THE EFFECTS OF HEPARIN BY LOCAL INJECTION ON THE BIOMECHANICAL PROPERTIES OF ACHILLES TENDON SYSTEM IN RABBIT

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The purpose of the present study on an animal model was to examine the mechanical changes and visco-elastic properties of the Achilles tendon in overuse injuries and to examine the effect of heparin on the mechanical and viscoelastic properties in Achilles tendon.

KEY WORDS: Achilles tendon, overuse injury, Heparin, biomechanics

INTRODUCTION: Achilles tendon calcanues (ATC) system injuries are the most frequently occurring sports injury. Most of the Achilles tendon injuries are related to peritendinitis. Achilles peritendinitis in acute cases is often accompanied by crepitating, due to the fibrin that is precipitated from fibrinogen-rich edematous fluid around the tendon. Sundquist encouraged by the good results with glycosaminoglycan polysulfate ($GAGP_S$) in jumper's knee, performed a double-blind study comparing local injections of $GAGP_S$ with high doses of oral indomethacin (IM), and again obtained an encouraging result (Sundqist, Forsskahl, & Kvst, 1987). The purpose of the present study in an animal model was to examine the mechanical changes and viscoelastic properties of the Achilles tendon in overuse injuries and to examine the effects of heparin by local injection around the Achilles tendon on the mechanical and viscoelastic properties in this tendon.

METHODS: Animal model and management. Thirty ten-months-old Japanese White rabbits (weight 4.10±0.23kg) were divided into a control group, an exercise group and heparin treatment group. The animals in exercise and heparin group were put in an electric cage with high pulsed voltage (15 Kv, duration 0.2 second, 10 times per minute). This cage causes the rabbits to jump and run (exercise) 360 times daily for 6 weeks. Having previously shown 3 weeks of exercise-chronic pathological changes in the rabbits' Achilles tendon (Liu, Yin, & Li 1995). It was decided to inject the heparin solution (468µl of Heparin Sodium Salt diluted into 0.4 ml 5% saline solution) into the peritendineum around the insertion of Achilles-calcaneus, two times per week for three weeks (from week 3 to week 6).
Biomechanical test. The cross-sectional area of middle portion of the tendon was determined with a soft tissue cross-sectional area micrometer. The preparation was first subjected to a single cycle of loading and unloading between 0 to 3.5% strain at an extension rate of 10mm/min and the peak values of load recorded. Cyclic testing between 0 load and the first cycle peak load was then performed for another 24 cycles with the computer acquiring load vs. deformation (hysteresis loop) and cyclic creep lengths vs. time data. Then the preparation was stretched to the first cycle peak load at a rate of 150 mm/min, and the stress relaxation vs. time data recorded for 600 seconds. The specimen was then unloaded. Subsequently, the preparation was stretched to failure at a rate of 150 mm/min and the failure mode noted. The stress and strain calculated according to the following equation adapted from Bulter (Bulter, 1978):

$$\sigma\Delta = F/A_{ref} \qquad \varepsilon = {}_{\Delta}l/L_{ref} \qquad E = KL/A_{ref}$$

Data processing. Fung (1981) proposed the use of a quasi-linear viscoelastic theory (QVL) to model the rheological properties of soft tissues, with a generalized reduced relaxation function as follows

$$G(t) = \textbf{Fehler!Fehler!}\frac{1+C[E_1(t/\ \tau_2 - E_2(t/\tau\ )]}{1+C\ln(\tau_2/\tau_1)}$$

where E_1 is the exponential integral, and C,$\tau_1$andτ_2 are constant parameters determined by

$$C = - dG(t) / [d\ln(t)\ \tau \otimes G(\infty)]$$

$$\tau_2 = t / \exp[(1 - c\gamma - G(t) / G(\infty)) / c]$$

$$\tau_1 = \tau_2 / \exp[(1/G(\infty) - 1) / c]$$

where γ is the Euler constant (γ =0.5772). In addition, an exponential expression was also chosen to represent the instantaneous stress-strain characteristics for the tendon (Woo, Johnson, & Smith, 1993). i.e. $\sigma^e = A(e^{B\varepsilon} - 1)$ where *A* and *B* are constant parameters determined using a nonlinear least-square curve fitting method in STATISTICA software.

RESULTS: Results of Creep. A typical cyclic and residual creep deformation vs. time curve for ATC is shown in Figure 1. Compared with the control group, the strains in the exercise and Heparin groups from 2nd to 25th cycle were found to be significantly larger (See Table 1).

