Intralesional injection of platelet-rich plasma followed by controlled exercise for treatment of midbody suspensory ligament desmitis in Standardbred racehorses

Martin Waselau, Dr med vet, MS; W. Wesley Sutter, DVM, MS, DACVS; Ronald L. Genovese, VMD; Alicia L. Bertone, DVM, PhD, DACVS

Objective—To determine outcome of Standardbred racehorses with moderate to severe midbody suspensory ligament desmitis (MSD) treated by means of ultrasound-guided intralesional injection of a single dose of platelet-rich plasma (PRP) followed by a program of gradually increased exercise.

Design—Nonrandomized clinical trial.

Animals—9 Standardbred racehorses.

Procedures—Following injection of PRP, horses were allowed a controlled, gradual return to exercise. Race records for the year prior to injury and for 3 consecutive years after horses returned to racing were reviewed. For comparison purposes, race records of 9 Standardbred racehorses with no history of MSD racing at the same time were also reviewed.

Results—All 9 horses with MSD returned to racing after treatment; median time to return to racing was 32 weeks. All 9 horses raced at least once during the first and second years after returning to racing, but only 5 raced during the third year. When number of starts, total earnings, and earnings per start were compared between case and comparison horses, the only significant differences were number of starts during the third year after case horses returned to racing and earnings per start during the first year after case horses returned to racing, with values being significantly lower for case horses than for comparison horses.

Conclusions and Clinical Relevance—Results suggested that horses with moderate to severe MSD treated by means of intralesional injection of a single dose of PRP followed by a program of gradually increased exercise had an excellent prognosis for returning to racing. (*J Am Vet Med Assoc* 2008;232:1515–1520)

Injury of the interosseous muscle, commonly known as suspensory ligament desmitis, is an important cause of lameness in horses, especially athletic horses.[1] The interosseous muscle is subdivided into a proximal portion, a body portion, and lateral and medial branches.[2] Injuries of the proximal portion of the interosseous muscle are common in all types of sport horses and typically represent chronic repetitive strain injuries.[3,4] A variety of medical and surgical treatments for proximal suspensory ligament desmitis have been described.[1,3,5–11] In contrast, MSD is typically an acute, career-limiting injury in racing Thoroughbreds and Standardbreds.[9] Treatment options for horses with MSD have been described less frequently,[9,12] and success rates for horses with moderate or severe MSD have been discouraging.[5,9]

Abbreviations

MSD	Midbody suspensory ligament desmitis
PRP	Platelet-rich plasma

Locally administered treatments to support ligament healing may offer advantages to systemic treatments and minimize the risk of systemic adverse reactions.[5] Although various intralesional treatments[13,a,b] for management of tendinitis have been studied and used clinically, results for only a few horses with suspensory ligament desmitis have been reported.[5,9,12,14]

In humans, PRP is frequently used to augment ligament, bone, and wound healing,[15–20] and preparation of PRP with various commercially available systems for use in experimental studies[21–25] of ligament healing in animals has been described. Platelet-rich plasma is an ultraconcentrate of platelets, which contain high concentrations of various growth factors that are released when the platelets are exposed to thrombin.[26] Of particular interest for ligament healing is the fact that transforming growth factor β, platelet-derived growth

From the Galbreath Equine Trauma, Intensive Care and Research Center, Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210 (Waselau, Sutter, Bertone); and Randall Veterinary Hospital, 20600 Miles Pkwy, Warrensville Heights, OH 44128 (Genovese). Dr. Waselau's present address is University of Zürich, Clinic for Large Animal Surgery, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland. Dr. Sutter's present address is Ocala Equine Hospital, 10855 N US Hwy, Ocala, FL 34482.

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Address correspondence to Dr. Bertone.

factor, insulin-like growth factor, vascular endothelial growth factor, and epidermal growth factor are released following platelet activation.[27–29] These factors act synergistically to accelerate neutrophil and macrophage infiltration, angiogenesis, fibroplasia, matrix deposition, and re-epithelization.[30] Thus, PRP would seem to have the potential to enhance or accelerate ligament healing in horses with MSD.[31,32]

The purpose of the study reported here was to determine outcome of Standardbred racehorses with moderate to severe MSD treated by means of ultrasound-guided intralesional injection of a single dose of PRP followed by a program of gradually increased exercise. Racing performance of treated horses was evaluated by comparing number of starts, total earnings, and earnings per start for affected horses with earnings for a comparison group of Standardbred racehorses without MSD matched with the affected horses on the basis of sex, age, and racing status.

