BASIC RESEARCH

High-energy extracorporeal shockwave therapy in a patellar tendon animal model: a vascularization-focused study

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OBJECTIVE: The aim of this study was to analyze the effect of high-energy extracorporeal shockwave therapy on tendon angiogenesis in the patellar tendons of rabbits. We sought to investigate whether different voltage and number pulses modify the angiogenesis pattern.

INTRODUCTION: High-energy extracorporeal shockwave therapy is an option in the treatment of orthopedic diseases such as chronic tendonitis. Despite its potential clinical applicability, there have been few studies on this technique that examine both its clinical effectiveness and its effect on angiogenesis.

METHODS: High-energy extracorporeal shockwave therapy was applied at the tibial insertion of the left patellar ligament in 30 rabbits that were separated into six groups that differed in terms of the voltage and number of pulses that were applied by high-energy extracorporeal shockwave therapy. The tibial insertion in the right legs of the animals was used as the control. After six weeks, we performed histological analysis on the region and quantified the number of blood vessels.

RESULTS: No significant differences in the number of blood vessels between the left and right patellar tendons were found within groups. Additionally, no significant differences in the number of blood vessels in the left patellar tendons were found between groups.

CONCLUSIONS: The application of high-energy extracorporeal shockwave therapy did not cause a change in vascularization in the patellar tendon in rabbits.

KEYWORDS: High-energy shock waves; Patellar ligament; Neovascularization; Animal model.

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INTRODUCTION

Treatment with high-energy extracorporeal shockwave therapy (HEST) is currently used for the treatment of musculoskeletal diseases.[1-5] Among its main indications are the treatment of nonunion, chronic insertional enthesopathies such as plantar fasciitis, lateral and medial epicondylitis, calcaneal tendon tendinitis, and calcareal tendinitis of the shoulder. It is a therapeutic option for cases in which conservative treatment has failed and surgery is potentially indicated.[1,4-7] There are some theoretical advantages of HEST in comparison to surgical treatment. Namely, HEST is noninvasive and fast, and it does not require hospitalization, has a low complication rate and high success rate, and has a relatively low cost.[6] The utilization of HEST for the treatment of musculoskeletal diseases began in 1986 when the first studies were performed regarding its effects on bone. Animal model studies have shown that HEST does not cause harmful changes to normal bone and increases the osteogenic potential through the activation of osteoblasts.[4]

HEST generates shockwaves that are transmitted through different tissues, and the processes by which this occurs are known.[21] Most experimental work has aimed to analyze the effect of HEST on bone.[8-10] In contrast, few studies have focused on the effects of HEST on soft tissue.[11,12] Thus, little is known about this subject.

To better understand HEST as a modality for the treatment of soft-tissue musculoskeletal disorders, basic science research should focus on local changes induced by HEST and seek to determine which are responsible for the improvements demonstrated in clinical studies.[13-16] One possibility for such an HEST-induced change is that HEST could stimulate the formation of new blood vessels in the

Table 1 - Distribution of groups in terms of energy level in kilovolts (kV) and the number of pulses applied.

Energy Pulses	14 kV	21 kV	28 kV
1000	Group 1	Group 2	Group 3
4000	Group 4	Group 5	Group 6

tendon and that it is this increased vascularization that leads to clinical improvement.[6,15,16]

There are few experimental studies showing changes in tendon vascularity after the application of extracorporeal shockwaves (ESW),[17,18] but there is evidence that HEST-induced tissue changes are dose dependent.[19] To find better evidence of HEST-induced angiogenesis in soft tissues, we designed this study to quantify the effects of HEST on tendons. The aims of this study were to examine the effect of HEST on the vascularity of the patellar tendon at its insertion at the anterior tibial tuberosity and to determine how varying the voltage and number of pulses applied changed this effect.

MATERIALS AND METHODS

A total of 30 six-week-old New Zealand white female rabbits were divided randomly into six groups of five animals. The groups received HEST that differed by voltage and the number of pulses (Table 1). The apparatus used for the study was the OssaTron® (Barueri, SP, Brazil), which was developed specifically for the treatment of musculoskeletal disorders. This apparatus uses electrohydraulic principles to generate shockwaves.

