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Mesenchymal stem cell treatment of suspensory ligament branch desmitis: post mortem findings in a 10 year old Russian Warmblood gelding - a case report

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Summary

Objective of the study was to compare the gross and histological appearance of one lateral suspensory ligament branch treated using autologous mesenchymal stem cells with one untreated lateral suspensory ligament branch in the same horse. Case records of a horse previously treated for lateral suspensory ligament branch desmitis using autologous mesenchymal stem cells were reviewed; the suspensory ligaments were dissected and the gross and histological findings recorded. Ultrasonographically, grossly, and histologically the treated suspensory ligament branch showed evidence of improved healing and formation of ligament-like tissue compared to the untreated suspensory branch in the contralateral limb. The implantation of cultured autologous mesenchymal stem cells into core suspensory branch lesions resulted in the formation of a matrix organisationally similar to normal suspensory ligament.

Keywords: Suspensory ligament; interosseous, stem cell; post mortem; histopathology

Mesenchymale Stammzellbehandlung einer Fesselträgerentzündung: Pathoanatomische Befunde bei einem 10-jährigen russischen Warmblutwallach

Ziel der Studie war der Vergleich anatomisch pathologischer und histologischer Befunde eines lateralen Fesselträgerastes, der mit mesenchymalen Stammzellen behandelt wurde, mit dem jeweils unbehandelten lateralen Ast der kontralateralen Gliedmaße. Es bestanden Erfahrungen von zuvor auf gleiche Weise behandelten Pferden. Die Fesselträgeräste wurden präpariert und die Befunde aufgezeichnet. Verglichen mit dem unbehandelten Fesselträgerast der kontralateralen Gliedmaße zeigt der behandelte Fesselträgerast sonographisch, pathoanatomisch und histologisch Anzeichen verbesserter Heilung und Bildung sehnenähnlichen Gewebes. Die Implantation kultivierter mesenchymaler Stammzellen in Kernläsionen entzündeter Fesselträgeräste führt zur Bildung einer Gewebematrix, die gleich der von gesundem Sehnengewebe ist.

Schlüsselwörter: Fesselträger, Interosseus, Stammzellen, mesenchymal, Pathologie, Histopathologie

Introduction

Injuries to tendons and ligaments are significant causes of lameness and financial losses in the equine industry. They account for nearly a third of all equine injuries that occur during racing, with a reported prevalence of 8–43% (*Dowling* et al. 2000, *Genovese* 1993, *Goodship* 1993, *Peloso* et al. 1994). These tissues heal extremely slowly and the repaired tissue is inferior in mechanical properties as compared to the original tissue, predisposing to poor running efficiency and recurrence and repeated injury in up to 80% of affected horses (*Bramlage* and *Hogan 1996*, *Madison* 1995, *Sawdon* et al. 1996, *Webbon* 1973). Tendon and ligament injuries may occur as result of acute overloading of the tendon or ligament or, more commonly, secondary to degenerative changes provoked by ageing and exercise (*Birch* et al. 1998, *Birch* et al. 1999, *Kannus* and *Jozsa* 1991).

Many different therapeutic methods have been used to promote ligament healing; results, however, are predominantly anecdotal. Over the last five years novel biological approaches, including the implantation of autologous suspensions of mesenchymal stem cells, have been used to attempt to facilitate suspensory ligament healing. This report describes the treatment of a case of bilateral suspensory ligament branch desmitis in a 10 year old Russian Warmblood gelding used for dressage and the post mortem findings following elective euthanasia.

Case Details

History

A 10 year old Russian Warmblood gelding presented with signs of a strain injury to the lateral branch of the right forelimb suspensory ligament. Following 4 months rest the lesion had failed to resolve prompting referral to the Sefton Equine Referral Hospital at the Royal Veterinary College, University of London.

Clinical Evaluation

On presentation the horse was sound at the walk and trot in a straight line. On palpation, there was swelling of the lateral branch of the suspensory ligament bilaterally but with no pain on palpation. Ultrasonographic examination (figures 1 and 2) revealed a heterogeneous lateral suspensory branch on the