Materials and Methods

Case selection—Medical records of Standardbred racehorses examined and treated at the Galbreath Equine Center at The Ohio State University during 2003 and 2004 were reviewed to identify horses in which moderate to severe MSD had been diagnosed. A diagnosis of moderate to severe MSD was made if ultrasonographic examination of the suspensory ligament revealed abnormal echogenicity and fiber alignment involving the entire midbody portion (zones 2A and 2B[33]) of the suspensory ligament with > 15% of the cross-sectional area of the ligament affected (**Figure 1**) and if severity of abnormal echogenicity and fiber alignment were both graded as grade 3 or higher on a scale from 0 to 4.[34] Horses with moderate to severe MSD were included in the study only if treatment had consisted of ultrasound-guided intralesional injection of a single dose of PRP followed by a program of gradually increased exercise and if race records for 3 consecutive years after horses returned to racing were available for review.

Comparison group selection—For comparison purposes, each case horse was matched with a single Standardbred racehorse that raced during the same time period. Comparison horses were identified by examination of the same electronic database[c] used to obtain race records for case horses and were matched with case horses on the basis of sex, year of birth, racing dates (ie, to be eligible for consideration, comparison horses must have raced during the same time period that case horses had raced prior to injury), and earnings per start (ie, to be eligible for consideration, comparison horses must have had earnings per start for the same time period as case horses prior to injury that were within 1 SD of mean earnings per start for case horses) and were randomly selected from all horses that met the matching criteria. Information on medical status of comparison horses was not available; therefore, it could not be determined whether these horses had ever had MSD. However, given the low incidence of MSD in Standardbred racehorses, for purposes of the present study, it was assumed that comparison horses had never had MSD.

Medical records review—Information recorded from medical records for case horses included in the study consisted of age, sex, affected limb, time between injury and treatment, platelet count, and results of ultrasonographic examination of the affected limb.

Ultrasonographic examination—In horses with MSD, the entire suspensory ligament was examined ultrasonographically in a transverse and a longitudinal direction with a 7.5-MHz linear array probe[d] after routine clipping. A transverse image of each core lesion was traced, and cross-sectional area of the lesion was calculated as a percentage of total cross-sectional area of the ligament. Fiber echogenicity was scored on the transverse image on a scale from 0 to 4, where 0 = no hypoechoic areas, 1 = minimal hypoechoic areas, 2 = mild hypoechoic areas, 3 = moderate hypoechoic areas, and 4 = marked hypoechoic areas, as described.[34] Fiber alignment was scored on a longitudinal image of the lesion on a scale from 0 to 4, where 0 = parallel longitudinal fiber alignment, 1 = minimal disruption of the parallel longitudinal fiber alignment, 2 = mild disruption, 3 = moderate disruption, and 4 = marked disruption, as described.[34]

Preparation of PRP—Platelet-rich plasma was obtained by means of the buffy coat method as described.[25] Blood samples were aseptically collected by means of right jugular venipuncture and processed with a commercially available kit.[e] The platelet concentrate that was obtained was transferred to a commercially available applicator set[f] for immediate intralesional injection. Platelets counts of the blood sample and the PRP were performed.

Treatment—For intralesional injection of PRP, horses were sedated with detomidine (1 µg/kg [0.45 µg/lb], IV) and butorphanol tartrate (0.02 mg/kg [0.009 mg/lb], IV), a high palmar or plantar nerve block was performed to anesthetize the distal portion of the limb, and the proposed treatment site was aseptically prepared. Under ultrasonographic guidance, 1.5-inch, 20-gauge needles were inserted perpendicular to the long axis of the ligament every 2 to 4 cm throughout the entire length of the lesion (**Figure 2**). The applicator set was attached to each needle, and PRP and bovine thrombin[g] (20 U/mL) were injected into the lesion in an 11:1 ratio at each site until resistance to injection was met, PRP leaked out another needle, or 3 mL was injected. Injections were repeated throughout the length of the lesion from proximal to distal. The total volume of PRP injected was recorded.

