Effects of extracorporeal shock wave treatment on equine tendon healing.

Ana Liz G. Alves, DVM, PhD; Brunna Patrícia Almeida da Fonseca DVM, Ms; Renée L. Amorim, DVM, PhD; Armen Thomassian, DVM, PhD; Carlos A. Hussni, DVM, PhD; José Luis M. Nicoletti, DVM, PhD

From the Faculdade de Medicina Veterinária e Zootecnia, São Paulo State University, Botucatu – SP (Caminoto, Alves, Colla, Amorim, Thomassian, Hussni, Nicoletti); Supported by Fundação de Apoio aos Hospitais Veterinários da Unesp (research support foundation of Unesp Veterinary Hospital).

Abstract

In the present study, the effects of the extracorporeal shock wave therapy (ESWT) was assessed in experimental equine tendinitis. Extracorporeal shock wave therapy may stimulate collagen fiber orientation permitting better reorientation of the repair tissue. Ten horses without health problems were used in this experiment. Superficial digital flexor tendinitis was induced in both front limbs of each horse by use of a collagenase injections in each superficial tendon. On the thirtieth day after the collagenase injection, the right front limb of each horse was treated with ESWT (3 treatments at 3-week intervals); the left front limb was not treated (control limb). Periodically during the study, the healing process was monitored ultrasonographically and the portions of tendons affected with lesions were assessed. On the 120th day of the experiment, biopsy specimens were collected from all tendons for histological evaluation. The present results indicate that ESWT improved scar remodeling and tendon wound collagen maturation.

Key words: Shock wave, healing, tendinitis, equine.

Introduction

Equine tendons are frequently hurt by different mechanisms and the lesions showed degenerative phenomena and inflammatory alterations of different degrees of intensity. The healing of these lesions is often unsuccessful concerning restoration of the morphologic and functional characteristics of the tendon, thereby compromising the future athletic performance or increasing the risk of recurrence of lesions in the affected horses.[1]

Many types of treatments have been used to facilitate healing, but there are currently few treatments that stimulate healing to proceed in a timely manner on a consistent basis.[2, 3] As one would suspect by the number of therapies that have been tried, none promote healing in a timely fashion to allow earlier return to normal function. Although long convalescence seems to be the most effective treatment, there is still high relapse when the horse returns to its normal workload.

New treatments such as extracorporeal shock wave therapy (ESWT) represent an option for the treatment of superficial digital flexor tendinitis in horses.[3] The ESWT goal is not just to get the horse back to work sooner but to promote a better healing i. e. normal collagen alignment of the tendon.[3,4] The use of ESWT in Veterinary is at the beginning. So far, there is little information about different indications, treatment regimens and long-term results. Recent studies have shown that shock waves induce neovascularization at the tendon-bone junction, which in turn relieves pain and improves tissue regeneration and repair.[5] Results of another recent study suggested that ESWT in horses with suspensory ligament desmitis resulted in increased amounts of collagen fibrils and extracellular matrix components and higher immunoreactivity for TGF≤-1 (which may possibly represent increased activity of fibroblasts) in affected ligaments.[6] Few controlled studies have been carried out to investigate the experimental use of the extracorporeal shock waves in horses. The purpose of this study was to evaluate the effects of ESWT on affected tendons of horses with experimentally induced superficial digital flexor tendinitis using ultrasonographic and histological techniques.

Materials and Methods

This study was approved by the Ethics in Animal Experimentation Committee of Unesp-Botucatu. Ten adult horses (5 males and 5 females) were included in this study. The animals were clinically healthy adult Arabian horses on average five years old, specifically selected in terms of a normal locomotor apparatus.

Superficial digital flexor tendinitis was induced in both front limbs of each horse by use the type I collagenase injections, 1ml, 2.5mg/ml, in the middle metacarpal third. For each collagenase treatment, the horses were each sedated with romifidine (0.1 mg/kg, IV) and 2% lidocaine administered in each front limb to provide local anesthesia of the palmar metacarpal and palmar nerves and the skin of

the lateral metacarpal region was prepared aseptically. According to ultrasound guidance, collagenase was injected into the lateral region of the metacarpus, reaching the center of the superficial digital flexor tendon, using disposable 30 X 8 hypodermal needle.