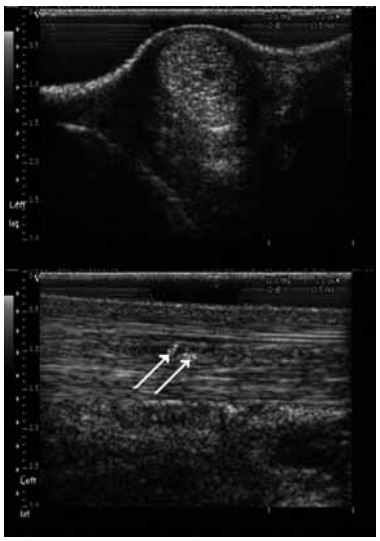

Fig 1 Left forelimb lateral suspensory ligament branch: transverse and longtitudinal ultrasonographs showing a small focal hypoechoic lesion. In the longitudinal view 2-3 small hyperechoic areas (arrowed) similar to those seen with mineralisation or ossification are visible within the hypoechoic lesion.
Lateraler Ast des Fesselträgers li vo.: tsonographischer Quer- und Längsschnitt mit kleiner fokaler hypoechogener Läsion. Im Längsschnitt innerhalb der echogenen Läsion 2-3 kleine hyperechogene Bereiche wie sie bei Mineralisation oder Ossifikation zu sehen sind.

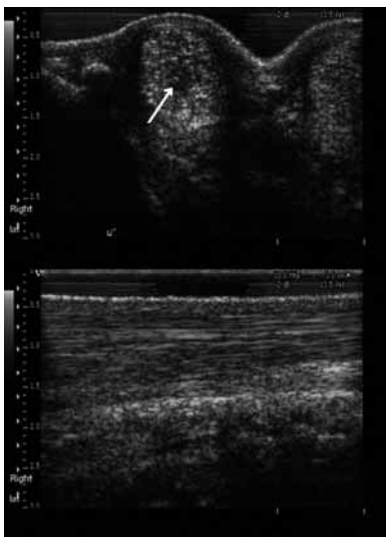

Fig 2 Right forelimb lateral suspensory ligament branch: transverse and longtitudinal ultrasonographs showing a large central core lesion (arrowed).
Lateraler Ast des Fesselträgers vo re: Sonographischer Quer- und Längsschnitt mit großer zentraler Läsion (Pfeil).

left forelimb which contained a small focal hypoechoic lesion. In the longitudinal view, this lesion was also visible and was found to contain 2-3 small pin-point hyperechoic areas similar to that seen with mineralisation or ossification although no acoustic shadowing was visible. On the right forelimb, there was a large central core lesion within the lateral branch. The medial suspensory ligament branches of both forelimbs were heterogeneous but did not contain any hypoechoic areas.

Treatment

The lesion in the lateral branch of the right forelimb suspensory ligament was deemed appropriate for treatment using cultured autologous mesenchymal stem cells. In view of the possible presence of mineralisation/ossification in the left forelimb lateral suspensory branch, it was not thought to be prudent to implant cells into this branch for fear of inducing more ossification.

Autologous mesenchymal stem cell collection, culture, and expansion

Bone marrow was collected from the sternum of the horse under standing sedation and local anaesthesia and transported chilled to the laboratory. The mesenchymal stem cells were separated cultured and expanded using standard techniques before being re-suspended in bone marrow supernatant and returned, chilled, 17 days post collection to the Sefton Equine Referral Hospital for implantation.

Autologous mesenchymal stem cell implantation

Implantation was carried out under standing sedation and local anaesthesia. $2x10^6$ cells suspended in 1ml of bone marrow supernatant were implanted into the lateral branch of the right forelimb suspensory ligament. At implantation they were found to spread to fill the lesion completely (see figure 3).

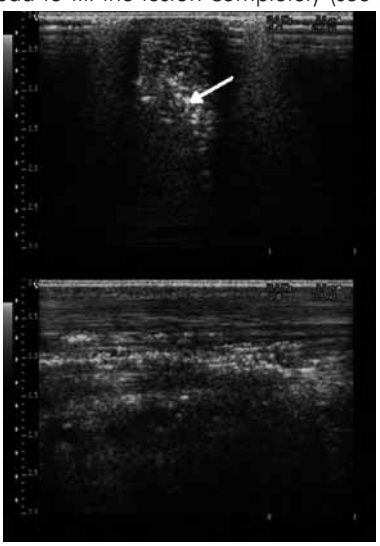

Fig 3 Right forelimb lateral suspensory ligament branch transverse and longtitudinal ultrasonographs immediately post-implantation with suspension of mesenchymal stem cells. Minute air bubbles within the suspension show up as hyperechoic areas (arrowed) within the ultrasonographs and demonstrate that the suspension has spread to fill the lesion completely.
Lateraler Ast des Fesselträgers re vo: Sonographischer Quer- und Längsschnitt unmittelbar nach Implantation einer Suspension mesenchymaler Stammzellen. Winzige Luftblasen in der Suspension stellen sich im Sonogramm als hyperechoge Bereiche dar (Pfeil) und zeigen, dass die Suspension die Läsion vollständig ausfüllt.