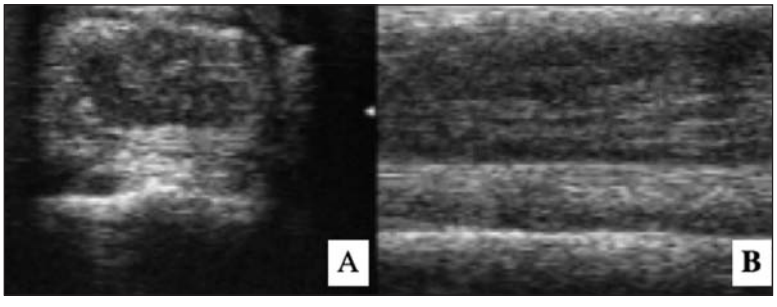

Figure 1—Transverse (A) and longitudinal (B) ultrasonographic images of the suspensory ligament in a Standardbred racehorse with MSD.

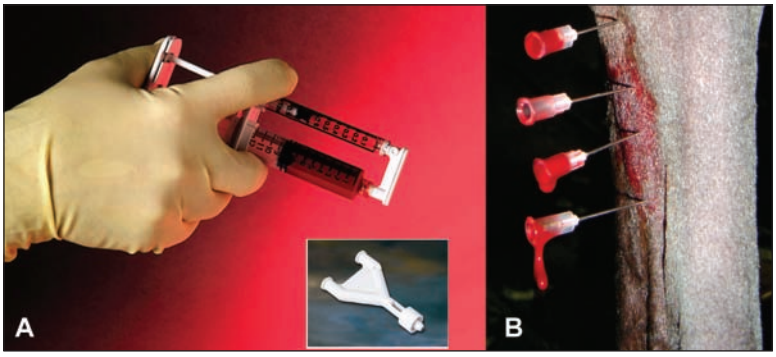

Figure 2—Illustration of a method for intralesional injection of PRP in a horse with MSD. A—Platelet-rich plasma and bovine thrombin were added to a commercial applicator set that could be used, by means of an adaptor (inset), to deliver the PRP and thrombin in an 11:1 ratio. B—Needles (1.5-inch, 20-gauge) were inserted under ultrasonographic guidance perpendicular to the long axis of the ligament every 2 to 4 cm throughout the entire length of the lesion, and PRP and thrombin were injected into each needle.

Following injection of PRP, a bandage was applied to the limb. Horses were hospitalized for 3 days after the procedure for observation and then discharged. Owners were instructed to change the bandage every other day for 2 weeks and to monitor the injection sites for swelling, drainage, and tenderness. Owners were also advised to not administer any nonsteroidal anti-inflammatory drugs for 1 week after the procedure.

Progression of healing was assessed by means of follow-up physical and ultrasonographic examinations performed at The Ohio State University or by the referring veterinarian (RLG) every 4 weeks. A standardized, controlled exercise program was initiated, consisting of stall rest for the first 14 days after treatment, walking in hand for 15 minutes daily on days 15 to 30, walking in hand for 30 minutes daily on days 31 to 60, walking in a bike for 30 minutes daily on days 61 to 90, any type of jogging on days 91 to 120, jogging plus training miles at a pace > 2 min and 20 s/mile on days 121 to 150, and return to racing any time between days 151 and 180. Each component of the exercise program was shortened or lengthened on the basis of ultrasonographic evidence of progression of healing (ie, filling of the defect) and severity of lameness.

Evaluation of racing performance—For horses with MSD, the ability to return to racing was considered evidence of recovery. For case horses, information on racing performance during the 12 months prior to injury and for 3 consecutive years after a return to racing was obtained from an electronic database.[c] For each comparison horse, information was obtained on racing performance for 3 consecutive years beginning at the date of return to racing for the matched horse with MSD. Raw data for number of starts and total earnings were obtained for each horse and summarized as 12-month intervals. For each 12-month interval, earnings per start was calculated as total earnings divided by the number of starts during that interval. For case horses, the date of the first race after injury and the date of injury recorded in the medical record were used to calculate time to return to racing.

