Eklem Hastalıkları ve Cerrahisi
Joint Diseases and Related Surgery

Experimental Study / *Deneysel Çalışma*

Joint Dis Rel Surg
2005;16(3):215-223

Comparison of early and late term effects of hylan G-F 20 in Achilles tendonitis: an experimental study

Aşil tendonitinde hylan G-F 20'nin erken ve geç dönem etkilerinin karşılaştırılması: Deneysel çalışma

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Objectives: The treatment of Achilles tendon disorders remains controversial. This study was designed to observe the local early and late term effects of intratendinous hylan G-F 20 injections on experimentally induced rat Achilles tendonitis.

Materials and methods: After inducing Achilles degeneration with local corticosteroid injections in the Achilles tendons, 36 male Wistar rats were divided into two main groups for early (group 1) and late (group 2) term observations. The right Achilles tendons served as the hylan injection group, and the left Achilles tendons injected with saline as the control group. Injections were given at 5-day intervals until day 60 of the experiment. The tendons and paratenons were excised at the end of 60 and 75 days in group 1 and at the end of four and six months in group 2 for histopathologic and statistical evaluations.

Results: Semiquantitative scoring of histopathological changes showed that histological appearances significantly differed between hylan and saline groups in the early term. Hylan-injected tendons and paratenons demonstrated significantly lower scores especially after 75 days. However, in the late period, the differences were mostly insignificant.

Conclusion: Hylan G-F 20 appears to have a promising curative effect on degenerated tendon processes in the early acute and subacute periods. Further studies, either biomechanical or biochemical, will show how useful this chemical agent is in treating tendon disorders.

Key words: Achilles tendon/pathology; hyaluronic acid/therapeutic use; injections, intra-articular; rats; tendon injuries/drug therapy; wound healing/drug effects.

Amaç: Aşil tendon patolojilerinin tedavisi halen tartışmalıdır. Bu çalışmada, sıçanlarda deneysel olarak oluşturulmuş Aşil tendonitinde lokal intratendinöz hylan G-F 20 enjeksiyonlarının erken ve geç dönem etkileri araştırıldı.

Gereç ve yöntem: Lokal intratendinöz kortikosteroid enjeksiyonları ile Aşil tendon dejenerasyonu oluşturulduktan sonra 36 erkek Wistar sıçanı erken (grup 1) ve geç (grup 2) dönem sonuçlar için iki gruba ayrıldı. Her sıçanın sağ Aşil tendonuna hylan, sol Aşil tendonuna serum fizyolojik enjeksiyonu (kontrol) uygulandı. Enjeksiyonlar 60. deney gününe kadar beşer gün arayla yapılacak şekilde sürdürüldü. Grup 1'deki sıçanların tendon ve paratenonları 60 ve 75. gün sonunda, grup 2'de ise dördüncü ve altıncı ay sonunda çıkartılarak histopatolojik ve istatistiksel olarak değerlendirildi.

Bulgular: Histopatolojik değişikliklerin semikantitatif skorlaması, erken dönemde hylan ve serum fizyolojik grupları arasında istatistiksel anlamda önemli bir fark olduğunu gösterdi. Hylan enjekte edilen tendon ve paratenonlara ait skorlar özellikle 75. gün sonunda anlamlı derecede düşük bulundu. Geç dönemde ise gruplar arasında anlamlı fark görülmedi.

Sonuç: Hylan G-F 20, akut ve subakut tendon dejenerasyonunda umut veren tedavi edici bir etki göstermiştir. Daha ileri biyomekanik veya biyokimyasal çalışmalar, bu kimyasal ajanın tendon patolojilerindeki yararını gösterecektir.

Anahtar sözcükler: Aşil tendonu/patoloji; hiyaluronik asit/terapötik kullanım; enjeksiyon, intra-artiküler; sıçan; tendon yaralanması; ilaç tedavisi; yara iyileşmesi/ilaç etkisi.

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The treatment of chronic Achilles tendon disorders is still controversial because of the difficulty and inadequacy of healing. Nonsurgical management has been accepted as the initial treatment option for most cases.[1,2] However, in severe cases, surgery may be indicated.[1-5] Although successful results from surgery have been reported[3,4] complications have also been noted.[4,5] The lesion throughout the tendon of the asymptomatic, macroscopically healthy areas may often be more extensive than may be expected, thus negatively affecting the benefits of surgical intervention.[6]

Because of these controversies in the treatment of chronic tendon disorders, investigators have tested clinically and experimentally the effect of local or systemic agents in curing Achilles tendon disorders.[1,7-9]

The purpose of this experimental study was to evaluate the local early and late term effects of intratendinous hylan G-F 20 injections on experimental Achilles tendonitis induced by locally administered corticosteroids.

