How to Select Cases and Use Autologous Conditioned Serum to Treat Proximal Suspensory Desmitis

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1. Introduction

The suspensory ligament (SL) can be divided into 3 regions subject to injury: the proximal region, the body, and the medial and lateral branches. In this presentation, only treatment of the proximal region is discussed.

Proximal suspensory desmitis (PSD) is a common injury of athletic horses causing pain and lameness. It can occur unilaterally or bilaterally and can affect the hind limbs, the fore limbs, or both. Clinical signs displayed by horses with PSD have been well documented and pain causing lameness can be localized to the proximal aspect of the suspensory ligament (PSL) by using a variety of local and regional anesthetic techniques. The PSL and adjacent bone is usually imaged by using ultrasonography and radiography. Nuclear scintigraphic examination of horses affected with PSD can be helpful, but magnetic resonance imaging (MRI) is the most defining method by which the ligament and surrounding structures can be imaged.

Most horses lame because of acute PSD of the fore limb, respond favorably to stall confinement and controlled walking exercise for 3 months, but for the 3-year-old Western performance horse, 3 months of confinement may end the horse’s show career, thereby significantly reducing the horse’s worth. The prognosis for return to soundness for horses lame because of PSD of one or both hind limbs is poor when affected horses are treated by confinement and incremental increase in exercise.

Besides stall rest, a myriad of adjunctive therapies have been recommended for horses lame because of PSD, including systemic and topical administration of an NSAID; periligamentous injection of a corticosteroid, hyaluronan, polysulfated glycosaminoglycans, extract of the pitcher plant, or a combination of these drugs; topical application of dimethyl sulfoxide (DMSO), with or without a corticosteroid or other drugs; extracorporeal shockwave therapy; and intralesional injection of 2% iodine in almond oil, extracellular matrix, mesenchymal stem cells, or platelet rich plasma.

NOTES
Surgical treatments of horses for PSD include splitting the ligament, lateral palmar/plantar neurectomy, neurectomy of the deep branch of the lateral palmar or plantar nerve, ulnar or tibial neurectomy, fasciotomy of the fascia overlying the ligament, and combinations of these surgical treatments.\textsuperscript{1} In this presentation, we describe periligamentous injection of autologous conditioned serum (ACS)\textsuperscript{c} for the treatment of horses lame because of PSD. Autologous conditioned serum is made by collecting the patient’s blood in a proprietary syringe containing glass beads soaked in chromium sulfate. The blood is incubated in the syringe for approximately 24 hours (18–26 h) and then centrifuged, filtered, and divided into dose-sized aliquots, which can be injected immediately or frozen for future use. The processing procedure is designed to specifically harvest anti-inflammatory cytokines,\textsuperscript{5} but anabolic cytokines and morphogenic proteins may be harvested, as well.\textsuperscript{6}

Commercial veterinary systems are available for the horse for patient-side production of ACS.\textsuperscript{c,d} Autologous, conditioned serum has been used in the horse for several years to modulate synovitis and has become an accepted mode of equine joint therapy.\textsuperscript{1,7–13} Autologous conditioned serum has also been investigated for modulation of the post-breeding inflammatory response in the mare’s uterus.\textsuperscript{14} Autologous, conditioned serum is used to treat humans for a variety of synovial and non-synovial orthopedic maladies,\textsuperscript{15,16} and experimental studies using rats have demonstrated improved tendon healing after ACS therapy.\textsuperscript{17,18}

The purpose of this report is to describe how we select horses lame because of PSD for treatment with ACS using the irap\textsuperscript{a} system. Our criteria for selection of horses for this treatment are based on our experience in treating 271 horses lame because of PSD.

2. Materials and Methods

Case Selection

Horses in this study were those determined to be lame solely because of PSD and that were treated with ACS by one of the authors\textsuperscript{a} between September 2004 and September 2013. All horses included in this study were lame at the time of treatment, showed signs of pain during palpation of the PSL, and had the pain causing lameness localized to the PSL by performing various techniques of diagnostic analgesia using 2\% mepivacaine hydrochloride (HCl). Only horses with a minimum follow-up time of 3 months, after the initiation of treatment were included in the study.

Fore Limb Blocks

Pain in the distal portion of the fore limb(s) was excluded as a cause of lameness by observing no improvement in lameness after administering a low four-point nerve block or after administering an abaxial sesamoid nerve block in combination with intrasynovial analgesia of the fetlock joint and, occasionally, intrasynovial analgesia of the digital flexor tendon sheath. Pain was isolated to the PSL by observing substantial improvement in lameness after desensitizing the PSL by anesthetizing the lateral palmar nerve at the level of the accessory carpal bone.

