Clinical and Ultrasonographic Findings of Collagenase Induced Tendinitis in the Horse

Mohammad Mehdi Dehghan*, DVSc
Hossein Kazemi Mehrjerdi1, DVM
Majid Masoudifard1, DVSc
Mohammad Reza Baghban Eslaminejad2, PhD
Davood Sharifi1, PhD
Ali Reza Vajhi1, DVSc

1 Department of Clinical Science, Faculty of Veterinary Medicine,
University of Tehran, Tehran, Iran.
2 Stem cell department, Royan Institute, Tehran, Iran.

Abstract

Objective- The aim of this study was to assess the clinical and ultrasonographic alterations in the superficial digital flexor tendon (SDFT) of horses with collagenase-induced tendinitis.

Design- Experimental study.

Animals- Five clinically normal adult horse.

Procedures- Two thousand units of collagenase were injected in the center of the SDFT of forelimbs of each horse under ultrasound guidance. Clinical examinations were performed to evaluate heat, response to palpation, presence of swelling, and lameness grade. Ultrasound images of the SDFT were recorded prior to injury and at 1, 3, 5, 7, and 14 days after injection. Images were digitized, and percentage of lesion, the echogenicity score (ES), and the fiber alignment score of the fibers (FS) at the maximum injury zone (MIZ) were measured. The sum of the core lesion from 8–20 cm distal to the accessory carpal bone was also measured.

Results- Collagenase resulted in anechoic core lesion involving 20%-35% of the tendon cross section at MIZ. There were not any significant difference in the size, ES, and FS between all the days except between the first day and the other days. There were no significant differences between observations made of each structure in the left and right forelimbs of the same horse.

Conclusions and Clinical Relevance- Collagenase-induced injury is ultrasonographically similar to naturally occurring injury and causes the same tendinitis in both left and right forelimbs and also in different horses. Tendon lesions using collagenase injection represent an experimental model for tendon injury in research trials.

Keywords- SDFT, tendinitis, collagenase, ultrasonography.

*Corresponding Author:
Mohammad Mehdi Dehghan
Department of Clinical Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
E.mail address: mdehghan@ut.ac.ir
Introduction

Injury to the superficial digital flexor tendon (SDFT) in racing horses has a reported incidence of 8% to 35% and is a significant cause of wastage. During athletic activity, repetitive forces on this structure predispose it to injuries, which culminates in partial or total rupture of the tendon. Acute tendinitis is characterized by collagen fiber damage and an increase in cross-sectional area (CSA) of tendon due to intratendinous hemorrhage and inflammatory fluid accumulation within the defect. These structural defects occur commonly in the center of the unsheathed portion of the SDFT and are commonly called core lesions. These core lesions have been defined as type 1–4 based on the ultrasound echogenicity of the lesion, although recording the CSA and length of the visible core lesion enhances the accuracy when determining severity of the lesion.

An accurate diagnosis of tendon injury and careful monitoring during treatment are important factors in the rehabilitation of the horse. The repair process can be monitored by noninvasive methods, such as ultrasonography. Ultrasonography is one of the most accurate tools to evaluate the tendon structure after an injury and during the healing process, and it is widely used in equine practice, for diagnostic purposes and follow-up. The ultrasonographic imaging provides the size, shape, location and severity of soft tissue lesions, based on the echogenicity and fiber alignment. Small changes in ultrasonographic size, echogenicity and fiber pattern are associated with relatively large changes in biomechanical strength. Close correlations have been also established between ultrasonographic observations of injured and normal tendons and their histopathologic appearance.

Many treatment modalities have been used to facilitate healing of these lesions, but currently there are not any treatments that enhance healing on a consistent basis. The methods that have been employed include prolonged periods of inactivity, controlled exercise program, anti-inflammatory therapy, peritendinous injection of counter-irritants, sclerosing agents, tendon splitting, annular ligament desmotomy, superior check ligament desmotomy, and numerous other therapies. Increasing attention is being to the intratendinous use of growth factors and/or cell-based therapies.