For the application of HEST, animals were anesthetized by intramuscular injection of 50 mg/kg ketamine hydrochloride and 5 mg/kg diazepam. After the animals had been anesthetized, the left knees were shaved. The application site, the anterior tibial tuberosity, was located with the aid of fluoroscopy and marked with a surgical marker pen. The animals were placed on a table in the supine position, and the device was positioned to direct the center of focus precisely to the marked site. Shockwaves were applied to the animal's left knee. After the procedure, intramuscular analgesics were provided to alleviate pain. The animals were sacrificed by carbon dioxide inhalation six weeks after HEST treatment, and the left and right patellar ligaments were harvested. The right patellar ligament was used as a control. The patellar ligaments were separated, fixed in 10% formalin, dehydrated in increasing alcohol concentrations, cleared in xylene, and embedded in paraffin in a manner that allowed the resulting blocks to provide an assessment of the entire ligament in the frontal plane. The paraffin blocks were cut into 5 μm sections that were stained with hematoxylin-eosin.

With the aid of a digital system *(Image Tool 3.0; Department of Dental Diagnostic Science at The University of Texas Health Science Center, San Antonio, Texas)*, we analyzed the entire length of the ligament insertion at the margin (Figure 1). To cover the entire length of the insertional ligament margin, sections were divided into fields (6–10 fields per section) and amplified 200 times. On the scanned images, all obvious vascular structures were marked, i.e., those with patent lumens, those lined by endothelium, or those containing red blood cells in the lumen. All of these structures were counted, and the end result was expressed in absolute numbers (Figure 2). Two independent observers, blinded to the distribution of the groups, examined the images.

Table 2 - Blood vessels per field from HEST-treated (left) and untreated (right) patellar tendons, indicating the differences between them.

Groups	N	Vessels/Field R	Vessels/Field L	R-L difference	*p*-value
	1	4.00	3.92	0.08	
	2	4.28	3.14	1.14	
1	3	7.19	8.81	-1.62	
	4	3.71	5.64	-1.93	
	5	-	7.57 *	-	0.465 (1)
	6	6.93	6.71	0.22	
	7	5.87	9.50	-3.63	
2	8	5.00	6.00	-1.00	
	9	7.43	7.14	0.29	
	10	4.56	9.50	-4.94	0.225 (1)
	11	-	3.50 *	-	
	12	8.22	6.37	1.85	
3	13	7.33	5.30	2.03	
	14	7.92	5.67	2.25	
	15	5.43	7.30	-1.87	0.273 (1)
	16	7.62	3.71	3.91	
	17	5.62	10.08	-4.46	
4	18	5.86	4.43	1.43	
	19	-	5.00 *	-	
	20	10.00	8.00	2.00	0.751 (1)
	21	7.28	7.17	0.11	
	22	-	5.37 *	-	
	23	7.21	10.57	-3.36	
5	24	9.00 *	-	-	
	25	10.86	11.00	0.14	0.285 (1)
	26	4.29	4.94	-0.65	
	27	4.10	6.44	-2.34	
6	28	2.62	4.50	-1.88	
	29	7.79	5.37	2.42	0.500 (1)
	30	4.57	5.00	-0.43	
					0.438 (2)

(*) Excluded from the statistical analysis because of the lack of a matching sample on the other side.
(1) Wilcoxon tests; (2) Kruskall-Wallis test.

Statistical Analysis

The data were analyzed first to evaluate the distribution. The Shapiro-Wilk test showed the data to be normally distributed *($p = 0.407$).* However, due to the small nature of the groups sampled, we chose to perform nonparametric testing. We calculated the mean number of vessels per slide, and this value was used to represent each subject. We determined the ratio between the total number of vessels and the total number of fields for each sample. This ratio (vessels per field) was used for statistical analysis. To standardize the groups, we calculated the difference in the number of vessels between the right and left patellar tendons of each animal. These differences were used to compare the groups. The nonparametric Wilcoxon test was used to compare the means of the number of vessels per field on the left and right legs within each group. The Kruskall-Wallis test was performed to compare groups when comparisons were made for more than two groups. In all statistical tests, an α of 5% ($p<0.05$) was considered significant.

The University's ethics research committee approved this study, which was conducted at the Department of Surgery of the Hand and Upper Limb, Department of Orthopedics and Traumatology, Escola Paulista de Medicina, Universidade Federal de São Paulo.

