THE EFFECTS OF EXTRACORPOREAL SHOCK-WAVE THERAPY ON THE ULTRASONOGRAPHIC AND HISTOLOGIC APPEARANCE OF COLLAGENASE-INDUCED EQUINE FORELIMB SUSPENSORY LIGAMENT DESMITIS

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Abstract—Extracorporeal shock-wave therapy (ESWT) may stimulate healing of desmitis in multiple species. The objective of this study was to evaluate the ultrasonographic and histologic appearance of collagenase-induced suspensory ligament (SUL) desmitis in untreated ligaments and ligaments treated with ESWT in horses. Four mature horses had SUL desmitis induced in both forelimbs. Beginning 3 weeks after induction of the lesions, one ligament per horse was treated 3 times at 3-week intervals with ESWT. The percent lesion, echogenicity and fiber alignment scores of the SULs were evaluated at 3-week intervals for a total of 15 weeks. At the completion of the study, the horses were euthanized and the SULs collected, fixed and processed for histopathologic evaluation. Ultrasonography showed treated SULs had a smaller percent lesion at the maximal injury zone (p < 0.001), and the total percent lesion (p = 0.01), total fiber alignment score (p = 0.0167) and total echogenicity (p = 0.0023) were all smaller in the treated ligaments. The fiber alignment score and echogenicity were not statistically different at the maximal injury zone. The treated SULs had a more concentrated area of metachromasia associated with healing than the untreated ligaments, where metachromasia appeared more diffuse throughout the ligament. ESWT improved the rate of healing as assessed ultrasonographically. (E-mail: mcclures@iastate.edu) © 2004 World Federation for Ultrasound in Medicine & Biology.

Key Words: Horse, Suspensory ligament desmitis, Extracorporeal shock-wave therapy.

INTRODUCTION

Extracorporeal shock waves are pressure waves generated outside the body that can be focused at a specific site within the body. Shock waves are characterized by high positive pressures, up to 100 MPa, and negative pressures of 5 to 10 MPa (Sturtevant 1996). The pressure increases rapidly with a rise time of 30 to 120 ns and a short 5-μs pulse duration (Sturtevant 1996). They are differentiated from ultrasound (US) by lower frequency, minimal tissue absorption and no thermal effects. The pressure waves travel through fluid and soft tissue, and their effects occur at sites where there is a change in impedance, such as the bone-soft tissue interface. The common clinical use for shock waves is to break renal and ureteral uroliths into fragments that can then be excreted (Fuchs and Patel 1996; Pettersson and Tiselius 1988). It is easily understood that, when the pressure wave meets an interface of different impedance, compressive and shear loads develop (Sturtevant 1996). Additionally, cavitation, which is the development of gas bubbles as a result of the rapid interaction between pressure and shear, occurs (Sturtevant 1996). The collapse of the gas bubbles leads to the development of fast flows or jet streams that contribute to the effect on the tissue. In addition to these mechanical effects, there are also cellular effects. Shock waves can increase cellular membrane permeability, stimulate cellular division and stimulate cytokine production by bone marrow cells differentiating toward osteoprogenitor cells (Wang et al. 2002b, 2002c). However, at this time, the mechanism by which shock waves stimulate tissue healing in vivo is unknown.

Shock waves are routinely used in Europe to treat insertional desmitis conditions in humans, including
planter calcaneal spurs (heel spurs) and lateral epicondylitis (tennis elbow) (Haupt 1997; Loew et al. 1995). Recent studies in dogs have demonstrated that shock waves induce neovascularization at the tendon-bone junction that, in turn, relieves pain and improves tissue regeneration and repair (Wang et al. 2002a). Extracorporeal shock-wave therapy (ESWT) has been approved by the US Food and Drug Administration (FDA) for treatment of heel spurs in the USA. In Europe (Crowe et al. 2001) and the USA (McClure et al. 2000; Scheuch et al. 2000), shock-wave therapy is being utilized to treat equine suspensory ligament (SUL) desmitis.

