups): In all groups right hindlimbs were constituted of study group and left ones were control group. TUS and phonophoresis were used in study groups. Transection of the achilles tendon and tenorrhaphy were applied in control groups as like as the study groups but TUS or phonophoresis were not applied. Twenty samples in our study and control groups were sent to histopathological evaluation and the other 20 samples were sent to biomechanical analysis.

In Group 1, TUS (0,5 W/cm², 1 MHz, 1:3 pulse mode, 5 minutes) was applied after tenorrhaphy, once a day for 10 days to right hindlimb and the samples were sent to histopathological evaluation. In Group 2, phonophoresis (0,5 W/cm², 1 MHz, 1:3 pulse mode, 5 minutes, with Gelfix) was applied after tenorrhaphy, once a day for 10 days to right hindlimb and the samples were sent to histopathological evaluation. In Group 3, therapeutical ultrasound (0,5 W/cm², 1 MHz, 1:3 pulse mode, 5 minutes) was applied after tenorrhaphy, once a

day for 10 days to right hindlimb and the samples were sent to biomechanical analysis and in Group 4, phonophoresis (0,5 W/cm², 1 MHz, 1:3 pulse mode, 5 minutes, with Gelfix) was applied after tenorrhaphy, once a day for 10 days to right hindlimb and the samples were sent to biomechanical analysis. The same device was used for the therapeutical ultrasound and phonophoresis (VetriCombi, Physiomed Elektromedizin AG, Germany).

Surgical procedures: Anesthesia was obtained by means of intramuscular injection of 0.04 mg/kg SC atropine (Atropin[®], Vetaş[®], Turkey), 5 mg/kg IM xylazine (Rompun[®], Bayer, Turkey) and 40 mg/kg IM ketamine (Bremar[®], Pharma GMBH[®], Germany) and maintained with 1-2% MAC inhaled isoflurane (Isoflurane, USP[®] Adeka, Turkey). Both of the hindlimbs of the rabbits were shaved from knee to phalanx and prepared aseptically.

Incision was made to medial side of distal tibia and soft tissues on the achilles tendon was removed. Transverse incision between insertion of calcaneus and the point of muscle to turn into tendon was made and the tips of the tendon was sutured with 3/0 absorbable suture material (Monocryl Plus[®], Ethicon[®], Scotland) by using locking loop technique. Subcutaneous tissue and skin was appropriately closed.

After the tenorrhaphy procedure of both hindlimbs were done, splint cast were applied to both hindlimbs in neutral position. A 1 x 2 cm window was constituted to median side of the splint cast of right hindlimbs of rabbits for TUS and phonophoresis.

Histopathological evaluation: At necropsy, right and left Achilles tendons were removed from starting point of gastrocnemius muscle behind the knee till the attached point to calcaneus. Samples fixed in 10% formalin, stained with hematoxylin-eosin and evaluated under light microscope. Sections were evaluated and scored, according to histopathological examination results for vascularization, fibroblast proliferation, inflammatory cell distributions and fibrosis. Severity of parameters present on each slide were converted into a numeric score as follows: 0, +1, +2, +3.

0: No inflammatory cell infiltration, fibroblast proliferation or fibrosis, 0 to 5 vascular structure in all sections. +1: Mild inflammatory cell infiltration and fibroblast proliferation, no fibrosis, 5 to 10 capillar vascular structure in all sections. +2: Mild, scattered inflammatory cell infiltration, mild to moderate diffuse fibroblast proliferation and mild fibrosis, 10 to 12 vascular structure in all sections. +3: Mild inflammatory cell infiltration, moderate to severe fibroblast growth, diffuse fibrosis, 12-15 vascular structure in all sections.

Biomechanical evaluation: For biomechanical pulling of tendon samples, ESIT TB 5000-C3 load-cell ESIT Data Logger (ESIT Electronics Co., Türkiye)

stretching machine was used. Tendon samples were placed to clamps and maximum tensile strength (Newton) was determined.

