Acellular Urinary Bladder Matrix in a Collagenase Model of Superficial Digital Flexor Tendonitis in Horses

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ABSTRACT

The objective of the present study was to determine the efficacy of urinary bladder matrix (UBM) in collagenase-induced superficial digital flexor (SDF) tendonitis by using clinical, ultrasonographic, and histologic data. A total of eight healthy adult horses were used in this study. Bilateral forelimb SDF tendonitis was created in the horses by injecting collagenase. After 14 days, one randomly selected forelimb SDF tendon was blindly treated with UBM and the opposite tendon was treated with a control (saline). Clinical and ultrasonographic parameters including lameness, lesion size, ultrasonographic fiber pattern, and echogenicity were measured throughout the study. After 84 days, horses were euthanized and SDF tendon lesions from the two groups were compared statistically using an analysis of variance with significance set at $P \leq .05$.Results showed that there were no significant differences between the treated and control tendons for any of the clinical, ultrasonographic, gross, or histologic variables. UBM does not appear to be an effective treatment for collagenase-induced SDF tendonitis. However, there may be differences in clinical tendonitis that might render the treatment more effective in the clinical setting.

Keywords: Urinary bladder matrix; Tendonitis; Horse; Collagenase

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INTRODUCTION

Tendonitis may result from a severe strain to the superficial digital flexor (SDF) tendon, because of excessive loading and overstretching of the tendon, repetitive overuse, or microdamage of the tendon associated with exercise.[1-5] Strain-induced injuries are believed to result following a phase of molecular degeneration or an inflammatory event that is neither clinically evident nor produces any reparative responses but instead progressively weakens the structure.[5] Tendonopathies often begin with degeneration of and minor changes in the structural integrity of the tendon, and thus predispose an already high-risk structure to injury. After the structural strength is overcome, physical disruption occurs within the tendon matrix. Various structural breakdowns are known to occur including fibrillar stretching with breakage of crosslinks, fibrillar rupture, or in some severe cases, separation of tendon tissue.[3] The tendon on healing produces a collagenous scar, predominantly of type III collagen, which is generally proportional to the severity of the tendon lesion.[6]

Tendonitis of the SDF tendon is a common cause of lameness in performance horses, especially race horses and event horses. Risk factors that contribute toward injury are the speed of exercise, the small cross-sectional area (CSA) of the SDF tendon, and the excessive load that is placed repetitively on the tendon during the early and mid-stance phase of the stride.[3,6] Additionally, predisposing factors for SDF tendonitis in other types of performance horses include inadequate training, muscle fatigue, uneven and slippery ground, sudden turning, excessive pastern slope, improper shoeing, and the long toe–low heel hoof conformation.[6]

Appropriate treatment of tendonitis after resolving the initial inflammatory stage is controversial. Treatment options that have been used for tendonitis include administration of intralesional hyaluronan and β-aminoproprionitrile fumurate, and intramuscular polysulfated glycosaminoglycans; tendon splitting with or without superior check ligament desmotomy; use of non-steroidal anti-inflammatory drugs; controlled exercise alone; and various physical therapy modalities such as ultrasound, laser, and magnetic therapies.[1,2,6-11] More recent treatment options include

extracorporeal shockwave and intralesional bone marrow therapy, insulin-like growth factor 1 hormone therapy, autogenous mesenchymal stem cells therapy, and administration of urinary bladder matrix (UBM) powder (ACell Vet Powder, ACell, Inc., Columbia, MD).[2,7,8,12,13]

UBM is a lyophilized powder derived from the extracellular matrix of the basement membrane of swine urinary bladder.[14-16] It is acellular in structure but is thought to recruit regenerative cells and other necessary growth factors from the circulatory system and local tissues for the purpose of tissue differentiation.[12,14-18] In other species, UBM has been found to produce a profound angiogenic response in the first 5 to 7 days post treatment.[15,17] The potential benefits of UBM to tendon healing include providing a scaffold for collagen deposition within the damaged tendon, recruiting growth factors to the site of injury, and minimizing excessive fibrous tissue formation.[12,14,16,18] Although little is known about the immunogenicity of UBM, a cross-species immune response may be possible, as this product originates from swine bladder.[18] However, to our knowledge, this has not yet been documented clinically.