Immediately before and 1 week after each collagenase treatment, ultrasonographic evaluation of the collagenase-induced lesions in each front limb was performed using a 7.5-MHz linear transducer. The extent of each lesion was calculated from the measurement of the area of the tendon and the area of the lesion.[8] Thirty days after the collagenase treatment, the horses were sedated with romifidine (0.1 mg/kg IV), and were treated in the right front limb with focused ESWT (G1). It involved applications of 500 shocks (5-mm focus) over the lateral aspect, 500 shocks (5-mm focus) over the medial aspect, and 500 shocks (35-mm focus) over the plantar aspect of the scanned region of superficial digital flexor tendon and the energy density was 0.15 mJ/mm^2. Every three weeks for three times, the horses were sedated and their right front limbs were treated with ESWT. The left front limb of each horse (G2) was not treated with ESWT (control group). The progression of the healing process in the ESWT-test and control front limbs were monitored ultrasonographically throughout the study. These evaluations were performed before each of the 3 treatments with ESWT (weeks 4, 7, and 10), and 2 and 4 weeks after the last ESWT (weeks 12 and 14). At the end of the study (120th day after the collagenase treatment), a tendon was biopsied (1.0 X 0.5-cm). After a 12-hour fast, each horse was premedicated with xylazine (1.1 mg/kg, IV) and anesthesia was induced with ketamine (2 mg/kg, IV) and diazepam (0.05 mg/kg, IV) and maintained with continuous IV infusion of guaifenesin (100 mg/kg), ketamine (2 mg/kg), and xylazine (1.1 mg/kg) to submitted to the surgical procedure for a tendon biopsy. Subsequently, horses were recovered from anesthesia and were kept on stall.

Histological analysis. The fragment collected for histology were fixed in 10% buffered formalin and routinely processed. Sections were stained with hematoxylin-eosin and Masson trichrome. Histological analysis was performed under the light microscope by an examiner who was unaware of the group to which the slides belonged. The following features were considered: number and characteristics of fibroblasts, presence of an infiltrate and vascularization, presence of collagen fibers and parallelism of collagen fibers scored on a scale from 0 to 3 (0=absence of parallelism of collagen fibers, 1+ discrete parallelism of collagen fibers, 2= presence of 50% of collagen fibers and 3= total parallelism of collagen fibers.

Statistical analyses were performed by using a Friedman rank point analysis of variance, Wilcoxon test for dependent qualitative samples, and Mann-Whitney tests for independent samples. The level of significance was set at $p = 0.05$.

Results

Seven days after the collagenase injections, ultrasonographic evaluations revealed 28.02 and 32.70 percentage of the tendon lesions on control and test groups respectively (**table 1**). Test and control groups weren't significantly different eather for ecogenicity or percentage of lesion area, except for the 90th day. Therefore the fiber alignment scores for test group were increasing faster than for control group which was statistically significant at the end of the experiment.

Few adverse effects such as small areas of hair loss and subsequent development of white hair after treatment.

Discussion

The collagenase model has previously been used for the evaluation of the tendon healing in horses. This model provides a

Table 1. Mean values of ecogenicity in the lesion area, percentage of the lesion area and parallelism score, observed in the experimental groups through ultrasonography examination on days 7, 30, 60, 90 and 120 of the experiment.

	7th	30th	60th	90th	120th
Ecogenicity					
G1*	3^{Aa**}	$2,5^{Aa}$	2^{ABa}	$1,5^{Ba}$	1^{Ba}
G2	3^{Aa}	2^{Aa}	3^{Aa}	2^{Aa}	1^{Ba}
Percentage of the lesion area					
G1	28.02^{Aa**}	18.68^{Ba}	12.45^{Ca}	6.42^{Da}	3.02^{Da}
G2	32.70^{Aa**}	24.00^{Ba}	11.37^{Ca}	9.53^{Ca}	6.85^{Da}
Collagen fibers parallelism					
G1	0^{Aa**}	1^{Aa}	0^{Aa}	$1,5^{Ba}$	3^{Cc}
G2	0^{Aa}	0^{Aa}	0^{Aa}	1^{Aa}	1^{Aa}