MATERIALS AND METHODS

The experiment was performed in 36 male Wistar white rats with ages ranging from 11 to 17 months (weight range 312 to 375 g). Both the right and left hind limb Achilles tendons (Fig. 1) were injected with 0.1 ml of betamethasone sodium phosphate (Diprospan) intratendinously by the same researcher (HT), with a 22-gauge needle, beginning at the insertion of the tendon to the calcaneus. Injections were given at 3-day intervals for 25 days. After a 5-day rest period, the second part of the experiment was started at Day 30. The right hind limb Achilles tendon of the rats received intratendinous hylan injection, while the left hind limb received intratendinous saline (control limb). The rats were divided into two groups. In group 1, the tendons were examined in the early-term, while in group 2 in the late-term. Both groups were divided into four subgroups to demonstrate the early- and late-term effects of the experiment. The subgroups 1.1 and 1.2/2.1 and 2.2 received hylan G-F 20 whereas the subgroups 1.3 and 1.4/2.3 and 2.4 received saline intratendinously. In both groups, injections were given at 5-day intervals until Day 60 of the experiment.

We used sterile hylan injectors containing 16 mg of hylan G-F 20 (Synvisc, Biomatrix, Inc., Wyeth, USA). The dosage was approximately 0.8 mg for each injection, which was equal to 0.1 ml. A single dose of saline was the same (0.1 ml). During the experiment, the rats were kept in individual cages and had freedom of movement and free access to food and water. At the end of the experiment, both Achilles tendons were exposed under general anesthesia induced by intraperitoneal injection of ketamine (10 mg/kg), and the tendons and paratenons were excised from the distal insertion site to the musculotendinous junction proximally, with maximum care given not to damage the tissue. This was done at the end of Day 61 in the subgroups 1.1 and 1.3, and at the end of Day 75 in subgroups 1.2 and 1.4 for the early-term effects (Table I). To observe the late-term effects, the biopsies were made at the end of four months in the subgroups 2.1 and 2.3, and at the end six months in the subgroups 2.2 and 2.4 (Table II).

The specimens were fixed overnight in buffered 10% formalin and embedded in paraffin after tissue processing. The paraffin blocks were cut into 5-µm sections, which were then stained with hematoxylin and eosin.

Light microscopic examination was performed at a magnification of 100. Histopathologic changes for tendon (staining affinity, nuclear appearance, fibrillar appearance) and for paratenon (thickness,

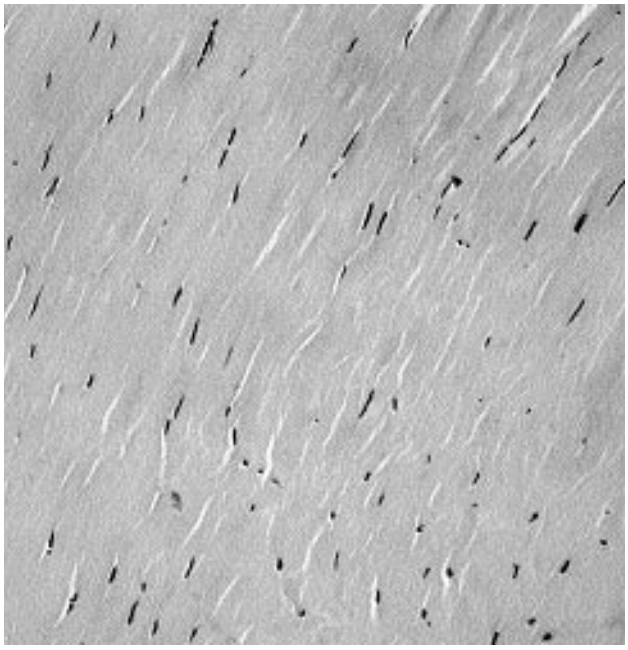

Fig. 1. Normal appearance of the tendon (H-E x 100).