In view of the fact that the ability to accurately induce uniform tendon injuries are necessary in studies designed to evaluate and compare new treatments, various methods such as tendon splitting, mechanical and cytokine injection were studied in the hope for finding an experimental model to standardize tendon injuries. Because of the lack of success in producing a tendinitis by this means, the possibility to induce tendon damage enzymatically with the use of a bacteria-derived collagenase was explored, resulting in lesions with similar characteristics to those occurring naturally.

The purpose of this study was to define the ultrasonographic, and clinical findings of collagenase induced tendinitis in acute phase in the horse.

Materials and Methods

Subject

Five normal adult horses, with a mean age of 4.3 years (range 2-6 years) and a mean weight of 368.5 kg (range 350–400 kg) were included in this study. The horses were examined clinically and ultrasonographically to rule out pre-existing tendinitis. The tendons were considered normal if there was no any clinical signs of tendinitis and ultrasonographically,
there was homogeneous echogenicity and normal fiber alignment, and their cross-sectional area did not exceed 1.2 cm$^2$. The SDFT was ultrasonographically evaluated at two centimeter intervals starting four centimeters distal to the accessory carpal bone (cm DACB) and continuing distally to 26 cm DACB. Horses were dewormed and hoof care was performed 2 weeks before the beginning of the project. The animals were confined to individual stalls, receiving water and balanced feeding consisting of concentrate and alpha alpha hay.

**Collagenase model**

Each leg was clipped and degreased with alcohol, and reference marks were made at 2 cm intervals starting four cm DACB, extending to 26 cm DACB with permanent markers. As the marks faded over the course of the study, fresh marks were made over the fading marks, using the accessory carpal bone as the reference point to ensure proper location of the marks. Filter-sterilized bacterial collagenase type I (Type I collagenase: C-0130, Sigma. USA) diluted in sterile water was injected in the center of the SDFT at 14 and 15 cm DACB. Collagenase administration was considered to be time zero of the experiment and was injected after animal sedation with xylazine 0.5 mg/kg by the intravenous route and blockade of the medial and lateral palmar digital nerves in the proximal metacarpal region with 2 ml lidocaine without a vasoconstrictor. The palmar region of the metacarpus of both limbs were prepared by shaving and antisepsis. A 27-gauge 1.25-cm needle was inserted into each tendon via its lateral border with ultrasonographic guidance (free hand technique) to confirm that the tip of the needle was exactly in the centre of the tendon, and 1000 IU (0.1 ml) of collagenase [2000 IU (0.2 ml) total] was injected in each tendon at the sites mentioned above, to create the lesions (fig. 1). Phenylbutazone, as 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 to prevent any discomfort following the procedure. Forelimbs were bandaged for 2 weeks after injection to reduce contamination and control swelling. Following the tendon injections the horses were housed in box stalls for two weeks.

![Ultrasound of the SDFT showing needle position within the tendon](image_url)
Clinical evaluation

Each horse was clinically evaluated on four separate occasions by one of the authors. These examinations were performed on days 0, 1, 3, 5, 7, and 14 after injection. At each time the observer scored each front limb on each horse for: Heat by palpation over the flexor tendons on a 10-point visual analogue scale with 0=no heat and 10=severe heat; Response to palpation over the SDFT on a 0–10 point visual analogue scale with 0=no response and 10=severe reaction; Presence of swelling in the SDFT region, and lameness score according to AAEP guidelines on a 0–5 scale \(^\text{17}\).