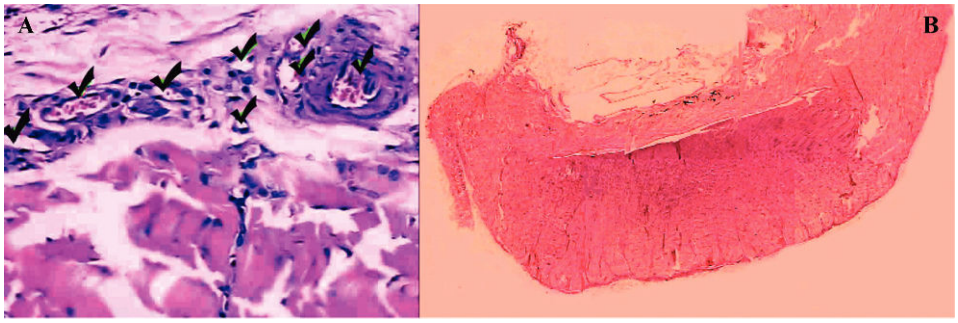

Figure - 1A (left): Histological section of a patellar tendon stained with hematoxylin-eosin (200×). Checkpoint arrows represent identified blood vessels. **Figure - 1B** (right): Patellar tendon section stained with hematoxylin-eosin (10×).

RESULTS

Twenty-five animals were included in the study's final analysis. Five animals were excluded because of problems related to histological analysis. Intergroup analyses indicated that there was some difference within the control groups (Kruskall-Wallis, $X2 = 13.52$; $p = 0.031$). This result led us to perform comparisons of the mean difference between the left and right patellar tendons of each animal (R-L difference). There were no statistically significant differences among groups in terms of the R-L difference (Table 2, Kruskal-Wallis; $X2 = 4.71$; $p = 0.438$).

DISCUSSION

It is known that the etiology of insertional enthesopathies is likely multifactorial, but the exact causes remain unknown.[5,8,19] It is thought that a degenerative process that is accompanied by inflammation is important in pathogenesis.[20] Authors have stated that the vascular changes and hyaline degeneration observed in surgical biopsies from insertional enthesopathy patients suggest that degeneration has a greater role than inflammation in disease pathogenesis and is the main etiological factor.[20,21] However, there is insufficient evidence to determine the best method for treatment of the disease. As such, there are a considerable number of options for treatment, including nonsteroidal anti-inflammatory and hormonal therapy, detention, infiltration, acupuncture, and surgery.[5,21]

Currently, we use ESW therapy as an alternative treatment for insertional enthesopathies and have achieved good results with this approach.[3,5,22-30] However, the mode of action behind this treatment is still unknown. It has been suggested that the pain in enthesopathies is the result of reduced vascularization in degenerative tendon tissue and that extracorporeal shockwave therapy could increase neovascularization and promote tissue regeneration.[21]

There is little in the literature regarding experimental research that is focused on understanding the effects of ESW on tendons. One author concluded that the effects of ESW are dependent on the amount of energy applied,[5,19] while others have observed an increased vascularization of treated tendons after ESW therapy.[1,31] Our work was designed to verify whether ESW causes changes in tendon vascularization, and if so, whether these changes would be dose-dependent.

The International Society for Musculoskeletal Shockwave Therapy advocates a normalization of the amount of energy to be applied in each case, based on the disease in question and the location of the ailment. Under these standards, the amount of energy to be applied for the treatment of most enthesopathies is 1000 pulses of 16 kV.[6] One experimental study cautioned about the harmful effects of using greater than 1000 pulses of 21 kV because there was evidence that such a dose could result in tendon tissue damage.[5] In our study, we used a broad spectrum of pulse-voltage combinations, with values both within the recommended range and above, to test the effects that HEST would have at different doses.

The tibial insertion of the patellar ligament was chosen as the application site for HEST because it is a structure that is easy to find and is relatively broad. These characteristics allowed for proper positioning of the device, which allowed us to focus the application of HEST correctly. In addition, because this ligament has a large insertion area and is well defined at one side, the demarcation of its margins was straightforward.

While it is not confirmed that the improvement of pain after ESW treatment of chronic enthesopathies is because of the induction of angiogenesis in the tendon, there are several reports that support this idea.[17,18] A histochemical study indicated that ESW appeared to induce the early release of vascular growth factors from the tendon. These growth factors induced angiogenesis, which led to increased blood supply to the tendon, which in turn supported tissue regeneration.[18] Hsu et al. studied the effects of ESW in a rabbit model of patellar tendinitis induced by the injection of collagenase and found an increase in vascularization at 16 weeks.[31] It is noteworthy that while all three of these studies showed an increase in tendon vascularization after ESW treatment, our results did not demonstrate such an increase. It should also be noted that only one of these three studies is comparable to our study in terms of the methodology used.