Statistical analysis: In evaluation of the histopathological scores for vascularization, fibroblast proliferation, inflammatory cell distributions and fibrosis, Kruskal-Wallis test was used.

GLM procedure in SPSS100 program was used for statistical analysis of tensile strength and ruptured point data. In statistical model we used, the group was listed as "fixed-effect" and the animal as "random factor".

Results

Histopathological results: In the ultrasound group, intense fibroblast tissue growth characterized scar tissues were determined and scattered, different size capillary blood vessels were seen in sections (Figure 1-a). Mild and scattered inflammatory cell infiltration consisting lymphocytes were observed around the same areas. Also non-uniform collagen sequence and hyalinized areas were seen intense fibrosis in scar tissue and collagen tissue growth in ultrasound group were more than phonophoresis and control groups (Figure 1-b, c, e).

In phonophoresis group, fusiform fibroblast percentage of this scar tissue was higher than ultrasound and control groups. Capillary vascularization percentage of scar tissue was higher than the ultrasound group and lower than the control group. In scar tissue and close

areas, scattered, mild lymphocyte rich mononuclear cell infiltration was seen. Fusiform fibroblast intense in scar tissue and improvement of collagen fiber in hyalin areas were higher than ultrasound but lower than control group (Figure 1-d).

In control group, especially fusiform fibroblast rich fibrous tissue which composed scar tissue was determined in sections as in ultrasound and phonophoresis groups (Figure 1-f). Fusiform fibroblast percentage in scar tissue in this group was same as TUS group and higher than Phonophoresis group. Flattened fibroblasts and collagen fiber ratio and pattern in the same scar tissue were more regular than the TUS and Phonophoresis groups and the collagen fiber sequencing in tissue sections was in irregular pattern. Capillary vessel structures ratio in scar tissue were lower than the TUS and Phonophoresis groups. Inflammatory cell infiltration was almost same in TUS and Phonophoresis groups.

Histopathological scores of control, TUS an phonophoresis groups are shown in Table 1, Table 2 and Table 3 respectively.

Biomechanical results: At the end of the biomechanic evaluation, maximum tensile strength of the 3 groups: 241,3 N mean value in TUS group, 172,7 N mean value in phonophoresis group and 104,7 N mean value in control group for were determined.

Statistical evaluation of the histopathological and biomechanic evaluations results are shown in Table 4.