Ultrasonographic examination

The tendons were evaluated using Sonosite Micromax Ultrasonographic Machine \textit{with a 10-13 MHz transducer and a 4 cm Foot-print} prior to injection, and at 1, 3, 5, 7, and 14 days after injection. Ultrasonographic images recorded prior to tendon injury served as control images. All ultrasonographic examinations were completed with the horses lightly sedated with xylazine 0.5 mg/kg IV and restrained in stocks. Palmar metacarpal regions were clipped, before the application of a water-soluble coupling gel. Transverse and longitudinal images were obtained every two cm from 4 to 26 cm DACB. All images obtained at resolution frequency and 2.7 cm depth of view. The images were captured digitally and Sigma Scan Software Version 5 was used to measure the: 1) percentage lesion (lesion CSA/tendon CSA \times 100) at the maximum injury zone (MIZ) and 2) the echogenicity score of the SDFT at the MIZ in the cross sectional image and 3) the fiber alignment at the MIZ in the sagittal image. None of the lesions extended proximal to 8 cm DACB and none of them were distal to 20 cm DACB, consequently the data were summed for each of the seven points from 8 to 20 cm DACB to determine the sum percentage lesions. The echogenicity was determined based on a scale of 0 to 3, suggested by Genovese et al and Reef (0: Parallel fiber pattern in 76-100% of fibers, 1: Parallel fiber pattern in 51-75% of fibers, 2: Parallel fiber pattern in 26-50% of fibers, and 3: Parallel fiber pattern in 0-25% of fibers) \(^\text{1,16}\). The evaluation of changes in fiber alignment, based on the linear arrangement of the echoes in the longitudinal images, were scored between 0 and 3 (0: Normal to near normal echogenicity, 1: Mostly echogenic (25-50% loss of echogenicity), 2: 50% anechoic and 50% echogenic, and 3: Mostly to completely anechoic) \(^\text{8,16}\).

Statistical analysis

Statistical analysis was performed using paired-sample t-test to compare two means. A value of P<0.05 was considered as significant.

Results

Clinically, there was notable swelling and heat at each of the injection sites, in the day after injection. The heat lasting for five to six days and swelling remained until the end of the study. The heat and swelling were confined to the palmar aspect of the midmetacarpal region (fig. 2). Pain was elicited on palpation for about 1 week. The horses remained comfortable at a walk and did not have a decreased appetite following the injection of collagenase. Lameness scores, ranging from 2 to 3, were observed in all horses 24 hours after induction of injury, decreasing gradually after this time. There were not any significant differences between the
animals with regards to heat by palpation, response to palpation, swelling, or lameness scores (p > 0.05).
Transverse and sagittal ultrasonographic images showed tendon lesions of variable size, shape and position, from hypo to anechoic, with loss of the linear pattern of fibers, being generally located in the core of the SDFT. One day after collagenase injection, ultrasonic examination revealed a structural defect surrounded by an area of decreased echoic intensity as well as swelling of the SDFT. There was also edema of the peritendinous tissue which enhanced the ability to discern the underlying SDFT by physically separating it from the skin.
The most severe lesions were identified between the 5th and the 7th day after the injection of collagenase. At this moment, the echogenicity ranged from 2 to 3 (Mean 2.6) and the fiber alignment from grades 2 to 3 (Mean 2.5). At the end of the study, diffuse or uniform hypo to anechoic areas characterized the lesions, with echogenicity and fiber alignment between grade 2 and 3 (Mean ES=2.4 and Mean FS= 2.5) (Figs. 3A, 3B).
A collagenase dose of 2000 IU of activity resulted in anechoic cavitations (Grade 3 or 4 lesions) involving 20%-35% of the tendon cross section at MIZ (Fig. 5) and 7-19% in 8 to 20 cm DACB. Ultrasonographically the tendon proximal and distal to the injection site was hypoechoic. The lesion size increased between the 1 and 7 days, and decreased between 7 and 14 days after injection. In ultrasonographic appearance, the echogenicity of the lesion during 1-7 days was decreased which showed increasing in pathologic grade, but during days 7 to 14 it was vice versa. The FS was also decreased during days 1 to 7, but was not changed from days 7 to 14. However, there were not any significant difference in the size, ES, and FS between all the days except between the first day and the other days (P<0.05). The MIZ was at the site of the injection or within 1 to 2 cm distal (figs. 4,5).
There were no significant differences between observations made of each structure in the left and right forelimbs of the same horse (P> 0.05).