*G1 = test group; G2 = control group.
For each group, medians followed by capital letters did not differ significantly ($p>0.05$). For each phase, medians followed by small letters did not differ significantly ($p>0.05$). The results obtained in the histopatological examination performed on the 120th day after the lesion, showed fibroplasia, as also reported in literature.1 We observed differences between groups in terms of degree of collagen fiber maturation based on the identification of a low number of fibroblasts per unit area with elongated nuclei, few residual neoformed vessels, a few traces of mononuclear inflammatory infiltrate and a parallel arrangement of collagen fibers in test group, observed under the microscope (**Table 2**).

controlled mechanism for paired comparisons and it has been considered an efficient model for tendinitis study.[7,8]

The repair process can be monitored by noninvasive methods, such as ultrasonography or by invasive methods, such as biopsy of tendons for histologic examination, allowing evaluation for the cellularity and the degree of disorganization of the extracellular matrix.[9]

In horses with collagenase-induced superficial digital flexor tendinitis used in the present study, ultrasonographic evaluations revealed that treatment of affected tendons with ESWT had decreased the mean percentage lesion on 90th and 120th days more than in control group and better ecogenicity score on 60th and 90th than control group. Our data corroborate from findings of other studies[6,10] as to the use of ESWT in horses with collagenase-induced suspensory ligament desmitis, except that they had significant differences.

The mean fiber alignment ultrasonography score differed significantly between test and control groups. The longitudinal tendon fiber alignment occurred on ESWT group (G1) is an important parameter to return the horses with superficial digital flexor tendinitis to the previous level of activity without relapse.[7]

Although the mean value of 494.5 fibroblasts per mm^2 in the ESWT test group did not differ significantly from 789.5 per mm^2 in control group, the treatment's value was close to 459 fibroblasts per mm^2 proposed as normal for horse tendons by authors.[1,7] These data allowed us to speculate that the lower number of fibroblast detected in the test group (G1) may represent a later remodeling phase in the repair process.

The small amount of residual neoformed vessels and mononuclear infiltrate observed in the group submitted to ESWT (G1) indicated a higher speed of maturation of the repair process compared with control (G2). Similar data were reported by Willians 9 who stated that the reduction of cellularity and vascularization suggests progressive maturation of granulation tissue.

The present results demonstrate that the parallel arrangement of collagen fibers in the ESWT test group (G1) was more evident than in control group (G2) (**table 2**). When comparing these results with those reported by Alves et al.[7] and Goodship and Birch,[1,11] it can be observed that during the process of tendon repair, collagen fibrils are irregularly deposited, with predominant longitudinal alignment as the process matures. In our study the process of collagen fiber remodeling was more efficient in test group than in the control one.

The fact that ESWT had stimulated the alignment of collagen fibers during the course of the repair process, led us to believe that the ESWT in the present experiment had a high beneficial effect on the quality of tendon repair.[1,11,12] Alves et al.[7] emphasized the importance of the high degree of parallelism of collagen fibers, demonstrating the correlation between this characteristic and the increased resistance of tendon fibers.

Table 2. Mean values of number of fibroblast cells in the lesion area and of parallelism score, observed in the experimental groups through histopathological examination on the 120th day of the experiment.

	G1	G2
Mean fibroblast number	494.5[a*]	789.5[a]
Median parallelism score	3[a]	1[b]

*G1 = test group; G2 = control group.
For each group, medians followed by lower case letters were did not differ significantly ($p>0.05$).

We concluded that the effect of ESWT on tendon lesions allowed for a significant improvement in the quality of the tendon repair and favorable prognosis for the horse principally due to the arrangement of the collagen fibers.

The next phase of a future study could be the investigation of the proportion of the two types of collagen fibers (I and II) in this repair tissue and the mechanical properties of the scar.

[a] Collagenase type I, Sigma, USA
[b] Sedivet, Boehringer Ingelheim, Brasil
[c] Lidocaína 2%, Cristália, Brasil
[d] SSD 900, Aloka, Japan
[e] Versatron, HMT, Swiss
[f] Sedazine, Fort Dodge, Brasil
[g] Vetanarcol, Konig, Brasil
[h] Valium, Roche, Brasil
[i] EGG, Henrifarma, Brasil

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