Hind Limb Blocks

Pain in the distal portion of the hind limb(s) was excluded as a cause of lameness by observing no improvement in lameness after administering a low four-point nerve block or after administering an abaxial sesamoid nerve block in combination with intrasynovial analgesia of the fetlock and, occasionally, intrasynovial analgesia of the digital flexor tendon sheath. Pain was isolated to the PSL by observing substantial improvement in lameness after desensitizing the PSL by anesthetizing the tibial nerve or by locally infiltrating the PSL with local anesthetic solution. For some horses, pain originating from the distal joints of the hock was excluded as a cause of lameness by observing little or no improvement in lameness after intrasynovial analgesia of the distal intertarsal and tarsometatarsal joints during lameness exam performed after the effects of local or regional analgesia had dissipated.

Imaging

The metacarpus (Mc III) or metatarsus (Mt III) of all horses were radiographically examined to exclude horses with substantial bone disease at the proximal aspect of Mc/Mt III, and all were examined sonographically in the palmar/plantar metacarpal/metatarsal region. Horses with a stress fracture of the proximal palmar/plantar Mc/Mt III were excluded from the study. Horses with substantial tissue disruption of the PSL or damage to other structures in the area such as the inferior check ligament were excluded. Horses that had enlargement of a PSL or edema of a PSL, as compared to the contralateral limb, were included (Fig. 1). For horses with bilateral PSD, assessment of enlargement and edema of the PSL was based on published guidelines.\textsuperscript{4}

ACS Harvest and Processing

Whole blood harvested from each horse was incubated in the proprietary syringe in which it was collected and processed according to the manufacturer’s guidelines. Some aliquots were used immediately after processing whereas others were stored in a $-20^\circ$C freezer for future use. Frozen aliquots were thawed in a water bath immediately prior to use, and the ACS was filtered through a 0.2 \(\mu\)m disk filter\textsuperscript{f} prior to injection.
Treatment

Injection Technique

All injection sites were prepared for aseptic injection, and the injector wore sterile gloves. The horse was sedated, and the affected fore limb or hind limb was held in flexion. A 20-gauge, 1.5 in needle, attached to a 6 mL Luer-lock syringe containing 5 mL of ACS, was inserted through the skin approximately 4.5 cm distal to the proximal end of the head of the lateral splint-bone. The needle was directed proximomedially and inserted deep to the PSL until its tip engaged the palmar/plantar cortex of Mc/Mt III axial to the axial border of the lateral splint bone in the space between Mc/Mt III and the PSL. The ACS was injected with little or no resistance if the needle was properly placed. If resistance to injection was encountered, the bevel of the needle was assumed to be within the periosteum of the Mc/Mt III, and the needle was withdrawn until the ACS could be injected with ease.

All horses received a single dose (1 mg/kg) of flunixin meglumine intravenously after injection of ACS. The limbs were not bandaged after injection.

Fig. 1. These sonograms of forelimbs (1A and 1B) and hindlimbs (1C and 1D) are examples of sonograms of horses described in this report. In these examples, the left ligament is from the limb exhibiting lameness, and the right is from the sound contralateral limb. Sonograms of the diseased left ligaments demonstrate increased cross-sectional area (swelling), poor definitions of margins, decreased echogenicity (edema), and lack of striations (i.e., loss of normal fiber pattern), whereas the right ligaments have a more normal appearance. A, Cross-sectional image of an injured (left) and non-injured (right) forelimb PSL. Note the poorly defined central hypoechoic area (HEA) denoted by arrow. B, Longitudinal image of the same forelimb PSL. Note: 1. Enlargement of PSL in left as compared to right. 2. Poor definition of dorsal and palmar margins in left as compared to right. 3. Loss of linear striation. C, Cross-sectional image of an injured (left) and non-injured (right) hindlimb PSL. Note: 1. Enlargement of PSL in left as compared to right. 2. Poor definition of peripheral margins in left as compared to right. D, Longitudinal image of the same hindlimb PSL. Note enlargement of PSL in left as compared to right.
Rest, Follow-Up Examination, and Retreatment Protocol