TABLE I

Semiquantitative scoring of histopathologic changes in group 1 after injections of hylan G-F 20 and saline

Rats	Day of biopsy (D)	Tendon			Paratenon					Average	
		Staining affinity	Nuclear appearance	Fibrillar appearance	Thickness	Fibrosis	Edema	Capillaries	Inflam.	T	*p*
Group 1.1:	D 61 (Hylan)									3	5.4
1.1.1		2	0	2	2	1	3	1	3		
1.1.2		1	0	1	1	0	2	1	2		
1.1.3		2	1	2	2	2	1	0	1		
1.1.4		1	3	2	2	1	2	2	0		
1.1.5		0	1	0	2	1	0	2	0		
1.1.6		0	0	1	2	0	2	3	1		
1.1.7		1	1	1	1	0	2	1	0		
1.1.8		1	2	2	1	0	0	0	0		
1.1.9		0	0	0	1	0	1	1	0		
Group 1.2:	D 75 (Hylan)									1.8	0.8
1.2.1		1	1	1	11	0	2	0	0		
1.2.2		1	0	1	0	1	1	0	0		
1.2.3		0	1	1	0	0	0	0	0		
1.2.4		0	1	0	0	0	1	0	0		
1.2.5		1	1	0	0	0	0	1	0		
1.2.6		0	1	1	0	0	0	0	0		
1.2.7		1	0	0	0	0	0	0	0		
1.2.8		1	1	0	0	0	0	0	0		
1.2.9		0	1	0	0	0	0	0	0		
Group 1.3:	D 61 (Saline)									5.2	4.4
1.3.1		1	3	2	1	1	0	0	0		
1.3.2		2	2	3	1	0	0	1	0		
1.3.3		1	2	1	2	1	1	2	1		
1.3.4		2	2	2	3	2	2	0	0		
1.3.5		1	1	1	2	1	1	0	0		
1.3.6		1	2	1	2	1	2	1	0		
1.3.7		2	2	2	2	2	2	1	1		
1.3.8		2	2	2	1	2	1	0	0		
1.3.9		2	1	2	1	1	1	1	0		
Group 1.4:	D 75 (Saline)									3.4	1.5
1.4.1		1	0	1	0	0	0	0	0		
1.4.2		2	3	2	1	0	1	1	0		
1.4.3		0	3	2	2	1	0	0	0		
1.4.4		0	1	2	0	1	0	0	0		
1.4.5		1	1	1	1	1	0	0	0		
1.4.6		1	1	1	0	0	1	1	0		
1.4.7		1	1	1	0	0	1	0	0		
1.4.8		1	1	1	0	0	0	0	0		
1.4.9		1	1	0	1	1	0	0	0		

0: No change; 1: Slight change; 2: Moderate change; 3: Marked change. D: Day; Inflam: Inflammation; T: Tendon; P: Paratenon.

fibrosis, edema, capillary changes, and inflammation) were assessed according to a semiquantitative scoring system.[10] Histopathologic changes in each group were scored by two pathologists with scores ranging from 0 to 3, indicating no change, slight change, moderate change, marked change, respectively.

For each parameter, a point between 0 and 3 was given; therefore, for each limb, a tendon with three parameters was scored between 0 and 9; a paratenon with five parameters was scored between 0 and 15. The scores were given synchronously by the pathologists who were blind to the rat groupings. The scores of each Achilles tendon

TABLE II

Semiquantitative scoring of histopathologic changes in group 2 after injections of hylan G-F 20 and saline