Data analysis—Repeated-measures ANOVA was used to compare measures of racing performance (number of starts, total earnings, and earnings per start for each 12-month period) between horses with MSD involving a forelimb versus a hind limb and to compare racing performance prior to injury with racing performance following a return to racing. Two-factor ANOVA (treatment and time) followed by the least significant difference test was used to compare racing performance between the case and comparison cohorts. A paired *t* test was used to compare platelet concentration in blood and PRP. All analyses were performed with standard software.[h] Values of $P < 0.05$ were considered significant.

Results

Nine Standardbred racehorses met the criteria for inclusion as case horses; this represented all horses examined during 2003 and 2004 because of MSD. Median age was 4 years (range, 2 to 5 years); there were 8 geldings and 1 stallion. All 9 horses were actively racing at various tracks at the time of injury, had sustained a single acute injury, were otherwise healthy without any concurrent orthopedic problems, and were immediately treated with stall rest after the injury occurred. Median time between injury and examination was 4 weeks (range, 1 to 28 weeks). Median cross-sectional area of the lesion at the time of initial examination, expressed as a percentage of the total cross-sectional area of the ligament, was 42% (range, 17% to 66%). Echogenicity score was 3 in 3 horses and 4 in the remaining 6; fiber alignment score was 3 in 7 horses and 4 in the remaining 2.

Platelet counts were available for 6 of the 9 horses. Mean platelet count in PRP (mean ± SD, 1.37×10^6 platelets/µL ± 1.11×10^4 platelets/µL) was significantly ($P < 0.02$) higher than mean platelet count in blood (1.55×10^5 platelets/µL ± 4.0×10^4 platelets/µL). No complications associated with intralesional injection of PRP were recorded, although minimal leakage of PRP through adjacent needles was seen during the procedure. Total volume of PRP injected was recorded in 7 horses (median, 9 mL; range, 7 to 12 mL).

All 9 horses with MSD returned to racing following treatment with PRP; median time to return to racing was 32 weeks (range, 26 to 68 weeks). All 9 case horses raced during the first and second years after returning to racing, but only 5 of the 9 case horses raced during the third year after returning to racing. For the 9 case horses, median number of starts during the first year of racing was 18 starts (range, 6 to 37 starts), median number of starts during the second year of racing was 22 starts (range, 3 to 26 starts), and median number of starts during the third year of racing was 9 starts (range, 0 to 25 starts). All 9 comparison horses raced at least once during each year of the 3-year follow-up period.

For the case horses, number of starts during the year prior to injury was significantly ($P < 0.03$) higher than number of starts during the first and third years after returning to racing, but was not significantly different from number of starts during the second year after returning to racing (**Table 1**). Also, number of starts during the third year after returning to racing was significantly ($P < 0.03$) lower than number of starts during the first or second year after returning to racing. Earn-

Table 1—Measures of racing performance the year prior to injury and each year for 3 consecutive years after returning to racing for 9 horses with moderate to severe MSD treated by means of ultrasound-guided intralesional injection of a single dose of PRP followed by a program of gradually increased exercise (case horses) and for 9 comparison horses racing during comparable periods (comparison horses).

	No. of starts		Total earnings ($)		Earnings per start ($)	
Year	Case	Comparison	Case	Comparison	Case	Comparison
Prior to injury	21* (5–46)	10 (5–19)	14,275 (1,862–127,851)	3,349 (1,785–19,584)	717 (133–6,729)	670 (167–1,780)
After returning to racing						
First year	18† (6–37)	21 (6–28)	8,738 (0–37,107)	9,668 (743–87,405)	339*,† (0–1,197)	644 (123–3,641)
Second year	22 (3–26)	23 (12–42)	8,424 (0–75,348)	5,428 (1,093–27,995)	560 (0–3,588)	379 (42–3,151)
Third year	9*,† (0–25)	20 (9–42)	2,563 (0–20,502)	7,955 (1,196–28,694)	233† (0–2,278)	392 (107–994)

Data are given as median (range).
*Significantly ($P < 0.05$) different from value for comparison horses at the same time interval. †Significantly ($P < 0.05$) different from value for case horses during the year prior to racing.

ings per start during the year prior to injury were significantly ($P < 0.04$) higher than earnings per start during the first and third years after returning to racing but was not significantly different from earnings per start during the second year after returning to racing. Total earnings during the first, second, and third years after returning to racing did not vary significantly from each other, and no significant differences were found among total earnings during the year prior to injury and the first, second, and third years after returning to racing.

