Injection of the navicular bursa with corticosteroids and hyaluronic acid has been reported to have an 80% short-term (mean, 4.6 months) and a 56% long-term (range, 1 to 3 years) success rate for treatment of navicular syndrome.

Numerous techniques have been described for injection of the navicular bursa; however, this therapeutic modality is technically more difficult than injection of the DIPJ and may induce inconsistent results, and radiographic guidance is advised to verify needle position.1–3 Many practitioners prefer to treat horses with navicular syndrome by injection of the DIPJ with the hope that the medication will diffuse into the adjacent navicular bursa.

Commonly used pharmaceuticals for injections of the DIPJ in treatment of navicular syndrome include triamcinolone acetonide and a combination of triamcinolone acetonide and hyaluronic acid.1,2,4 Corticosteroids, such as triamcinolone acetonide, have a potent anti-inflammatory effect when injected into a synovial structure. The combination of hyaluronic acid and corticosteroid decreases the intensity of signs of pain more rapidly than treatment with hyaluronic acid alone5–7; however, it is not known whether hyaluronic acid will affect diffusion of corticosteroid.

Multiple studies8–10 using latex injections, various dyes, contrast arthrography, magnetic resonance imaging,...
aging, and computed tomography reveal that there is rarely direct communication between the DIPJ and the navicular bursa. However, experimentally, mepivacaine injected into the DIPJ diffuses into the bursa.\textsuperscript{11,13} More recently, Pauwels et al\textsuperscript{13} detected diffusion of triamcinolone (a metabolite of triamcinolone acetonide) and methylprednisolone from the DIPJ to the navicular bursa. Because diffusion of a substance across a biological barrier is in part related to its molecular size,\textsuperscript{14} the molecular weights of such substances (latex, > 1,000 Da; contrast medium, 821 Da; dyes, 492 to 738 Da; methylprednisolone acetate, 374.5 Da; triamcinolone, 394.5 Da; and mepivacaine, 246 Da) play a role in determining permeability. Because triamcinolone acetonide has a molecular weight of 434.5 Da, which is greater than those of triamcinolone and methylprednisolone acetate but less than those of dyes, it is probable that triamcinolone acetonide will move from the DIPJ into the navicular bursa. Hyaluronan, however, is unlikely to diffuse between synovial structures because of its molecular weight (2.92 $\times$ 10\textsuperscript{5} Da),\textsuperscript{15} but its effect on diffusion of corticosteroids is not well established.

The purpose of the study reported here was to determine whether triamcinolone acetonide diffuses from the DIPJ to the navicular bursa, diffusion is direct or systemic, and addition of hyaluronan has an effect on diffusion in horses. We hypothesized that there would be local diffusion of triamcinolone acetonide from the DIPJ into the navicular bursa and that hyaluronan would not have an effect on diffusion of triamcinolone acetonide.

Materials and Methods

Procedures—This study was performed in 2 phases. In the preliminary phase, 7 mixed-breed horses (mean age, 5.1 years; range, 2 to 15 years) donated for reasons other than forelimb lameness were used to determine when the concentration of triamcinolone acetonide reached a peak in the navicular bursa after injection into the DIPJ. Lameness evaluation included physical examination, hoof tester response, evaluation at the walk and trot, and flexion tests. No obvious forelimb lameness was detected. Both forelimb DIPJs received an injection of 10 mg of triamcinolone acetonide\textsuperscript{6} plus 2 mL of LRS. This volume of LRS was chosen to equal the volume of hyaluronan used in the primary phase. Synovial fluid samples were collected from the navicular bursae at 0.5, 1, 3, 6, and 9 hours (2 samples at each point), and the concentration of triamcinolone acetonide was determined by use of HPLC-MS/MS.

Eleven mature horses (mean age, 11.8 years; range, 4 to 27 years) of various breeds (5 American Paint Horses, 4 Arabians, and 2 Thoroughbreds) were used in the primary phase of the study. Seven horses were from the University of Minnesota teaching herd, and 4 horses were donated for reasons other than forelimb lameness. There were 7 mares and 4 geldings, and the mean weight was 470.3 $\pm$ 38.9 kg. Horses were evaluated and excluded from the study if forelimb lameness detected during a subjective lameness evaluation, pain response to hoof testers applied across the palmar third of the hoof, and improvement of lameness after administration of palmar digital or abaxial sesamoid nerve blocks.\textsuperscript{16} Lateromedial, dorsopalmar, 60\textdegree dorsoproximal-palmarodistal oblique, and palmoproximal-palmarodistal oblique radiographic views from each forelimb were obtained, and each navicular bone was scored on a scale of 0 to 4.\textsuperscript{17} Any horse with a combined radiographic score of ≥ 2 was excluded from the study.

One randomly selected forelimb DIPJ of each of the 11 horses was treated with an injection of 10 mg of triamcinolone acetonide plus 20 mg of hyaluronan\textsuperscript{4} (group 1), and the other forelimb DIPJ was treated with an injection of 10 mg triamcinolone acetonide plus 2 mL of LRS (group 2). Distal interphalangeal joint synovial fluid samples were collected from both joints prior to injection, and 20-$\mu$L aliquots of each sample were pooled for analysis. On the basis of peak concentration data from the preliminary phase, synovial fluid was collected from both forelimb navicular bursae and 1 hind limb navicular bursa (systemic control group) of each of the 11 horses at 6 hours after injection. The use of all horses was approved by the University of Minnesota Institutional Animal Care and Use Committee.