Clinical Course

At re-examination one month after implantation the horse was sound at the walk and trot. There was still swelling of both lateral branches of the suspensory ligament but with no pain on palpation. There was no oedema present and the branches felt supple. On ultrasonographic examination, there was no change to the ultrasonographic appearance of the lateral suspensory branch of the left forelimb. However, the hypoechoic lesion within the right forelimb lateral suspensory branch had filled in (see figures 4 and 5). Nineteen months post implantation, after completing rehabilitation and returning to full work, the horse was euthanased for unrelated reasons.

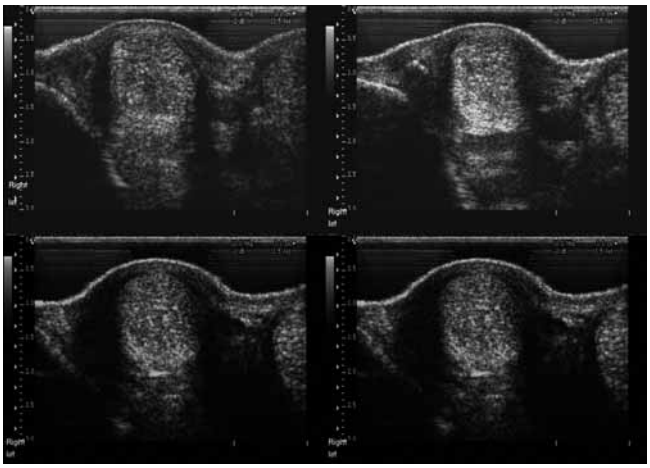

Fig 4 Right forelimb lateral suspensory ligament branch: transverse ultrasonographs prior to implantation (above) and one month after implantation (below) showing filling in / resolution of the lesion-
Lateraler Ast des Fesselträgers vo re: Das transversale Sonogramm vor der Implantation (oben) und einen Monat nach Implantation (unten) zeigt die Füllung und Auflösung der Läsion.

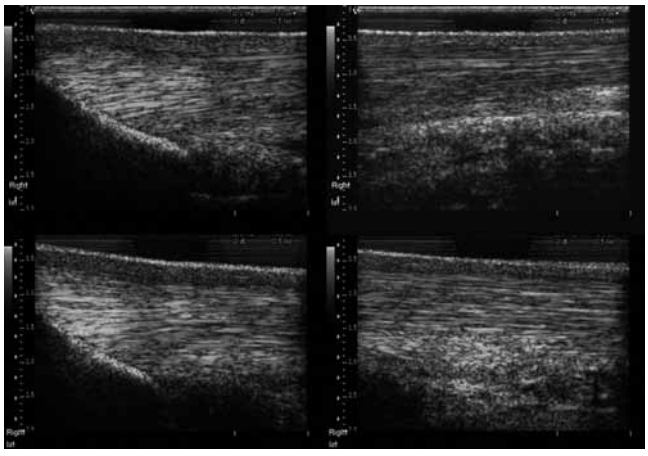

Fig 5 Right forelimb lateral suspensory ligament branch: longtitudinal ultrasonographs prior to implantation (above) and one month after implantation (below) showing filling in / resolution of the lesion
Lateraler Fesselträgerast vo re: Der sonographische Längsschnitt vor der Implantation (oben) und einen Monat danach (unten) zeigt die Füllung und Auflösung der Läsion.

Post mortem examination

The two affected ligaments (lateral suspensory branches from right and left forelimbs) were obtained and subjected to post mortem examination. Figures 6 and 7 show the gross appearance of the ligament branches and demonstrates better healing of the treated right forelimb (Figure 7) compared to the left (Figure 6). Histological examination confirmed this gross appearance. In the untreated left forelimb lateral suspensory branch the fascicles were poorly defined with increased amounts of branching interfascicular tissue (endotenon) containing fibroblastic cells and dense multifocal infiltrates of plasma cells, lymphocytes with smaller numbers of eosinophils (Figure 5). At margins of the interfasicular tissue, small areas were noted to be encroaching on and replacing the fascicular matrix (Figure 5). Within the fascicles, fibroblastic cells predominantly had plump nuclei (type 2 cells; *Smith* and *Webbon 1996*: The physiology of normal tendon and ligament) and were increased in number particularly immediately adjacent to inflamed areas. No evidence of mineralization or ossification was noted. contains evidence of persistent inflammation with multifocal infiltrates of plasma cells, lymphocytes and eosinophils. In contrast the right forelimb lateral suspensory branch which had been treated with stem cells contained better defined fasciclesshowed good longitudinal orientation of fascicles, with slightly increased amounts of interfascicular tissue containing only occasional scattered lymphoid cells (Figure 6). The and a collagenous matrix of fascicles which exhibited a clearly defined crimp pattern under polarized light that was characteristic of ligament rather than scar tissue. Only occasional scattered plasma cells and lymphocytes were noted.