All horses were discharged to the owner/trainers care immediately after the injection was completed. The owner or trainer was instructed to confine the horse to a stall and to walk the horse in hand for 20 minutes daily for 14 days. The horses were reexamined for lameness at 14 days, and degree of the lameness was recorded. The limb(s) affected with PSD were re-injected with ACS in similar fashion, the horse was discharged, and the owner or trainer was instructed to confine the horse to a stall and to walk the horse in hand for 20 minutes daily for another 14 days. At 28 days after the initial injection, the horse was reexamined for lameness, and structures palmar/plantar to the Mc/Mt III were examined ultrasonographically. The horse was then administered a third treatment with ACS. If the horse was judged to be sound during examination and sonographic quality of the PSL was within normal limits or if the edema and/or cross-sectional area of the PSL were reduced, the owner or trainer was instructed to continue confining the horse to a stall but to walk the horse in hand for 20 minutes daily for 5 days and then, on the sixth day, to ride the horse using a regime of gradually increasing, controlled exercise. The owner or trainer of those horses that did not meet the criteria to enter a controlled, ridden exercise program was instructed to continue to confine the horse and to walk it in hand until the horse became sound and had no sonographic abnormalities, or an alternate treatment plan was instituted.

Rehabilitation Exercise Program

During the first week of controlled, ridden exercise, the horse was walked daily for 15 minutes and then trotted for 5 minutes. For each subsequent week, the daily trotting time was increased by 5 minutes until the horse was able to trot without lameness for 15 minutes for 14 consecutive days (2 weeks). Beginning the fifth week, if the horse was not lame, loping/cantering was added to the exercise regimen. The time of loping/cantering initially was 2 1/2 minutes daily, and increased weekly by increments of 2 1/2 minutes. After the horse was able to walk for 15 minutes, trot for 15 minutes, and lope for 15 minutes daily for 2 weeks without lameness, the horse was reintroduced to its normal training regimen at the same level of training it had been receiving prior to injury. The treatment protocol was considered successful if the horse entered controlled, ridden exercise at approximately 33 days from initial injection, maintained soundness without deviation from the described protocol of controlled, ridden exercise, entered full training by at least 110 days, and trained at least 1 month without recurrence of lameness.

3. Results

Two hundred seventy-one horses met the criteria required for inclusion in the study, and of these, 47% (127/271) were lame because of PSD of one or both fore limbs, and 53% (144/271) were lame because of PSD of one or both hind limbs. A total of 352 limbs were injected with ACS. No horse suffered an adverse reaction to any treatment with ACS.

Horses Treated for PSD of One or Both Fore Limbs (127/271)

Of horses lame because of PSD of one or both fore limbs, 83% (105/127) were affected unilaterally, and 17% (22/127) were affected bilaterally. Failure of a horse affected bilaterally to return to soundness in either limb was considered a failure of treatment.

Horses Treated for PSD of One Fore Limb (105/127/271)

Of the horses treated for PSD of one fore limb (105), 85% (89/105) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Horses Treated for PSD of Both Fore Limbs (22/127/271)

Of the horses treated for PSD of both fore limbs (22), 82% (18/22) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Horses Treated for PSD of One or Both Hind Limbs (144/271 Total)

Of horses lame because of PSD of one or both hind limbs, 59% (85/144) were affected unilaterally, and 41% (59/144) were affected bilaterally. Failure of a horse affected bilaterally to return to soundness in either limb was considered a failure of treatment.

Horses Treated for PSD of One Hind Limb (85/144/271)

Of the horses treated for PSD of one hind limb (85), 78% (66/85) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Horses Treated for PSD of Both Hind Limbs (59/144/271 Total)

Of the horses treated for PSD of both hind limbs (59), 71% (42/59) were judged to have become sound, able to complete the exercise schedule prescribed, and to have maintained soundness for at least 30 days after reentering full training.

Confine ment coupled with controlled exercise was continued for many of the horses for which treatment was considered to have failed (i.e., those horses unable to sustain the scheduled rehabilitation and train without lameness for at least month), and although many of these horses eventually became sound and were trained successfully, we have not determined the percentage that did.

4. Discussion

Proximal suspensory desmitis is a common cause of lameness of performance horses of many disciplines...
and other categories of horses as well. In the practice of one of the authors, PSD is the number one cause of fore limb lameness of young, Western performance horses that precludes a horse from competing in a major event for which it has trained and ranks in the top 3 causes of fore limb lameness overall. In the same practice, PSD is also one of the 3 most common causes of lameness in the hind limb of the Western performance horse.

Treatment of horses for PSD typically includes a long, costly period of confinement and inactivity (i.e., 3–12 months) and is often the cause of missing a major event for which they were bought and trained. When a young, Western performance horse misses a major event such as the discipline’s major futurity, the worth of the horse is reduced. Horses with PSD of one or both hind limbs have a guarded prognosis for return to full athletic function at all.