Suspensory ligament (interosseous medius) desmitis is a common and complex disease causing primary and compensatory lameness in the sport horse (Dyson et al. 1995). Suspensory ligament desmitis can cause long-term lameness, restricting the horse’s ability to perform at the level of competition achieved before the onset of lameness. Current medical treatment options include confinement and rest, controlled exercise protocols, intralesional injections, corticosteroid therapy, anti-inflammatory therapy, periligamentous injection of counter irritants and sclerosing agents, bone marrow injections and corrective shoeing (Dyson 1991; Dyson et al. 1995; Reef 1998).

The object of this study was to assess the effects of ESWT on the repair of collagenase-induced lesions in the body of the suspensory ligament of normal horses by ultrasonographic and histopathologic evaluation.

MATERIALS AND METHODS

Four quarter-horse type horses free of lameness with a mean age of 7.25 years (range 3 to 12 years), mean weight of 430 kg (range 383 to 473 kg) and ultrasonographically normal forelimb suspensory ligaments (SULs) were utilized for the study. Suspensory ligament bodies were considered ultrasonographically normal if there was no disruption of the echogenicity or fiber alignment, and their cross-sectional area did not exceed 1.5 cm² (Reef 1998). Horses were vaccinated, dewormed and routine dental and hoof care was performed 2 weeks before the beginning of the project. Horses were fed ad lib grass-alfalfa mix hay and had access to salt, mineral and water. Horses were confined to a 5 × 5 meter stall and were allowed free paddock (40 × 80 m) exercise for 2 h, 6 days a week, except for the day lesions were induced and on treatment days. This study protocol was approved by the Iowa State University Animal Care and Use Committee.

All ultrasonographic examinations and shock-wave treatments were completed with the horses lightly sedated with detomidine hydrochloride 0.01 mg/kg (0.005 mg/lb) IV (Dormosedan; Pfizer Animal Health, Exton, PA) and restrained in stocks. For all ultrasonographic evaluations and ESWT, the palmar metacarpal regions were clipped, shaved and degreased with alcohol before the application of a water-soluble coupling gel. All examinations were performed by one person (S. McClure) who was not “blinded” as to treatment group. The suspensory ligament body was ultrasonographically evaluated with an 8-MHz linear transducer (Falco 100; Pie Medical, Indianapolis, IN). Both transverse and longitudinal images were obtained at 8, 10, 12, 14 and 16 cm distal to the accessory carpal bone and captured digitally (Fig. 1). The cross-sectional area and dorsopalmar thickness of the ligament and lesion were measured from the cross-sectional and longitudinal views, respectively, by the software package in the US machine; therefore, the percent lesion and the fiber alignment scores were obtained by direct measurement. The echogenicity and fiber alignment scores were similar to those previously described for superficial digital flexor tendons, with a score of 0 being normal and a score of 3 being 25% or less of normal echogenicity or fiber alignment (Rantanen et al. 2003) (Figs. 2 and 3). For each ligament, the percent lesion was measured and an echogenicity and fiber alignment score assigned to the lesion at each 2-cm interval for five measurements (two proximal, two distal and one centered where the lesion was created). At the maximal injury zone (MIZ), the percent lesion (%Les-MIZ), echogenicity score (Echo-MIZ), and fiber alignment score (FiAS-MIZ) and the sum of each of these parameters at each site measured (total percent lesion, tot%Les, total echogenicity, totEcho, and total fiber alignment score,
totFiAS) were recorded for each forelimb during each ultrasonographic evaluation.

To induce the lesions, the horses were anesthetized, placed in dorsal recumbency with the forelimbs suspended and the palmar metacarpal region aseptically prepared. A focal desmitis of both forelimbs was induced in the center of the body of the suspensory ligament (SUL) 12 cm distal to the accessory carpal bone by injecting 4000 IU of collagenase (Collagenase Type 1-S, c1639; Sigma-Aldrich, St. Louis, MO) in 0.1 mL volume using a US-guided needle biopsy attachment and a 22-gauge 8.25-cm spinal needle. Phenylbutazone, a nonsteroidal anti-inflammatory agent, (4.4 mg/kg IV, every 24 h) was administered immediately before collagenase injection and continued for a total of 3 days.