Fig 6 Gross appearance of the untreated left suspensory ligament branches (lateral branch on left) showing gross evidence of scar tissue (arrowed) at site of lesion.
Der anatomische Querschnitt durch den unbehandelten Fesselträger (lateraler Ast links) zeigt Narbengewebe (Pfeil) im Bereich der Läsion.

Fig 7 Gross appearance of the treated right suspensory ligament branches (lateral branch on left) showing no gross evidence of scar tissue at site of lesion.
Der anatomische Querschnitt durch den behandelten Fesselträger (lateraler Ast links) zeigt im Bereich der Läsion kein Narbengewebe.

Thus in spite of the most severely aeffected branch having been treated with stem cells, the resulting ligament branch

had an almost normal appearance while the 'control' branch on the contralateral limb had persistent inflammationexpansion of interfascicular tissue containing persistent inflammatory cell infiltrates, with resultant poor organisation of fascicles and poor quality matrix organisation. Large numbers of "active" desmocytes as indicated by their plump nuclei suggested persistent cellular reaction within the fascicles themselves.

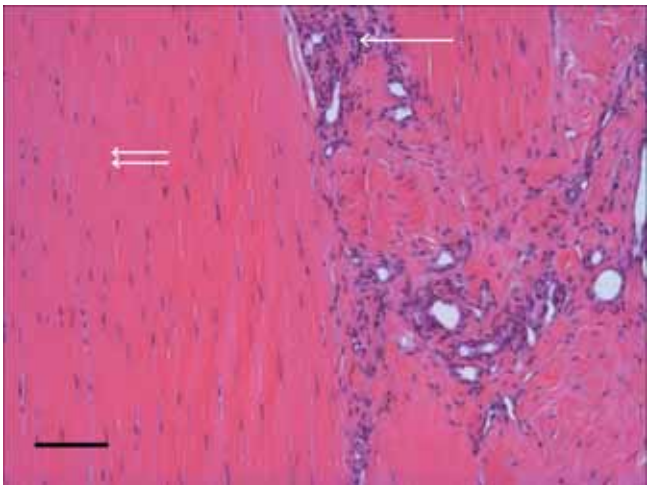

Fig 8 Treated right forelimb, lateral suspensory branch of the suspensory ligament. There is slight expansion and branching of interfascicular tissue on the right (single arrow) , with few lymphoid cells. Fascicular tissue on the left (double arrow) has a relatively normal appearance. Haematoxylin and eosin, Bar = 50 µm.
Lateraler Fesselträgerast einer rechten Vordergliedmaße: auf der rechten Seite leichte Ausdehnung und Verästelung des interfaszikulären Gewebe mit wenigen lymphoiden Zellen (Pfeil). Das faszikuläre Gewebe links erscheint relativ normal (Doppelpfeil).

Discussion

Desmitis of the lateral branch of the suspensory ligament (superior sesamoidean ligament or interosseous muscle) is a relatively common injury, occurring in all types of horses (*Dyson* 1996). The clinical signs, including localised heat and swelling, depend on the degree of damage and the chronicity of the lesions. Swelling is often due to enlargement of the branch per se and peri-ligamentous oedema or peri-ligamentous fibrous material and lameness is variable and may be absent Diagnosis is based on clinical signs and ultrasonographic examination. The prognosis for minor lesions is good for a return to athletic function following conservative management but suspensory ligament lesions vary considerably with their ability to resolve ultrasonographically. The resolution of clinical and ultrasonographic signs can be very slow and can persist for over 18 months. Some horses, however, do successfully resume work despite the persistence of an ultrasonographically detectable lesion although there is a significant risk of re-injury in these cases.