Fig. 2: Swelling of the palmar aspect of the midmetacarpal region, 5 days after collagenase injection
Fig. 3A: Transverse images of SDFT show changes overtime

Fig. 3B: Longitudinal images of SDFT show fiber pattern changes overtime
Fig. 4A: The graphs show the changes over time in the percentage lesion (lesion area/tendon area ×100) at the maximum injury zone (MIZ). There were significant differences only between the first day and the other days.

Fig. 4B: The graphs show the changes over time in the fiber score at the maximum injury zone (MIZ). There were significant differences only between the first day and the other days.

Fig. 4C: The graphs show the changes over time in the echogenicity score at the maximum injury zone (MIZ). There were significant differences only between the first day and the other days.
Discussion

Injection guided by ultrasound proved to be an accurate method for confirming needle placement and intratendon injection in all cases. The volume of injected fluid, 0.1 ml, was always seen on injection, and the injected material diffused along the plane of tendon fibers, affecting more than the injection site (Fig. 1).

At the present study all horses showed pronounced lameness 24 hours after collagenase injection. The remission of this clinical sign occurred rapidly, but was not absent on day 14. This finding are close to those described by Silver et al. (1983), Williams et al. (1984), Spurlock et al. (1989), Foland et al. (1992), Keg et al. (1992) and Alves (2001) who noticed lameness few hours after collagenase application, until a period of 60 days on average, in similar model. A significant increase in pain and heat in the tendon were observed in the first day in all horses, noticeable by palpation until day 8. These observations are agree to those described previously by Silver et al. (1983), Williams et al. (1984) and Spurlock et al (1989), who, although verifying an increase in pain and heat immediately after collagenase injection, observed the total remission of these signs in seven to 14 days.

The acute swelling, matrix destruction, and increase in tendon CSA are similar to those seen in naturally occurring tendon injuries. And all horses showed moderate to severe swelling around the site of collagenase injection, increased tendon CSA, and loss of normal fiber pattern in the core lesion on ultrasound examination.

The local swelling was attributed to the formation of hematoma and edema, as part of the inflammatory process, remaining visible in all horses until the end of the study. Reports of clinical observations presented by Keg et al. (1992) and Alves (2001) corroborate these findings. In a research performed by Williams et al. (1984) about the pathogenesis of experimental tendinitis, local swelling was still present 14 months after induction with collagenase.

Tendon core lesions initially increased in size, in a pattern similar to that seen in clinical cases of tendinitis. In clinical tendinitis, enzyme degradation by endogenous collagenase and proteases are responsible for lesion enlargement beyond the initial mechanical injury to
collagen fibers. When using collagenase to induce lesions, the enzyme takes some time to create the defect and then for the lesion to stabilize. In order to avoid further enzymatic digestion causing changes in the size of the tendon and lesion we suggested that the treatment should not be initiated until the lesions were stable, which occurred some days after injection. In this study, all lesions increased in size from 1 to 7 days following collagenase injection, and finally began to decrease between 7 and 14 days (Fig. 4).

In the most severe stages the echogenicity and the fiber alignment ranged from grade 2 to 3. According to preliminary studies, the anechoic and hypoechoic lesion observed at this moment correspond to areas of hemorrhage, edema and fibril disruption. The collagenase-induced model is superior to the cytokine-induced model, because it produces a degenerative rather than an inflammatory model of tendinitis. Additionally, the lack of histologic changes in the tendon matrix of cytokine-induced tendinitis are not consistent with clinical lesions, particularly those void lesions for which mitogenic and matrix enhancing treatment modalities such as growth factor, and stem cell therapies would be targeted.