Treatment of horses for acute or chronic PSD has encompassed a vast array of therapies, perhaps because no single therapy is effective for most horses affected with PSD. Almost every therapy used to combat pain and inflammation or stimulate healing has been used to treat horses for PSD. The authors believe that although some traditional therapies, such as regional injections of a corticosteroid, initially reduce pain, swelling, and inflammation, they inhibit healing by slowing metabolism, growth, and replication of fibroblasts important in repair of the injured ligament. Some therapies, such as internal blisters of 2% iodine in almond oil or intralesional injection of extracellular matrix may lead to production of non-elastic scar tissue within or around the PSL. Other therapies, such as extracorporeal shock wave therapy, may only reduce ligamentous pain, rather than stimulate healing, thereby potentially perpetuating damage to the ligament when the horse is returned to training.

Our goal for using ACS to treat horses lame because of PSD was to not only rapidly reduce pain, and therefore lameness, by reducing inflammation in the ligament but also to augment healing to return the ligament to its original form and function. Treatment with ACS shortened the time of lay-up of horses lame because of PSD as compared to other published reports, thereby allowing many horses to compete in events they otherwise would have missed, thus preserving the horse’s worth and reducing expense to the owner. Numerous cases that the authors considered failures for this report continued to have further treatments of ACS and controlled exercise and many of them too eventually reentered training.

Autologous conditioned serum has been administered most commonly intrasynovially to reduce inflammation of synovial structures, but this study and others have shown that ACS can be used effectively to modulate the repair phase of non-synovial structures. Meier et al theorized that cytokines, such as interleukin-1 (IL-1), played an important role in degenerative musculoskeletal diseases and originally attributed the therapeutic qualities of ACS to the production of anti-inflammatory cytokines in the serum, particularly interleukin-1 receptor antagonist protein (IL-1ra). Hraha et al determined that other cytokines besides IL-1ra, such as interleukin-10 (IL-10), insulin-like growth factor-1 (IGF-1), transforming growth factor-beta (TGF-β), tumor necrosis factor-alpha (TNF-α), and interleukin-1b (IL-1b) were present in higher concentrations in ACS than in control serum. They theorized that these cytokines may play a previously understated role in clinical improvements seen when ACS has been used to alleviate clinical signs of osteoarthritis and suggested that these cytokines, rather than IL-1ra alone, might be involved in ACS’s efficacy.

Autologous conditioned serum has historically been considered to be an anti-catabolic treatment by virtue of its ability to block the inflammatory cascade initiated by IL-1. By blocking inflammation, ACS might have an indirect, anabolic, pro-healing effect. However, ACS also contains the growth factors listed above, and perhaps others, that are released by platelets activated during clotting while whole blood is incubated with the glass beads. These growth factors may be directly beneficial in healing, and, therefore, ACS may also have a direct, anabolic effect on healing. Numerous studies have documented the beneficial effects of individual growth factors found in ACS, such as vascular endothelial growth factor (VEGF), TGF-β, platelet-derived growth factor (PDGF), growth and differentiation factors, IGF-1, basic fibroblast growth factor (FGF-2, also bFGF), and bone morphogenetic proteins (BMP-12 and BMP-13), on tendon healing using animal models. Exposure, in vitro, of patellar tendon fibroblasts to bFGF was found to affect cellular proliferation and collagen type III expression, both early events in tendon healing. In addition, bFGF was found to stimulate angiogenesis.

Majewski et al showed that ACS, when injected into the rat Achilles tendon, accelerated the rate of organization of repair tissue. Specifically, it increased collagen mRNA expression, collagen deposition, as reflected by tendon thickness, and accelerated collagen fiber maturation, an aspect the authors believe may be important in treating horses for PSD. Majewski et al also showed that stiffness of the ACS-treated tendons improved significantly over that of controls by week 4 post-treatment and theorized that this effect was likely due to reduction in time required for collagen cross-linking and remodeling of the repair site. This time frame coincided with our time frame of implementing controlled work in horses post ACS treatment of PSD. Majewski et al noted that remodeling of tendinous and ligamentous tissue is a slow process as evidenced by their finding that tendon thickness remained elevated even by week 8, but PSD of the horses in our study was accompanied by minimal
loss of fiber. Majewski, et al observed that ACS treatment might shorten recovery time because ACS-aided tendon repair is characterized by repair with well-organized, strong collagen fibers, suggesting that the healed tendon should contain fewer imperfections than in repair tissue formed after spontaneous healing. They believed such tissue would be less prone to re-injury, and they acknowledged that the biologically active component(s) in ACS that were responsible for the accelerated healing of the injured Achilles tendons remain to be identified.18