Rats	Month of Biopsy (M)	Tendon			Paratenon					Average	
		Staining affinity	Nuclear appearance	Fibrillar appearance	Thickness	Fibrosis	Edema	Capillaries	Inflam.	T	*p*
Group 2.1: M 4 (Hylan)										1.3	2.4
2.1.1		0	0	1	1	1	1	1	1		
2.1.2		1	0	1	1	1	1	1	1		
2.1.3		0	0	1	1	1	0	0	1		
2.1.4		1	0	1	0	0	0	0	0		
2.1.5		1	0	0	0	0	0	0	1		
2.1.6		1	0	0	0	0	1	0	0		
2.1.7		1	0	0	1	0	2	1	0		
2.1.8		1	0	1	0	0	0	0	0		
2.1.9		0	1	0	2	0	1	0	0		
Group 2.2: M 6 (Hylan)										0.5	3.2
2.2.1		0	0	0	2	0	2	1	0		
2.2.2		1	1	0	1	1	1	0	0		
2.2.3		0	0	1	1	1	0	0	0		
2.2.4		1	0	1	0	0	0	0	0		
2.2.5		0	0	0	1	2	0	1	1		
2.2.6		0	0	0	0	0	1	0	0		
2.2.7		0	0	0	1	0	1	1	1		
2.2.8		0	0	0	1	0	1	1	0		
2.2.9		0	0	0	1	1	1	2	1		
Group 2.3: M 4 (Saline)										1.9	3.9
2.3.1		0	0	1	1	1	1	1	1		
2.3.2		1	0	1	1	1	0	0	1		
2.3.3		1	0	1	2	1	1	1	1		
2.3.4		0	1	1	0	0	1	0	1		
2.3.5		2	1	1	2	1	1	1	1		
2.3.6		2	0	0	0	0	1	1	0		
2.3.7		1	0	1	0	0	1	0	0		
2.3.8		0	0	0	0	0	1	0	0		
2.3.9		2	0	0	1	1	0	1	0		
Group 2.4: M 6 (Saline)										0.9	4.1
2.4.1		1	1	0	1	1	0	0	1		
2.4.2		0	0	1	1	1	0	1	1		
2.4.3		0	0	1	1	1	0	0	0		
2.4.4		0	0	1	2	1	1	1	1		
2.4.5		0	0	1	0	1	0	0	1		
2.4.6		0	0	1	2	1	1	1	1		
2.4.7		0	0	1	2	1	2	1	1		
2.4.8		0	0	0	1	1	0	1	1		
2.4.9		0	0	0	1	0	1	1	0		

0: No change; 1: Slight change; 2: Moderate change; 3: Marked change. M: Month; Inflam: Inflammation; T: Tendon; P: Paratenon.

are presented in Table I and Table II. Statistical analysis of the histopathologic scores was performed using the Mann-Whitney U-test .

Ethical considerations. The study was approved by the local ethics committee of our faculty and was performed in accordance with the ethics standards noted in "Principles of Laboratory Animal Care."

RESULTS

Semiquantitative scoring of histopathologic changes in the early-term showed that histologic appearances differed between the hylan and saline groups and between the two hylan groups (groups 1.1 and 1.2). The average score in group 1.1 (hylan G-F 20, excised at Day 61) was 3 in the tendon and

5.4 in the paratenon (Fig. 2, 3); in group 1.2 (hylan G-F 20, excised at Day 75), the corresponding values were 1.8 in the tendon and 0.8 in the paratenon. The scores in paratenons between groups 1.1 (Fig. 2) and 1.2 (Fig. 4) showed a statistically significant difference (p=0.001). Inflammatory changes in the paratenon decreased with hylan between Days 61 and 75. However, there was no statistical difference between groups 1.1 and 1.2 with respect to tendon changes (Table I, III).

At the end of Day 61, the scores in the tendon for group 1.1 (hylan) were lower than for group 1.3 (saline), but did not reach statistical significance (p=0.06) (Table I, III).

At the end of Day 75, a statistically significant difference was evident in the tendon between groups 1.2 (Fig. 4) and 1.4 (p=0.02) (Fig. 5), the average scores being 1.8 and 3.4, respectively. The difference in the paratenon scores between these groups was statistically insignificant (Table I, III).

Semiquantitative scoring of histopathologic changes in the late-term demonstrated a significant difference between the groups 2.1 and 2.2 for tendon in favor of the former (p=0.035). There