Backman et al. modified models created by Obolenskaja and Goljanitski and Raise by using electrical stimulation and passive range of motion to produce an eccentric load on the tendon, thus causing tendinitis. Histologically, changes in tendon structure was seen in this model within 1 month; however, by current standards for evaluating tendon disorders, it appeared to be model of tendinosis and was not similar to the lesion representing the majority of cases seen in clinical practice.

This study utilized both forelimbs so that paired comparisons could be used to eliminate intra-horse variation. It is possible that if the lameness in one limb was severe it could affect the contra-lateral limb by changing the loading between limbs. However, we did not see a significant difference in the heat by palpation, response to palpation, swelling or lameness between limbs.

Injection volumes of 0.3 ml used by other investigators resulted in excessive leakage of collagenase from within the paratenon into the subcutaneous space. In this study, total dose of 2000 IU per tendon divided into 2 volumes of 0.1 ml at each injection site was used. We were able to consistently reproduce an adequate tendon lesion based on ultrasonographic examination by using a smaller injection volume and dose of collagenase per tendon.

In conclusion, it was seen that 2000 IU collagenase injection in the SDFT of horses causes the same tendinitis in both left and right forelimbs and also in different horses. The characteristic of this tendinitis is closely resembles the naturally developing condition in clinical and ultrasonographic aspects. Therefore, tendon lesions using collagenase injection represent a model for tendon injury in clinical and research trials.

References


چکیده:
یافته هایی بالینی و اولتراسونوگرافی تاندونتی حاد ایجاد شده
متعاقب تزریق کالزانز در اسب

دکتر محمد مهدی دهقان، دکتر حسین کاظمی مهرچری، دکتر مجید مسعودی فردا، دکتر محمد رضا یافضای اسلامی نژاد، دکتر داوود فرشی، دکتر علی‌رضا وجه.

گروه علمی درمانگاه، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

۱)
پژوهشگاه رویان، قسمت سلوئیه بنیادی ویژنیک، پژوهشگاه رویان، تهران، ایران

هدف: بررسی تغییرات بالینی و اولتراسونوگرافی تاندونتی حاد ایجاد شده در تاندونت خم کننده سطحی بندهای انگشت در اسب متعاقب تزریق کالزانز

طرح: مطالعه تجارب

جریان‌ها: ۵ راس اسب سالم بالغ

روش کار: در هر یک از اسب‌ها تحت هدایت سونوگرافی در مرکز تاندونتی مبتنی بر نتایج سونوگرافی در مقطع عرضی تاندونت در اثر تزریق کالزانز، دو اندازه‌گیری سونوگرافی انجام گردید.

نتایج: تزریق کالزانز باعث ایجاد جراحت مکزیکی فاقد اکوزنیتیشین شد که در حدود ۲۰ الی ۲۵ درصد سطح مقطع عرضی تاندون را در بر گرفته بود. همگی این خاصیت معنی داری از نظر آنالیزی معنی‌داری داشتند که میزان اکوزنیتیشین و توان معکوسی رشته‌های طولی بین روزهای مختلف به استثنای روز اول با بقیه روزها وجود نداشت. در تمامی ارزیابی‌های انجام شده هیچ گونه اختلاف معنی‌داری بین اندازه گرفته راست چپ اسکان سه‌دی نشود.

نتیجه گیری: با توجه به نتایج سونوگرافی تاکید شد که ایجاد شده توسط کالزانز شامل قطعه درمانگاه برای توانایی روش که به طور طبیعی در تاندونتی این اتفاق می‌دهد و باعث ایجاد تاندونت نسبتا مشابهی در خود ادامه قدمای اسب‌ها و همچنین دیگر جراحی ممکن در مرکز تاندونتی برقرار می‌خشد.

کلید واژگان: تاندونت، خم کننده سطحی انگشت، تاندونتی، اولتراسونوگرافی.