When values for measures of racing performance (number of starts, total earnings, and earnings per start) were compared between case and comparison horses, the only significant differences were number of starts during the third year after case horses returned to racing ($P < 0.003$) and earnings per start during the first year after case horses returned to racing ($P < 0.01$), with values being significantly lower for case horses than for comparison horses.

Measures of racing performance (number of starts, total earnings, and earnings per start for each 12-month period) were not significantly different between horses with MSD involving a forelimb (n = 5) and horses with MSD involving a hind limb (4).

Discussion

Results of the present study suggested that horses with moderate to severe MSD treated by means of intralesional injection of a single dose of PRP followed by a program of gradually increased exercise had an excellent prognosis for returning to racing, with all 9 horses eventually returning to racing. No complications were reported in association with this treatment. Because of a lack of previous reports on results of treatment of MSD in racehorses, we cannot compare our results with results of other treatments. However, previous authors[5,9] have suggested that severe MSD is a career-limiting or career-ending injury in racehorses and that horses with this injury are frequently retired for economic reasons. Importantly, horses were included in the present only if they had moderate or severe MSD, with cross-sectional area of the lesion, expressed as a percentage of the total cross-sectional area of the ligament, ranging from 17% to 66%.

All horses with MSD in the present study started at least 6 races after recovery, with 1 returning to racing as early as 26 weeks after treatment. One gelding did not race during the first 12 months after treatment, but returned to racing 68 weeks after treatment and competed more frequently than the other horses later on. Importantly, horses with MSD had as many race starts during their first 2 years after returning to racing as did a comparison group of horses racing at the same time, suggesting that affected horses were able to return to athletic function.

We could not determine why horses with MSD had fewer starts during the third year after returning to racing than did horses in the comparison group. However, it was unlikely that they were retired for breeding because all but 1 were geldings. The lower earnings per start during the first year after returning to racing may have represented a conservative return to a full race schedule. Horses may have been competing at a lower level with smaller purses or placing lower. The gradual decrease in earnings per start over the 3-year follow-up period for both groups may have represented a normal pattern for aging racehorses.

Reviewing race records is a well-accepted method for determining treatment outcome in Standardbred racehorses. For the present study, we selected a comparison group of horses matched with case horses on the basis of age, sex, and racing status to allow us to compare racing performance for horses that had been treated for moderate to severe MSD with racing performance for horses that had apparently never had this injury. Unfortunately, we were not able to match case horses with comparison horses racing at the same tracks, and this may have affected our results.

The goal of intralesional treatment in horses with ligament injuries is to enhance healing while minimizing adverse systemic effects,[5] with previously reported treatments involving administration of bone marrow and autologous stem cells. Previous studies[21,22] involv-

ing experimentally induced ligament injures have suggested that PRP can enhance ligament healing, and it has been documented that PRP can contain a variety of growth factors, such as transforming growth factor β1 and β2 and insulin-like growth factor 1.[25]

Simultaneous injection of PRP with thrombin is suggested to enhance platelet degranulation[21,22,25] and, possibly, release of growth factors. For horses in the present study, bovine thrombin was injected at the same time as PRP to promote retention of platelets in the lesion and enhance platelet degranulation. Although there is still a debate whether thrombin is required to activate platelets, it has been shown that addition of thrombin to PRP will trigger release of growth factors such as transforming growth factor β.[35] Therefore, we elected to use bovine thrombin as directed by the PRP kit manufacturer.

All horses with MSD in the present study underwent a controlled exercise regimen after treatment with PRP. Therefore, we could not determine how much each of these components of the treatment regimen contributed to the successful outcome or whether results would have been similar with PRP alone or with controlled exercise alone. Platelets positively influence the early phases of regeneration,[36] and improved early healing may permit earlier exercise. It is believed that a return to exercise promotes appropriate ligament healing by ensuring correct fiber alignment and increased strength of the healed ligament, reducing the risk of reinjury.[5] Thus, we believe that both PRP and the controlled exercise program were important in the outcome of these horses. However, additional studies are needed to confirm our findings.

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