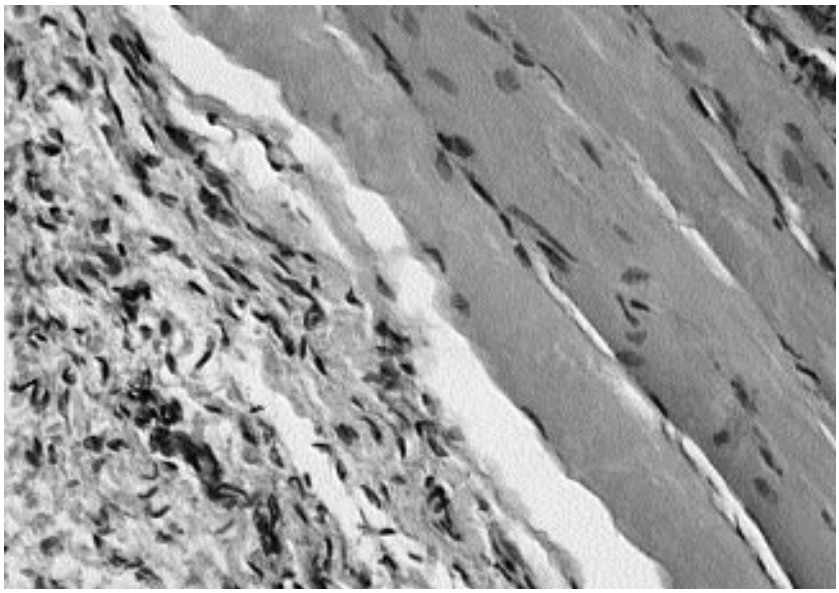

Fig. 2. Thickening of the paratenon with the occurrence of fibrosis, edema, increased capillaries, and inflammatory cell infiltration in group 1.1 rats (H-E x 100).

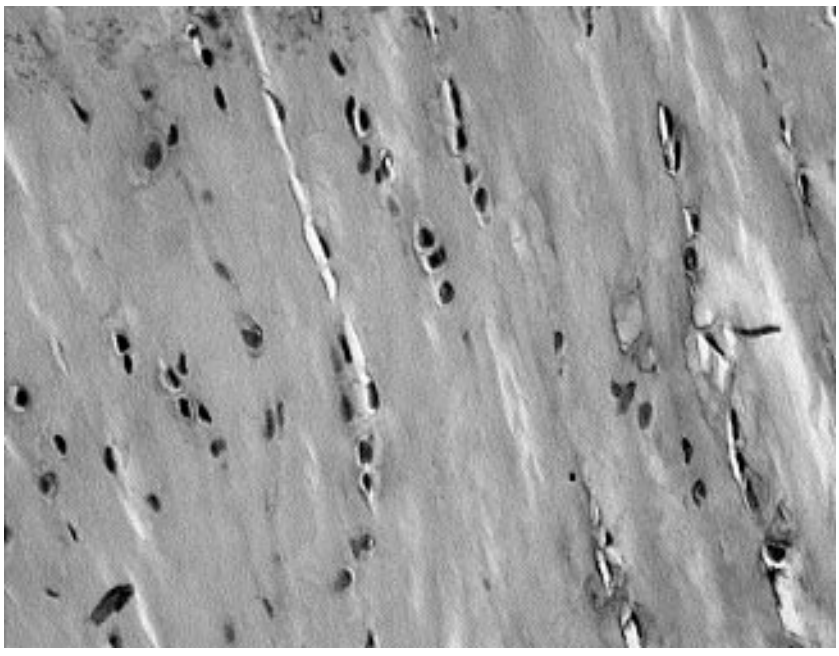

Fig. 3. The nuclei of the tendons exhibiting various sizes and contours in group 1.1 rats (H-E x 100).

were no other statistically significant differences in tendon and paratenon between the late-term groups (Table II, III).

When the early- and late-term scores were evaluated, it was observed that there was a significant difference between the groups 1.1 and 2.1 for paratenon in favor of healing (p=0.01). Significant differences also existed between the groups 1.2 and 2.2 for tendon in favor of healing (p=0.009), and for paratenon in favor of degeneration (p=0.01), and between the groups 1.1 and 2.2 for tendon in favor of healing (p=0.009) (Table III).

When the saline groups were compared, it was observed that there was a statistically significant difference between the groups 1.3 and 2.3 (p=0.001) for tendon in favor of healing, and between the groups 1.4 and 2.4 both for tendon in favor of healing (p=0.000) and for paratenon in favor of degeneration (p=0.005) (Table III).

DISCUSSION

Corticosteroids and heparin have been used locally or systematically in animal experiments and in clinical practice for tendon disorders. However,