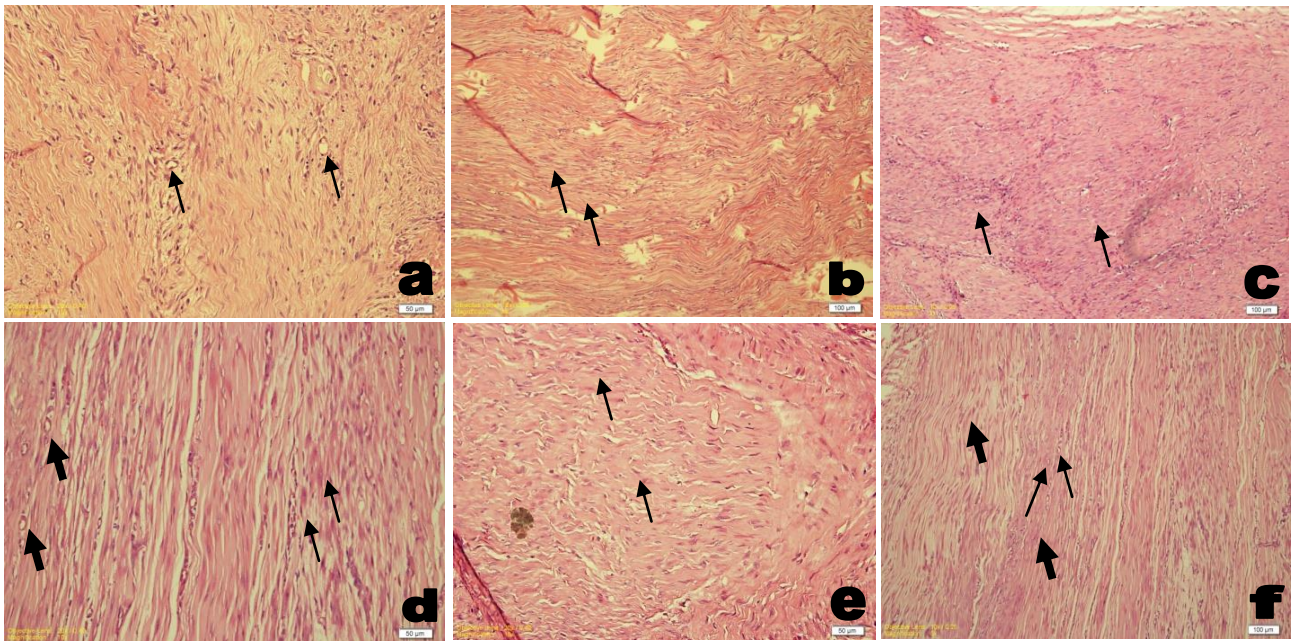


Figure 1: **a**, Vascularization +3, (black arrows); **b**, fibrosis +2, (black arrow); **c**, intense fibrosis +3, (black arrows); **d**, fusiform fibroblast intense in scar tissue (black arrows) and vascularization (thick arrows); **e**, fusiform fibroblast growth +1, (black arrows); **f**, vascularization +2, (black arrows) and fusiform fibroblast growth +2, (thick arrows).

Şekil 1: **a**, Damarlaşma +3, (siyah oklar); **b**, fibrozis +2, (siyah oklar); **c**, yoğun fibrozis +3, (siyah oklar); **d**, skar dokusu içinde işçi fibroblast yoğunluğu (siyah oklar) ve damarlaşma (kalın oklar); **e**, işçi fibroblast üremeleri +1, (siyah oklar); **f**, damarlaşma +2 (siyah oklar) ve işçi fibroblast üremeleri +2 (kalın oklar).

Table 1: Histopathological evaluation results of Control group (0, +1, +2, +3)

Tablo 1: Kontrol grubunun histopatolojik değerlendirme sonuçları (0, +1, +2, +3)

Animal Number	Inflammatory cell distribution	Vascularization	Fibroblast proliferation	Fibrosis
1	+1	+2	+2	+1
2	+2	+1	+3	+1
3	+2	+1	+1	+1
4	+1	+2	+2	+1
5	0	+1	+1	0
6	+2	+1	+1	+1
7	+2	+1	+2	+2
8	+1	+2	+2	+2
9	+1	+2	+1	+2
10	+1	+1	+2	+2
11	+2	+1	+1	+1
12	0	+2	+1	+1
13	+1	+2	+2	+1
14	+1	+1	+2	+1
15	+2	+1	+1	+2
16	+2	+2	+2	+1
17	0	+2	+2	+1
18	+2	+2	+2	+1
19	+1	+1	+2	+1
20	+1	+1	+1	+1

Table 2: Histopathological evaluation results of TUS group (0, +1, +2, +3)

Tablo 2: Ultrason grubunun histopatolojik değerlendirme sonuçları (0, +1, +2, +3)

Animal Number	Inflammatory cell distribution	Vascularization	Fibroblast proliferation	Fibrosis
1	+1	+2	+2	+1
2	+1	+2	+2	+2
3	+1	+1	+1	+2
4	+1	+3	+3	+2
5	0	+2	+1	+2
6	+2	+2	+1	+1
7	+1	+1	+2	+1
8	+1	+1	+1	+1
9	+2	+2	+2	+2
10	+1	+1	+2	+2

Table 3: Histopathological evaluation results of P group (0, +1, +2, +3)

Tablo 3: Fonoforez grubunun histopatolojik değerlendirme sonuçları (0, +1, +2, +3)

Animal Number	Inflammatory cell distribution	Vascularization	Fibroblast proliferation	Fibrosis
11	+1	+1	+2	+2
12	+1	+1	+3	+2
13	+1	+2	+2	+2
14	+1	+1	+1	+2
15	+2	+2	+2	+1
16	+2	+2	+2	+1
17	0	+2	+3	+1
18	+2	+1	+2	+1
19	+1	+2	+3	+1
20	+3	+2	+1	+1

Table 4: Statistical evaluation of the both histopathological and biomechanical investigation results.