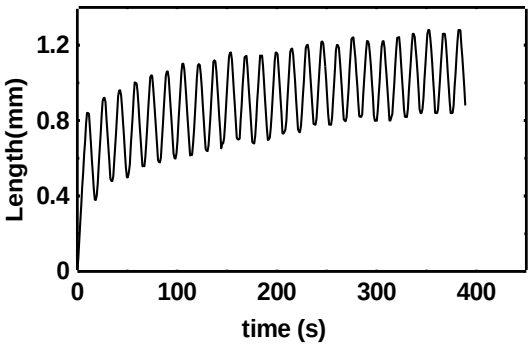

Figure1 - The typical cyclic creep curve from rabbit.

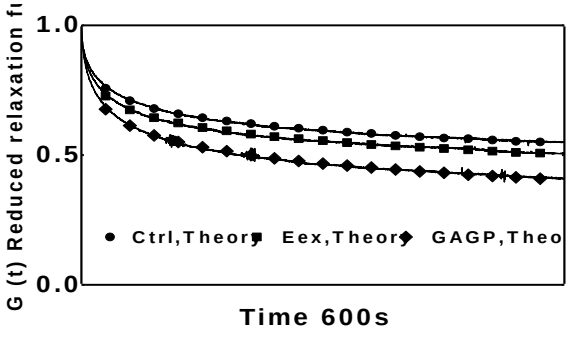

Figure 2 - The reduced relaxation function of rabbit ATC in different treatment groups.

Table 1 The Properties of Cyclic, Residual Creep of ATC Preparations (x±sd)

	Ctrl. n=20		Ex, n=12		Heparin, n=18	
Cyc. No.	Cyc.Creep %	Re.Creep %	Cyc.Creep %	Re.Creep %	Cyc.Creep %	Re.Creep %
1	3.56±0.17	1.39±0.42	3.54±0.07	1.68±0.31	3.76±1.02	1.72±0.60*
5	3.94±0.16	1.84±0.39	4.14±0.17*	2.24±0.35*	4.45±1.36	2.41±0.97*
10	4.10±0.24	2.11±0.44	4.40±0.25*	2.59±0.40*	4.84±1.59*	2.88±1.21*
15	4.25±0.36	2.27±0.57	4.60±0.27*	2.79±0.48*	5.05±1.67*	3.16±1.38*
20	4.34±0.49	2.38±0.66	4.62±0.34*	2.81±0.55*	5.29±1.70*	3.40±1.40*
25	4.44±0.59	2.53±0.81	4.80±0.44*	3.04±0.62*	5.53±1.84*	3.68±1.53*

*:P<0.05, Compared with the control group.

Results of Hysteresis. As shown in Table 2, the normalized area of hysteresis (the area for each cycle / the area of first cycle) from the exercise and heparin groups were significantly reduced in the most cycles (except 1~2th, 8th, 14th, 20th cycle in exercise group, and 1st, 12th cycle in heparin group).
Results of stress relaxation. It can be seen that G (t) curve fitted the experimental results very closely (see Figure 2) with the constants of C, τ_1, τ_2 for control and experimental groups listed in Table 4. From Figure 2 and Table 4, it can be seen that the stress relaxation reduced more quickly in the exercise and heparin groups.

Table 2 The normalized teresisareaof ACT (x±sd)

Cycle No.	Control	Exercise	Heparin
1	100	100	100
5	54.10±10.14	45.96±5.67*	46.23±7.46*
10	57.56±11.25	46.82±7.30*	44.54±12.75*
15	53.77±10.16	44.72±9.66*	42.42±11.32*
20	50.13±16.23	43.34±6.19	40.55±10.00*
25	54.42±9.33	43.28±7.31*	42.17±10.52*

*P<0.05, Compared with the control group

Table 3 The Normalized Stress Relaxation

Time(Sec.)	Control(%)	Exercise(%)	Heparin(%)
0s	100	100	100
90s	68.28±4.97	64.30±6.67*	58.35±9.61*
180s	63.11±6.12	58.73±6.92*	2.10±10.57*
360s	58.03±7.37	53.96±7.44	46.31±10.75*
480s	56.28±7.28	52.42±7.67	44.19±10.95*
600s	54.75±7.88	50.58±8.61	42.43±10.47*

*P<0.05, Compared with the exercise group

Table 4 The Constants of C,τ_1,τ_2 for each groups (x±sd)

	Control	Exercise	Heparin
C ($\times 10^{-2}$)	13.48±4.94	15.57±5.05	25.40±14.39*
τ_1 (s)	1.997±1.30	21.545±.843	1.923±1.141
τ_2 (s)	1029.65±138.82	1050.61±418.63	1079.32±411.60

Table 5 The Constant A,B of Stress-strain for ATC (x±sd)

	A	B
Control	1.94±.77	1.31±.13
Exercise	1.87±.76	1.35±.17
Heparin	2.36±1.22	1.35±.15