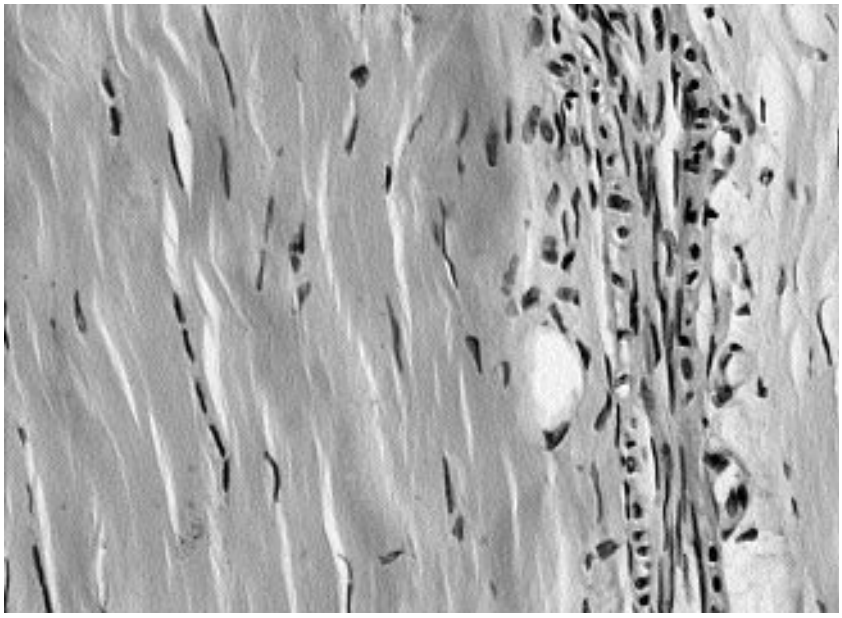

Fig. 4. Improved appearance of the tendon and paratenon in group 1.2 rats at the end of Day 75 (H-E x 100).

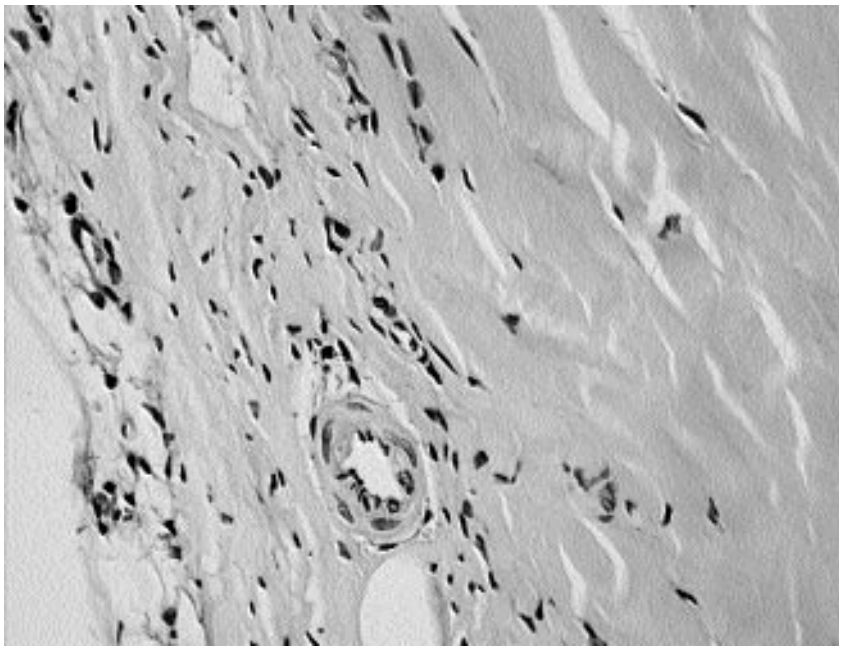

Fig. 5. Varying staining affinity and loss of fibrillation in the tendons of group 1.4 rats at the end of Day 75 (H-E x 100).

TABLE III

Differences between the mean scores by Mann-Whitney U-test

Compared groups	Tendon	Paratenon
1.1 *vs.* 1.2	0.24	0.001*
1.1 *vs.* 1.3	0.06	0.5
1.2 *vs.* 1.4	0.02*	0.37
2.1 *vs.* 2.2	0.03*	0.42
2.1 *vs.* 2.3	0.1	0.39
2.2 *vs.* 2.4	0.23	0.39
1.1 *vs.* 2.1	0.09	0.01*
1.2 *vs.* 2.2	0.009*	0.01*
1.1 *vs.* 2.2	0.009*	0.05
1.2 *vs.* 2.1	0.13	0.06
1.3 *vs.* 2.3	0.001*	0.09
1.4 *vs.* 2.4	0.000*	0.005*

*: Statistically significant values.

they are abandoned today because of their side effects described in some studies.[1,7-9] Therefore, tendon disorders need more effective treatment modalities. In our opinion, hylan G-F 20 is an agent worthy of consideration.