The SULs were ultrasonographically evaluated at weekly intervals for 3 weeks. ESWT was started 3 weeks after lesion induction. One SUL of each horse was randomly assigned as a control. The contralateral SUL was treated with a focused extracorporeal shock-wave generator (Equitron; High Medical Technologies, Lengwil, Switzerland) at 0.14 mJ/mm² 3 times at 3-week intervals. The peak positive and negative pressures at this setting are 45.3 MPa and −5.9 MPa, respectively. A total of 500 pulses were administered from the palmar aspect of the limb with a 35-mm probe and 500 more from palmar-lateral and palmar-medial with a 5-mm probe for a total of 1500 pulses. The shock waves were distributed over a 10-cm length of the SUL centered over the lesion with the horses standing on the limb. The lesions were evaluated and images recorded ultrasonographically at 3-week intervals starting 3 weeks after induction of lesions to the completion of the project at 18 weeks. On the 3 days when horses were both treated and examined ultrasonographically, the US examinations were completed before treatment.

At the completion of the project at week 18, horses were euthanized with a barbiturate overdose. The SULs were collected and placed in 10% neutral buffered formalin solution. They were transected in the frontal plane to facilitate dehydration, which was achieved through increasing percentages of ethanol, cleared in xylene and infiltrated with polymethyl methacrylate (PMMA). The samples were then sectioned with a computerized microtome (Polycut; Reichert-Jung, distributed by Leica, Bannockburn, IL, USA) to 20-μm sections that were mounted on glass slides. Sequential sections were produced and every other slide deplasticized and stained with toluidine blue. Selected unstained sections were deplasticized and stained for type III collagen with Gridley’s reticulum technique. Slides were evaluated by an orthopedic pathobiologist (D. VanSickle) without knowledge of the treated or control groups.

Statistical analysis

A previous tendon-healing collagenase model resulted in a 42 mm² difference in lesion size between treated and control limbs (Henninger et al. 1992). Using these data for sample size calculation, a sample size of 4 with α = 0.05 and a conservative expected mean cross-sectional lesion difference of 20 mm² between groups, with an SD of 8 mm² would result in a power of 0.91. Statistical analysis was performed on the six outcome variables (%Les-MIZ, Echo-MIZ, FiAS-MIZ, tot%Les, totEcho, totFiAS). Each variable was measured before the initial treatment and was repeated at 3-week intervals for a total of five time points; therefore, the data are repeated measures. To test the difference between treatment and control groups for each of the six variables, two methods of analysis were used. Echo-MIZ and FiAS-MIZ are ordinal scores (0–3), which do not satisfy assumptions required for continuous data repeated-measures analysis methods; therefore, each ligament score is summarized over time by the slope coefficient of a linear regression (Echo-MIZ or FiAS-MIZ score vs. time). The slope coefficient represents the trend of the variable over time for each ligament. The resulting summaries (four slope coefficients per group) were used in t-tests to compare Echo-MIZ and FiAS-MIZ for each treatment and control. The totEcho and totFiAS alignment scores

Fig. 3. The echogenicity scores were assigned with 0 being normal and a score of 3 being 25% or less of normal echogenicity.
are sums of the echogenicity and fiber alignment scores with a possible range of the totals (0–15) sufficient to treat these variables as continuous variables. The %Les-MIZ and tot%Les lesion are continuous variables. Multivariate analysis of covariance (MANCOVA) was used to compare treatment and control groups for continuous variables. MANCOVA accounts for within-subject correlation across time, and controls for differences in baseline data across groups (Tabachnick and Fidell 2001) Data were analyzed with a commercially available software program (JMP Statistical Software; SAS Institute, Cary, NC). A p value of < 0.05 was considered significant.

RESULTS

At 12 h following injection of collagenase, there was a diffuse swelling on the palmar metacarpus. Pain was elicited on palpation for about 1 week. Within 2 weeks, no lameness was consistently observable. The lesion size determined ultrasonographically did not change in size between the second and third weeks. The lesions were considered to be stable and treatment was started at that time (day 0). The MIZ was at the site of the injection or within 1 to 2 cm distal.