Definitive information on the benefits of intralesional UBM therapy in horse tendon injuries is scarce. Anecdotally, it has shown promise with both tendonitis and desmitis in clinical cases. In a recent report of 53 horses treated with intralesional UBM, 81% of the horses $\geq$6 months post-treatment were found be sound, healthy, and capable of carrying out work.[12] As compared with more conventional treatments, tendon and ligament healing was thought to occur more rapidly with better quality of the repaired tissue being visible ultrasonographically. Less scarring and permanent enlargement of the treated tendons and ligaments were thought to occur clinically.[12] However, no controlled studies evaluating the efficacy of intralesional UBM in the treatment of soft-tissue injuries in horses have been performed.

The purpose of our study was to determine the efficacy of intralesional UBM in horses with collagenase-induced tendonitis, using clinical, ultrasonographic, and histologic data. We hypothesized that as compared with the saline control, UBM would promote better quality tissue repair in the collagenase-induced tendonitis model.

MATERIALS AND METHODS

The study protocol was approved by the Colorado State University Institutional Animal Care and Use Committee. For this study, eight healthy adult horses free from lameness and musculoskeletal abnormalities were selected on the basis of baseline physical and lameness examinations. The cohort consisted of 5 females, 2 stallions, and 1 gelding, with a mean age of 4.25 years (range, 2.5–5) and a mean body weight of 395 kg (range, 350–425 kg). Baseline ultrasound was performed on the front limbs, and CSA was measured in five zones (6 cm, 8 cm, 9 cm, 10 cm, and 12 cm distal to the palmar aspect of the carpometacarpal joint as assessed with ultrasound, respectively). Baseline limb circumference measurements were taken at 9 cm distal to the palmar aspect of the carpometacarpal joint as assessed with ultrasound, using a flexible tape measure, and calipers were used to measure the medial-lateral width of the SDF tendon at the same level. On day 14, after pretreating with flunixin meglumine (1.1 mg/kg IV) and performing a high 4-point nerve block with bupivacaine, tendonitis was induced in the front limbs. Collagenase (Bacterial Collagenase Type I, Sigma-Aldrich Co, St. Louis, MO; 2,100 units) diluted in 0.3 mL sterile water was injected using a 27 gauge 1/2″ needle into the body of the SDF tendons, under ultrasonographic guidance at two sites (ie, 8 cm and 10 cm) distal to the carpometacarpal joint, as has been described in previous studies.[9,13,19] Injections were administered at the medial aspect of the tendon so as to visualize the entrance of the needle into the thicker portion of the tendon and its advancement to the center of the tendon under ultrasonographic guidance from a palmar transverse view. After 12 hours, a high 4-point block with bupivacaine was repeated, and each horse was treated with phenylbutazone (4.4 mg/kg IV q 12 h for 2 doses, then 2.2 mg/kg PO q 12 h for 5 days). The limbs were then hosed with cold water for 15 minutes once daily for 3 days and bandaged until treatment to minimize swelling and inflammation. On the basis of pilot studies, horses were housed in stalls for the initial 2 weeks after collagenase injection to attempt to allow for lesion stabilization.

After 14 days (day 0), lameness examinations were performed by using the American Association of Equine Practitioners lameness scale.[20] The severity of pain on palpation of the SDF tendon was determined by using a grading scale ranging from 0 to 4 (0 = no pain, 1 = slight pain, 2 = mild pain, 3 = moderate pain, and 4 = marked pain). Front limb circumference and SDF tendon width at the site of collagenase injections were measured. Ultrasound evaluations were performed in both the transverse and longitudinal planes to document the size of the hypoechoic lesion and severity of SDF tendonitis. The CSA of the lesion calculated as a percentage of CSA of the tendon was determined in the transverse plane, and the length of lesion was determined in the longitudinal plane. Ultrasound scores were recorded separately for fiber alignment and echogenicity on a scale ranging from 0 to 4 (0 = normal, 1 = slight disruption/hypoechogenicity, 2 = mild disruption/hypoechogenicity, 3 = moderate disruption/hypoechogenicity, and 4 = severe disruption/hypoechogenicity).[21]

On day 0, one SDF tendon from each horse was randomly assigned to the treatment group (UBM) and the opposite SDF tendon to a sham treatment (saline). The investigators were blinded to the treatments. Using a 22