Hylan G-F 20 is a polysaccharide chain made of repeating disaccharide units of N-acetylglucosamine and glucuronic acid.[11] Its much-known effect is viscosupplementation, with the viscous and elastic properties in the osteoarthritic knee joint.[12-15]

Besides orthopedic applications of hylan G-F 20, the effect of exogenous hylan applied locally to tympanic membrane ruptures or cutaneous wounds and as an adjunct in cataract surgery has been examined in limited investigations with successful results. Healing of cutaneous ulcers in a few patients treated with hylan has been demonstrated by Italian investigators.[12] These results encouraged us to use and observe the effect of this agent in Achilles tendonitis.

The effect of hylan G-F 20 on tendon healing and adhesion formation has been demonstrated in both animal and human studies[11,16-20] and the results have usually been good. Unlike those studies, the present one concentrated on the healing process of degenerated tendons and paratenons, not on the prevention of adhesion formation.

In the present study, we observed a statistically significant difference in the early-term in favor of healing between the scores obtained from hylan G-F 20 and saline injections into the tendons and paratenons. However, the mechanism of this effect is unclear. It may be the result of the anti-inflammatory effect of this agent. Alfredson et al.[21] reported high levels of glutamate, but not prostaglandin E2 in Achilles peritendinous fluid of four patients with chronic Achilles tendinosis; this suggests that hylan G-F 20 can be used mainly in acute and subacute tendonitis.

Sodium hyaluronate has been shown to inhibit vasculogenesis in chicken embryos and in endothelial cell cultures. Formation of granulation tissue was also inhibited by application of sodium hyaluronate, although this may be the result of decreased vascular influx.[11] The same mechanism must have played a role in our experiment, although this is contrary to the evidence that there is a reduced vascular volume of 3 to 6 cm above the calcaneal insertion, which is likely to play a role in chronic Achilles tendinosis and spontaneous rupture. This controversy is worthy of examination in future studies.

When we compared late-term groups, we only observed a statistically significant difference for tendon in favor of healing between the groups evaluated at the end of 4 and 6 months; but it was difficult to explain this, because the other mean tendon scores had reached almost normal values in the late-term both in the study and control groups. This may be due to invalid effect of local corticosteroid injections after all that time.

In contrast, paratenon scores of the latest period had increased with a statistically significant difference between the early- and late-term groups. However, insignificant results obtained between the study and control groups in the late-term do not allow to evaluate the healing or degenerative effects of hylan and saline at that stage.

In our experiment, we preferred a locally administered corticosteroid-induced degeneration because of our experimental findings in a previous study,[22] which indicated that tendon and paratenon degeneration could be obtained with local corticosteroid injections. In our opinion, this experimental model works well, although it is not widely used as a model for Achilles tendonitis.

In the literature, digital flexor tendons are most frequently used to observe the effect of hylan G-F

20 on the prevention of adhesions.[11,16,18-20] In contrast, we used the Achilles tendon because it would be more rational to observe the histologic findings in a tendon which is more vulnerable to load bearing and tensional forces than the digital flexor tendon.[21] During the excisional biopsy of the specimens, no adhesion of the tendon or paratenon to the subcutaneous tissue was demonstrated. This finding can partly explain the effect of hylan in preventing adhesion formation.

The statistical results arouse the question whether the decreasing scores for the tendon and paratenon between the study and control groups in the early-term are the result of the beneficial effect of hylan G-F 20 or self-cure of the tendon with no medication after 15 days . When the scores of this study are evaluated in comparison with our findings of a previous study with heparin,[7] it can be observed that the scores of the tendons and paratenons after hylan G-F 20 injections at the end of 75 days are much lower than those obtained by heparin injection. This observation may be considered in favor of the healing effect of hylan G-F 20.

In conclusion, hylan G-F 20 appears to have a promising curative effect on degenerated tendon processes in the early term. The results of this study are encouraging for physicians who may consider hylan G-F 20 injections in tendon disorders experimentally or clinically. Further studies, either biomechanical or biochemical, will show how useful this chemical agent is in treating tendon disorders, including chronic overuse syndromes.

Acknowledgments

Pharmaceutical support of this study was supplied by Wyeth Pharmaceuticals, İstanbul, Turkey, which is the importer of Synvisc manufactured by Biomatrix, Inc., Wyeth, USA. We thank Prof. Dr. Ataman Güre and Associate Prof. Dr. Osman Yılmaz, Directors of the Experimental Research Unit of Dokuz Eylül University, School of Medicine, for their support.

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