The treatment and control groups were both significantly different for %Les-MIZ (p = 0.001), tot%Les (p = 0.01), totFiAS (p = 0.0167), and totEcho (p = 0.0023), with the lower means corresponding to the treatment group. (Figs. 4 and 5). The FiAS-MIZ was not statistically significant over treatment and control (p = 0.0584), but the treatment group had a lower average slope (−0.45, 0.1) than the control group (−0.1, 0.1). That is, the FiAS-MIZs for the treatment group were decreasing faster than for the control group. The Echo-MIZ was not statistically significant (p = 0.8) among the groups.

Histologically, metachromasia from proteoglycan deposition was more focal in treated SULs compared to control SULs. All eight ligaments had either focal or diffuse metachromasia (Table 1). The remaining histologic findings were not present in all of the ligaments. Other indications of fibroblastic activation, such as large round cells, doublet cells and increased numbers of fibroblasts, as well as metachromasia in perivascular connective tissue were similar between treated and control SULs. Staining for type III collagen was similar between the treated and control SULs. Hemosiderin was seen in two treated and two control ligaments.

DISCUSSION

The collagenase model has previously been used for the evaluation of tendon healing in the horse (Henninger et al. 1992; Redding et al. 1999). This model provides a controlled mechanism for paired comparisons that decreases the number of animals needed for the study. Naturally occurring SUL desmitis may respond differently to ESWT and may have a different histologic appearance; however, this model provides a mechanism to evaluate the therapy with fewer variables. The collagenase was injected 12 cm distal to the accessory carpal bone because the site was as proximal as possible to allow imaging of the entire area of the lesion proximal and distal to the point of injection. The induced lesions in the body of the SUL resulted in a focal desmitis. The area

Fig. 4. The mean percent lesion (%Les), fiber alignment score (FiAS) and echogenicity (Echo) at the maximum injury zone (MIZ) for the (●) treated and (■) control suspensory ligaments are charted over time, with the p value showing the statistical significance of the difference between the groups. Error bars are 1 SEM. ESWT was done on days 0, 21 and 42.
evaluated ultrasonographically was centered on this area with images at 2-cm intervals. This is different than the standard 4-cm zones described for the metacarpus; however, it permitted a thorough examination of the lesion.

Multiple authors report differences in the healing, clinical signs and lameness of SUL depending on location (proximal, midbody, branch) and limb (fore, rear). Controlled exercise alone can result in up to 86% of forelimb proximal suspensory desmitis cases resuming full work in contrast to rear limb proximal suspensory desmitis, which was found to have a guarded prognosis (Dyson 1991). Clinically, lesions of the body and branches are more apparent because they exhibit localized heat, pain and swelling. Lesions of the body are more common in forelimbs in all horses except Standardbreds, and are more common in race horses than sport horses (Dyson et al. 1995). In Thoroughbreds, desmitis of the body of the SUL is less common than proximal or branch desmitis and also has a poor prognosis. In Standardbreds, desmitis of the body can result in a chronic enlargement of the ligament, but the horse can often be managed and continue to perform. Clearly, the prognosis and healing of suspensory desmitis is related to location and activity. This study evaluated only the ultrasonographic and histologic appearance of forelimb body suspensory desmitis without long-term outcome.

The number of pulses and treatment intervals were based on what one of the authors (S. McClure) has developed for clinical cases, which is similar to protocols developed by others (Kreling 2001; Scheuch et al. 2000). The energy setting of 0.14 mJ/mm² was what is used clinically. The energy settings and number of pulses are important. Too low energy or too few pulses may not create the desired therapeutic effect. Alternatively, excessive energy or pulses can result in tendon damage. In rabbit Achilles’ tendons, energy flux density exceeding 0.28 mJ/mm² resulted in increased tendon fiber diameter, fibrinoid necrosis and peritendinous inflammatory reaction (Rompe et al. 1998). In a gastrocnemius tendon study in turkeys, an energy flux density of 1.2 mJ/mm² decreased the tensile strength of the tendon (Maier et al. 2001).