A variety of local, medical and cellular therapies are used to treat suspensory branch desmitides with the goal of controlling acute inflammation and optimising ligament healing; there are, however, limited studies assessing the efficacy of these therapeutic options. Most therapies have the goal of healing the injured ligament by creating scar tissue whereas some newer approaches, including the use of mesenchymal stem cells, aim to repair the ligament with regenerated tendon-like or ligament-like tissue.

The transplantation of mesenchymal stem cells (MSCs) into injured skeletal tissues can promote healing (*Caplan* et al. 1993, *Ferrari* et al. 1998, *Young* et al. 1998, *Awad* et al. 1999, *Herthel* 2001, *Smith* et al. 2003) and the use of autologous cells has a potential added benefit that they do not incite an immune response from the host (*Hildebrand* et al. 2002). *Young* et al. (1998) used a rabbit model to demonstrate Achilles tendon injury repair by MSCs. MSCs were seeded onto a biodegradable scaffold which was implanted into an excised section in the centre of the tendon. *Awad* et al. (1999) used a similar rabbit model but investigated injury to the patellar tendon. The results from the *Awad* et al (1999) study indicated that the MSC mediated repair improved histological appearance (including more cells and mature collagen fibres) and hence it was postulated that biomechanical properties were improved. *Hankemeier* et al. (2005) demonstrated that the implantation of mesenchymal stem cells induced a better quality and stronger repair in rat patella tendon. Each of these models used laceration injuries. Equine digital flexor tendon or suspensory ligament branch injuries, however, have different aetiopathogeneses to laceration and provide a central core lesion, which can naturally retain implanted MSCs. *Smith* et al. (2003) demonstrated that it was feasible to implant autologous MSCs into equine tendons with over-strain injuries, and that post-implantation ultrasonographic evaluation revealed no disruption to the healing tendon by the implantation, and showed evidence of rapid infilling of the defect.

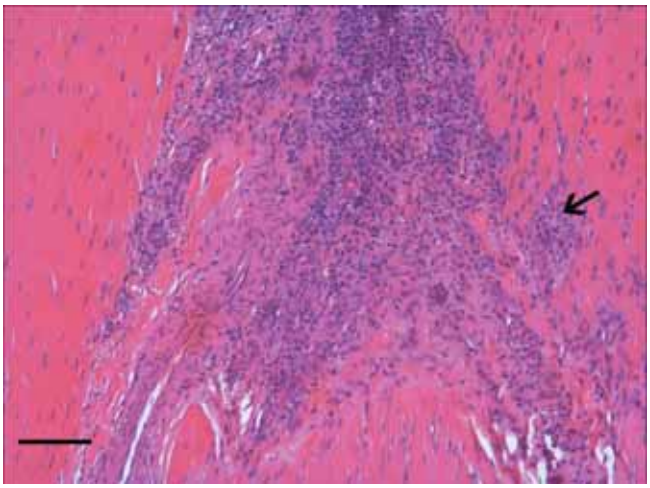

Fig 9 Untreated left forelimb, lateral suspensory branch of the suspensory ligament. Interfasicular tissue is markedly expanded by fibroblastic cells and inflammatory cells. Small areas of reactive interfasicular tissue are replacing the fascicular collagenous matrix (arrow). Haematoxylin and eosin, Bar = 50 µm.
Lateraler Fesselträgerast einer unbehandelten linken Vordergliedmaße: Ausgeprägte Erweiterung des interfaszikulären Gewebes durch Fibroblasten und Entzündungszellen. Kleine Bereiche reaktiven interfaszikulären Gewebes ersetzen die faszikuläre Kollagenmatrix (Pfeil).

Since 2003 some 400 horses have had cultured autologous mesenchymal stem cells implanted in tendon and ligament lesions1. The post mortem assessment of treated lesions may indicate whether treated lesions have healed with scar tissue or whether they show evidence of tendon-like or ligament-like tissue regeneration. In this single case of bilateral suspensory ligament branch desmitis there appears ultrasonographically, grossly, and histologically to have been evidence of improved

healing and the formation of ligament-like tissue in the suspensory ligament branch implanted with autologous mesenchymal stem cells compared to the untreated suspensory branch in the contralateral limb.

It is hypothesised that the formation of a more-ligament-like tissue post injury, rather than scar tissue, will facilitate improved clinical outcomes. The observation that implantation of cultured autologous mesenchymal stem cells into core suspensory branch lesions can result in evidence of the formation of a tissue organisationally similar to ligament provides preliminary support for the clinical use of stem cells in the treatment of equine suspensory ligament injuries.

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