The authors believe the horses selected to treat with ACS for PSD suffered from acute or chronic SL strains rather than major tearing of the SL. Strain is damage to elastic fibers from stretching of the fibers beyond their elastic limit without visual disruption of fibers. This results in pain, swelling, and inflammation within the strained structure as well as potentially causing compressive damage to adjacent nerves.20 Tóth, et al21 clearly showed that horses with PSD of the hind limbs could also have pathologic changes of the deep branch of the lateral plantar nerve caused by compression from swelling of the PSL. They theorized that this nerve damage could be one potential reason for the poor long-term prognosis of PSD in the hind limbs of horses.21 While ACS could have an indirect effect on nerve pathology by reducing compression on the nerve by reducing inflammation and thus swelling of the PSL, there is evidence that ACS can have a direct effect on pain from compressed nerves. Becker, et al demonstrated that epidural injections of ACS to treat lumbar radicular compression in humans were associated with clinically remarkable positive outcomes, potentially superior to the same treatment method using triamcinolone.20

The authors believe the acutely lame horses in this study were ideal candidates for treatment with ACS because the ligaments of these horses had no ultrasonographically discernible major disruption of fibers. Lameness of some horses may go undiagnosed causing it to become chronic if lameness of a fore limb is mild, if the lameness involves both fore limbs, or if the lameness affects one or both hind limbs. The authors believe that as long as major disruption of fibers is not ultrasonographically evident, even horses chronically lame because of PSD are good candidates for treatment with ACS, as it may be possible to return the tissue to normal size and function and heal or treat pain from potential neuropathies. Periligamentous injection, as described in this report, rather than intrasional injection, is indicated for horses without a core lesion. Forcing ACS into ligamentous or tendinous tissue in which there is no tissue void forces fibers apart, disrupting important cross-linking, this may worsen lameness and lengthen time of healing.

Short-comings of this study are the short follow-up time and lack of control groups. However, it has been the experience of one of the authors, in many cases of PSD in the young Western performance horse, that if we can get a horse to train a month without immediately getting lame, we have a chance of successfully keeping that horse in training long-term. With that stated, however, many of the horses will get lame again at some point from the same cause albeit usually less severely. When this occurs, we reevaluate sonographically, and unless we have tissue disruption of some sort (core lesion, etc.), we have been able to re-inject most of those and only give them short lay-offs of 5 to 7 days and resume training. Many do this several times in their careers and get into a routine with a fairly consistent interval. As these young Western performance horses mature, however, the intervals between flare-up usually widens until they reach 6 years of age in which many seem to mature out of the syndrome. We hypothesize that this is partly due to a reduced training schedule and amount of work needed to stay in top form (i.e., the horse knows its job) but also because the horse begins adaptive remodeling, which becomes complete by the time it is 5 to 6 years old. Occasionally, it was observed that the ligament begins to fail requiring that the horse be removed from training and subjected to a different treatment type or prolonged rest period.

An important consideration when using regenerative products, such as ACS, is that differences may occur between commercially available products. The concentration of IL-1 receptor antagonist protein and IL-1 itself, as well as anabolic growth factor concentrations, may vary among manufacturers. The concentrations may also vary both between and within patients. Hraha, et al tested 2 different commercially available veterinary systems, irap20 and Arthrex IRAP II, using whole blood in a clot tube as a control. They compared each system using the blood of 5 horses and found that IL-1ra was up-regulated by both systems, and within the control, clot tube, but that the IL-1 to IL-1ra ratio was only increased compared to serum in Arthrex IRAP II, thus illustrating differences. The authors used one of the systems and did not measure IL-1ra or other factors of interest. The authors believe caution should be used when extrapolating this data to other systems used to produce ACS.

The authors believe that the large number of horses with PSD treated with ACS demonstrates the safety and effectiveness of this treatment when horses are selected appropriately. There were no control groups of horses with PSD that received other therapies to which can be compared to the effects of treatment with ACS, but this experience with other treatments of horses for PSD as well as the published results of other clinicians indicates that horses treated with ACS are able to be returned successfully to training much sooner than horses receiving other treatment. Treatment with ACS has allowed many young Western performance horses to resume training in time to complete their
schedule of competitions. Further, the authors believe that this treatment should be evaluated in blinded clinical trials and that work should be done to define the exact mechanism of action.

Acknowledgments

Conflict of Interest

The Authors declare no conflicts of interest.

References and Footnotes

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