Skeletal muscle is less echogenic than the SUL, but is typically bilaterally symmetrical, so comparison of both forelimbs allows the ultrasonographer to separate normal ligament from one with desmitis. Interpretation of the histology of the suspensory ligaments was difficult because of the skeletal muscle cells present in some ligaments. In one horse, it appeared that the collagenase injection was at least partially injected into some muscle tissue that resulted in replacement of the skeletal muscle cells with adipose tissue. Fortunately, the cellular components were similar bilaterally, resulting in an even

**Table 1. The frequency of each of the histologic findings in the four treated and four control limbs**

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<tr>
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<th>Treated</th>
<th>Control</th>
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<tr>
<td>Diffuse metachromasia</td>
<td>1</td>
<td>4</td>
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<tr>
<td>Focal metachromasia</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Perivascular large cells, doublets and increased fibroblasts</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Metachromasia in perivascular connective tissue</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hemosiderin</td>
<td>2</td>
<td>2</td>
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Fig. 5. The %Les, FiAS, and Echo for each of the five sites evaluated were added for a total for each leg (tot) and the mean for the (●) treated and (■) control suspensory ligaments are charted over time, with the p value showing the statistical significance of the difference between the groups. Error bars are 1 SEM.
comparison. The variation on the amount of muscle within the suspensory ligament may affect comparisons of healing between horses, so comparison between left and right forelimb in a horse is a good mechanism to evaluate healing (Wilson et al. 1991). The presence of muscle tissue may affect healing of the lesions. Rear limbs have greater percentage of muscle tissue in Standardbreds and Thoroughbreds and rear limb suspensories tend to heal slower with a poorer prognosis. Interestingly, the muscle fiber would have a greater cellularity and one would usually expect it to heal faster.

The ultrasonographic evaluation of these horses was not a “blinded” evaluation. The ligament and the lesion diameters and FiAS were objectively measured; however, the tracing involves some subjectivity. The echogenicity is a subjectively assigned score. “Blinding” of the evaluator and obtaining grey-scale measurements would have improved the quality of the study. Finally, the FiAS and Echo scoring scales were designed for superficial digital flexor tendinitis but, due to the lack of a proven mechanism to score SUL desmitis, these scales were utilized for this study.

The metachromasia was more focal in the treated SULs than the control. The focal metachromasia was interpreted as accompanying the healing of the ligament and would be one of the initial phases of healing represented by increased proteoglycan content of the ligament. Determining how to interpret global metachromasia in the control SUL and the more focal metachromasia in the treated SUL is speculative. It is possible that the treated ligaments had already passed through the more diffuse global stage and the noted response was more focused on the area of the original lesion. Alternatively, the treatment may have prevented the global response that was seen in the control SULs. The perivascular connective tissue matrix of both ligaments of each animal had increased metachromasia, indicating increased cellular metabolic activation. Because this was similar in treated and control ligaments, it is likely due to the inflammation associated with the collagenase-induced desmitis.

The mechanism by which ESWT caused the differences seen in this study is unknown. The focal activation of fibroblasts in the treated ligament could result in a more rapid filling of the defect as seen by ultrasonography. Shock-wave therapy was found to stimulate neovascularization at the Achilles’ tendon-bone junction in dogs (Wang et al. 2002a). At the site of the lesions in the body of the SUL in these horses, notable neovascularization was not seen. However, the bone-ligament junction was not specifically treated or evaluated in this study. An area of current research interest is in the direct cellular stimulation following ESWT. The focal response seen in the treated ligaments may be the result of direct cellular stimulation. Another potential factor on the ultrasonographic evaluation is the potential mechanical factor of ESWT. The pressure waves could potentially help physically break down or move the hematoma or fluid from the lesion to allow the fibroblasts to more rapidly fill the defect. The objective of this study was to evaluate outcome and not mechanism.

Ultrasonographic evaluation of the ligament is only one mechanism by which healing can be evaluated. Ultimately, the important factor is returning the horses with SUL desmitis to the previous level of activity, fully sound and without recurrence of the disease. This study did not evaluate long-term return to function. This study did not address the mechanical strength of the ligament. Future studies and clinical evaluation will provide insight into the potential for recurrence of the injury and strength of the ligament.

Further investigations and clinical evaluations will be needed to assess long-term outcome in naturally occurring suspensory desmitis. In this study, 1. ESWT resulted in faster filling of the defect as assessed by ultrasonography when compared with control limbs, and 2. the activation of the fibroblasts was more focal in the treated limbs than in the control limbs.

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REFERENCES