Each type of tissue has a particular impedance value. The impedance of bone is much greater than that of water, and therefore, bone absorbs large amounts of energy from shockwaves. However, the impedance of a healthy tendon is very similar to that of water, and thus, a tendon absorbs less energy than bone. However, a calcified tendon has a higher level of impedance because of the presence of calcium crystals. This could explain why HEST is more effective in cases of chronic enthesopathies because the tendons in these cases tend to be tougher and more calcified than in acute enthesopathies.[6]

We believe that more reliable results could be obtained if we use an experimental model of induced calcification in the tendon that simulates cases of chronic, calcified enthesopathies. There is definitely a need for more research on this topic.

CONCLUSION

Within the energy range tested, high-energy shockwave therapy did not cause significant changes in the vascularity of insertional patellar tendons in a rabbit model.

REFERENCES

1. Wang CJ. An overview of shock wave therapy in musculoskeletal disorders. Chang Gung Med J. 2003;26:220-32.
2. Loew M, Jurgowski W, Thomsen M. [Effect of extracorporeal shockwave therapy on tendinosis calcarea of the shoulder. A preliminary report]. Urologe A. 1995;34:49-53.
3. Thiel M, Nieswand M, Dorffel M. The use of shock waves in medicine–a tool of the modern OR: an overview of basic physical principles, history and research. Minim Invasive Ther Allied Technol. 2000;9:247-53.
4. Haupt G. Use of Extracorporeal Shock Waves in the Treatment of Pseudarthrosis, Tendinopathy and Other Orthopedic Diseases. The Journal of urology. 1997;158:4-11, doi: 10.1097/00005392-199707000-00003.
5. Rompe JD, Küllmer K, Vogel J, Eckardt A, Wahlmann U, Eysel P, et al. [Extracorporeal shock-wave therapy. Experimental basis, clinical application]. Orthopade. 1997;26:215-28.
6. Coombs R, Schaden W, Zhou S. Muskuloskeletal Shockwave Therapy. Greenwich Medical Media Ltd. London. 2000.
7. Ramos LAo, Carvalho RrTd, Garms E, Navarro MS, Abdalla RJ, Cohen Ms. Prevalence of pain on palpation of the inferior pole of the patella among patients with complaints of knee pain. Clinics. 2009;64:199-202, doi: 10.1590/S1807-59322009000300009.
8. Delius M, Draenert K, Al Diek Y, Draenert Y. Biological effects of shock waves: in vivo effect of high energy pulses on rabbit bone. Ultrasound Med Biol. 1995;21:1219-25, doi: 10.1016/0301-5629(95)00030-5.
9. Wang CJ, Wang FS, Yang KD. Biological effects of extracorporeal shockwave in bone healing: a study in rabbits. Arch Orthop Trauma Surg. 2008;128:879-84, doi: 10.1007/s00402-008-0663-1.
10. Gollwitzer H, Roessner M, Langer R, Gloeck T, Diehl P, Horn C, et al. Safety and effectiveness of extracorporeal shockwave therapy: results of a rabbit model of chronic osteomyelitis. Ultrasound Med Biol. 2009;35:595-602, doi: 10.1016/j.ultrasmedbio.2008.10.004.
11. Bosch G, Lin YL, van Schie HT, van De Lest CH, Barneveld A, van Weeren PR. Effect of extracorporeal shock wave therapy on the biochemical composition and metabolic activity of tenocytes in normal tendinous structures in ponies. Equine Vet J. 2007;39:226-31, doi: 10.2746/042516407X180408.
12. Qin L, Wang L, Wong MW, Wen C, Wang G, Zhang G, et al. Osteogenesis induced by extracorporeal shockwave in treatment of delayed osteotendinous junction healing. J Orthop Res. 2010;28:70-6.
13. Steinacker T, Steuer M. [Use of extracorporeal shockwave therapy (ESWT)in sports orthopedics]. Sportverletz Sportschaden. 2001;15:45-9, doi: 10.1055/s-2001-14817.
14. Zwerver J, Dekker F, Pepping GJ. Patient guided Piezo-electric Extracorporeal Shockwave Therapy as treatment for chronic severe patellar tendinopathy: A pilot study. J Back Musculoskelet Rehabil. 2010;23:111-5.