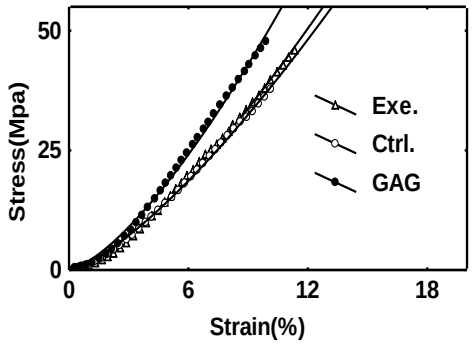

Figure 3 - The stress-strain curve from rabbit ATC preparation

Table 6 The Mechanical Properties of Preparations (x±s)

	Control	Exercise	Heparin
Sectional Area (mm2)	8.47±0.43	8.17±0.66	8.29 ±1.26
Failure load (N)	8.66±84.00	504.63±60.98	567.90 ± 137.32*
Yield load (N)	388.37±83.60	458.33±75.21*	489.73 ± 134.25*
Linear stiffness(N/mm)	164.13±29.00	167.25±26.98	202.25±36.61*, #
Failure Energy(N.mm)	948.35±372.69	1071.97±266.94*	1245.57± 473.46*
Yield Energy(N.mm)	501.81±200.45	714.07±266.94*	694.88 ±317.04*
Failure Stress(Mpa)	57.68 ± 9.56		62.08 ± 9.38 69.18±21.00*
Yield Stress(Mpa)	45.79±9.58	56.37±10.80*	60.14±20.42*
Fail. Strain Energy	4.43±1.34	5.42±1.31*	6.18±2.40*
Yield Strain Energy	2.35 ± 0.96		3.60 ± 1.22* 3.51±1.75*
Elastic Module(Mpa)	490.68 ± 83.93		497.49 ± 95.63 600.76±147.09*, #

*P<0.05, Compared with the control group. #P<0.05, Compared with the exercise group.

Results of tensile properties. The constants A and B of the stress-strain curve for each group is shown in Table 5. In terms of the constants A in the elastic responses, the values obtained for the Heparin preparations tended to increase, but no statistical significant difference between the exercised and the control preparations was found. Compared to the control and exercise preparations, the stress-strain curve was straighter in the Heparin group (Figure 3).

The mean values for yield load and failure energy, yield energy, and yield stress and strain energies from the exercised preparations were higher than those of the controls (see Table 6). All of the mechanical properties from the heparin treated preparations were improved significantly except the cross sectional area (see Table 6). The elastic module and linear stiffness were significantly greater for the heparin treated preparations than either the control or exercised preparations.

DISCUSSION: The beneficial effects of heparin intravenous injections for acute Achilles peritendinitis and the good results of local injections for jumper's knee and chronic

peritendinitis with $GAGP_S$ have been reported by several laboratories (Sundqist et al. 1987; Rais 1961). There are only a few studies to investigate the mechanical properties in Achilles tendon with overuse injury. Additionally, most studies focused on effects of exercise on the mechanical properties of Achilles tendon. Reproduction of typical chronic peritendinitis with the exercise program in the present experiment has been simulated in the present study's laboratory (Liu et al. 1995). There is a close relationship between the structure and function of tendon. Both the viscoelastic and mechanical results obtained from present study showed a change in the preparations of exercise and considerable change in the heparin group. The changes in viscoelastic behavior of the exercised preparations in the present study may be an adaptation of the tendon-bone complex to intensive exercise according to Wolf's law. These changes may be beneficial for the tendon-bone complex to bear strenuous alternate loading and unloading during exercise training and sports competitions.

$GAGP_S$ has been shown to inhibit the formation of thrombin and fibrin, which is considered to be an initiating mechanism in the formation of immature connective tissue, with delayed collagen formation and the formation of hypertrophic scar tissue (Sundqist, et al. 1987). The heparin, combined with a α glubular protein in the plasma, turned into a complex, which has been shown to inhibit the formation of fibrinogen from the fibrin. The transition of FactorXIII from Factor $XIII_a$ also can be blocked by the heparin. All of these factors can restrain the formation of stable fibrinogen polymer (Torngren, Kuttunen, & Lahtinen, 1984). The $GAGP_S$ treatment has heparin-like effects and its suppression of the fibroblast proliferation and fibrosis in wound healing is apparently due to fibrinolytic effects (Kvist, Lehto, & Jozsa, 1988). The findings from present study of increased creep and stress relaxation in the preparations treated by heparin indicate a beneficial effect for releasing the adhesion and scar tissue in Achilles peritendinitis. The changes of viscoelastic behavior for the heparin preparations in present study may also be due to the fibrinolytic effects of heparin.

CONCLUSION: The results showed that the tensile strength and viscoelastic properties of tendon-bone complex were improved by management using Heparin. It is indicated that the local injections of Heparin at the location of peritendineum and tendon-bone insertions are beneficial to the viscoelastic and tensile behaviors of the Achilles tendon system.

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