Tablo 4: Histopatolojik ve biomekaniksel inceleme sonuçlarının istatistiksel olarak değerlendirilmesi.

Parameter	Gruplar						Significance
	Control (n=20)		TUS (n=10)		P (n=10)		
	Mean	SE.	Mean.	SE.	Mean.	SE.	
Inflammatory cell distribution	1,20	,156	1,10	,180	1,40	,267	P > 0,05
Vascularization	1,40	,112	1,90	,233	1,60	,163	P > 0,05
Fibroblast proliferation	1,65	,131	1,60	,221	2,10	,233	P > 0,05
Fibrosis	1,20	,117	1,60	,163	1,40	,163	P > 0,05
Rupture Point	104,70 ^b	15,18	241,3 ^a	26,29	172,70 ^{ab}	26,29	P = 0,004

n: Animal number in a group, **mean:** mean value, **SE:** Standart Error

a,b between the mean values of the different letters on the same line is statistically significant (p<0,01)

Discussion and Conclusion

Achilles tendon ruptures is generally occurred because of traumatic reasons and repeated injuries also result with ruptures after a certain period (8, 13, 15, 16, 23).

Healing process of Achilles tendon ruptures in small animals, depends on it's stage. Early diagnosis and appropriate management of the rupture is very important for optimum healing. Since there is not a widely accepted treatment protocol, approach to treatment of Achilles tendon ruptures is depending on the surgeon's preference.

Researchers have tried to find new methods which have better functional results after tendon injuries because of long recovery time after operation. Implant application, using of silicone sheet around repaired tendon as a ring to prevent restricting adhesions, tendon treatment with fibrin glue without suture are a few of them (4, 25, 26). Today, variety of physical therapy techniques are used after operative treatment of tendon ruptures and injuries. Effectiveness of both TUS and Phonophoresis are evaluated methods in experimental trails and clinical studies. However, there are different opinions about dose range and duration of application.

After the surgical procedures, it's the fact that TUS is accelerating the healing both in intermittent and continuous mode (7). According to Enwemeka (11) and Christine et al. (7) the continuous mode of TUS is successful alone. On the other hand, some researchers reported that, use of continuous mode of TUS in early healing period results with slow improvement but intermittent mode of TUS provides better and faster healing (8, 16). Acceptable dose range for biomechanical evaluation in TUS is 0.5 W/cm², 1 MHz, 5 minutes (11). Our results are analyzed in terms of biomechanics, results of using intermittent mode TUS 0.5 W/cm², 1MHz dose, 5 minutes duration is compatible with literature.

Selected mode and dose range in TUS for phonophoresis were determined as intermittent mode 0.5 W/cm², 1 MHz, 5 minutes duration (17). According to results of the studies about effectiveness and absorption characteristics of steroid and non-steroid drugs, phonophoresis is more effective than the TUS (17). In our study, results of the statistical analysis of the biomechanical measurement data showed that; Gelfix usage in Phonophoresis after TUS is more satisfactory than the control group but in biomechanical evaluation results of the TUS group were best (9,13, 28).

In conclusion, TUS group was found to be statistically significant in biomechanical evaluation. Difference between individuals were important in TUS and Phonophoresis groups when compared to the control group in histopathological examination but were not statistically significant. According to biomechanical evaluation results, TUS group was superior than the other groups and TUS can be effectively used after surgery of

tendon injuries and ruptures in veterinary practice. Different form of collagen containing lyophilized pad, which we used in Phonophoresis, should study further in different studies.

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