15. Rasmussen S, Christensen M, Mathiesen I, Simonson O. Shockwave therapy for chronic Achilles tendinopathy: a double-blind, randomized clinical trial of efficacy. Acta Orthop. 2008;79:249-56, doi: 10.1080/17453670710015058.
16. Vulpiani MC, Trischitta D, Trovato P, Vetrano M, Ferretti A. Extracorporeal shockwave therapy (ESWT) in Achilles tendinopathy. A long-term follow-up observational study. J Sports Med Phys Fitness. 2009;49:171-6.
17. Wang CJ, Huang HY, Pai CH. Shock wave-enhanced neovascularization at the tendon-bone junction: an experiment in dogs. J Foot Ankle Surg. 2002;41:16-22, doi: 10.1016/S1067-2516(02)80005-9.
18. Wang CJ, Wang FS, Yang KD, Weng LH, Hsu CC, Huang CS, et al. Shock wave therapy induces neovascularization at the tendon-bone junction. A study in rabbits. J Orthop Res. 2003;21:984-9, doi: 10.1016/S0736-0266(03)00104-9.
19. Rompe JD, Kirkpatrick CJ, Kullmer K, Schwitalle M, Krischek O. Dose-related effects of shock waves on rabbit tendo Achillis. A sonographic and histological study. J Bone Joint Surg Br. 1998;80:546-52, doi: 10.1302/0301-620X.80B3.8434.
20. Regan W, Wold LE, Coonrad R, Morrey BF. Microscopic histopathology of chronic refractory lateral epicondylitis. The American Journal of Sports Medicine. 1992;20:746-9, doi: 10.1177/036354659202000618.
21. Maier M, Milz S, Wirtz DC, Rompe JD, Schmitz C. [Basic research of applying extracorporeal shockwaves on the musculoskeletal system. An assessment of current status]. Orthopade. 2002;31:667-77, doi: 10.1007/s00132-002-0328-7.
22. Geddes LA, Tacker WA, Rosborough JP, Moore AG, Cabler PS. Electrical dose for ventricular defibrillation of large and small animals using precordial electrodes. J Clin Invest. 1974;53:310-9, doi: 10.1172/JCI107552.
23. Loew M, Jurgowski W, Mau HC, Thomsen M. Treatment of calcifying tendinitis of rotator cuff by extracorporeal shock waves: a preliminary report. J Shoulder Elbow Surg. 1995;4:101-6, doi: 10.1016/S1058-2746(05)80062-X.
24. Haake M, Sattler A, Gross MW, Schmitt J, Hildebrandt R, Muller HH. [Comparison of extracorporeal shockwave therapy (ESWT) with roentgen irradiation in supraspinatus tendon syndrome–a prospective randomized single-blind parallel group comparison]. Z Orthop Ihre Grenzgeb. 2001;139:397-402, doi: 10.1055/s-2001-17981.
25. Maier M, Steinborn M, Schmitz C, et al. Extracorporeal shock-wave therapy for chronic lateral tennis elbow–prediction of outcome by imaging. Arch Orthop Trauma Surg. 2001;121:379-84, doi: 10.1007/s004020100261.
26. Schmitt J, Tosch A, Hunerkopf M, Haake M. [Extracorporeal shockwave therapy (ESWT) as therapeutic option in supraspinatus tendon syndrome? One year results of a placebo controlled study]. Orthopade. 2002;31:652-7, doi: 10.1007/s00132-002-0325-x.
27. Buchbinder R, Green S, White M, Barnsley L, Smidt N, Assendelft WJ. Shock wave therapy for lateral elbow pain. Cochrane Database Syst Rev. 2002:CD003524.
28. Peers KH, Lysens RJ, Brys P, Bellemans J. Cross-sectional outcome analysis of athletes with chronic patellar tendinopathy treated surgically and by extracorporeal shock wave therapy. Clin J Sport Med. 2003;13:79-83, doi: 10.1097/00042752-200303000-00003.
29. Gerdesmeyer L, Wagenpfeil S, Haake M, Maier M, Loew M, Wörtler K, et al. Extracorporeal shock wave therapy for the treatment of chronic calcifying tendinitis of the rotator cuff: a randomized controlled trial. JAMA. 2003;290:2573-80, doi: 10.1001/jama.290.19.2573.
30. Harniman E, Carette S, Kennedy C, Beaton D. Extracorporeal shock wave therapy for calcific and noncalcific tendinitis of the rotator cuff: a systematic review. J Hand Ther. 2004;17:132-51, doi: 10.1197/j.jht.2004.02.003.
31. Hsu RW, Hsu WH, Tai CL, Lee KF. Effect of shock-wave therapy on patellar tendinopathy in a rabbit model. J Orthop Res. 2004;22:221-7, doi: 10.1016/S0736-0266(03)00138-4.