Sample collection—Horses were sedated with xylazine hydrochloride\textsuperscript{6} (0.3 to 0.5 mg/kg, IV) or detomidine hydrochloride\textsuperscript{6} (0.01 to 0.02 mg/kg, IV) and restrained with a nose twitch if necessary. Bilateral forelimb abaxial sesamoid nerve blocks were performed with 2 mL of mepivacaine\textsuperscript{12} at each site to facilitate sample collection. The synovial injection sites were clipped and aseptically prepared. Arthrocentesis of the DIPJ was performed with an 18-gauge, 1.5-inch needle via the dorsal approach. If fluid was not obtained, the palmar approach was used.\textsuperscript{18} Successful placement of the needle was determined by appearance of synovial fluid in the hub of the needle. A synovial fluid sample was collected, and each forelimb received an injection as described. Horses were walked for 5 minutes after injection and then placed in a stall. Navicular bursal fluid samples were collected at 6 hours after injection. Horses were sedated, and centesis sites were clipped and aseptically prepared. Abaxial sesamoid nerve blocks were performed in both forelimbs and 1 hind limb by use of 2 mL of mepivacaine at each site. For navicular bursal theco-centesis, the forefeet were placed on 4-inch wooden blocks and an 18-gauge, 3.5-inch needle was inserted midway between the bulbs of the heel, to 2 to 3 mm proximal to the coronary band.\textsuperscript{19} The needle was advanced along the sagittal plane of the foot, aiming for a point on the coronary band halfway between the most dorsal and palmar aspects of the coronary band. The needle was advanced until the tip of the needle contacted bone. A lateral radiograph was obtained to establish correct needle placement. The foot was then elevated and the toe flexed to facilitate synovial fluid collection. Immediately after sample collection, radiographic contrast medium\textsuperscript{7} (1 mL of contrast medium plus 2 mL of LRS) was injected into the forelimb navicular bursa and a lateral radiograph was obtained to verify that there was no direct communication between the DIPJ and the navicular bur-
sa. Forelimb DIPJ synovial samples were obtained as described. All samples were divided into aliquots and stored in cryovials at −80°C until analyzed.

**Extraction and analysis**—Each sample was thawed on ice. For liquid-phase extraction, 10 µL of internal standard (TAC<sub>6</sub> at 1 ng/µL concentration), 80 µL of PBS solution, and 10 µL of sample were mixed before the addition of 400 µL of ethyl acetate. The mixture was vortexed and kept on a rotary shaker for 5 minutes. Triamcinolone-<sup>6d</sup>, acetonide-<sup>6d</sup>, and triamcinolone acetonide partitioned into the top layer (organic phase). A 250-µL aliquot was removed from the top layer and transferred to a new tube. An additional 300 µL of ethyl acetate was added to the original tube, vortexed, and agitated before removal of another 250 µL from the top layer. The 250-µL aliquots from each addition of ethyl acetate were pooled and dried in a speed vacuum for 30 minutes. The triamcinolone acetonide samples were rehydrated in 75 µL of load buffer (water:acetonitrile:formic acid:trifluoroacetic acid [98:2:0.1:0.02]) and sonicated for 1 minute. The sample was incubated at 21°C for 30 minutes and stored at −20°C. At the time of sample loading for HPLC-MS/MS analysis, 25 µL of sample was diluted with load buffer to 100-µL total volume and 5 µL was used for the analysis.

Reversed-phase HPLC-MS/MS was used to detect and quantify triamcinolone acetonide concentrations in synovial fluid. The extracts were analyzed by use of a capillary HPLC and autosampler<sup>4</sup> connected to the nanospray<sup>4</sup> source of a mass spectrometer by use of a linear gradient.<sup>4</sup> Instruments were controlled via software<sup>1</sup>. To quantify mass spectrometry data, known amounts of triamcinolone acetonide were added to DIPJ synovial fluid samples prior to liquid-phase extraction. The mass transitions monitored were as follows: 435.2 → 397.1 and 435.2 → 399.1 for triamcinolone acetonide and 442.2 → 404.1 and 442.2 → 340.1 for TAC. The peak areas under the transition ions, 397.1 and 404.1, were used in quantification. Samples were loaded onto a carbon 18 cartridge.<sup>4</sup> After 10 minutes of loading, the valve was switched to elute triamcinolone acetonide and TAC from the cartridge onto the column. Samples were separated over 12 minutes at a column flow rate of 500 nL/min by use of a self-packed carbon 18<sup>4</sup> column, with a linear gradient from 40% to 90% solvent B. High-performance liquid chromatography solvents were as follows: water:acetonitrile:formic acid (98:2:0.1) as solvent A and water:acetonitrile:formic acid (2:98:0.1) as solvent B. The mass spectrometer was operated in positive mode using multiple reaction monitoring mode. Additional mass spectrometer parameters were as follows: ion spray voltage, 2,400 V; curtain gas, 10 units; collision gas, high; nebulizer gas, 9 units; ion source gas, 20 units; interface heater, 150°C; declustering potential, 60 V; entrance potential, 10 V; and collision energy, 25 V. The quadrupoles were operated at unit resolution. The data obtained were processed with software<sup>1</sup>. For method validation, extraction of 1 sample was performed and 3 aliquots were analyzed over 3 consecutive days. Additionally, 3 extractions from the same sample were performed and an aliquot from each extraction was analyzed. A software program<sup>6</sup> was used to analyze validation data.

**Statistical analysis**—In the preliminary phase, the mean concentration of triamcinolone acetonide at each time period was calculated. In the primary phase, triamcinolone acetonide results were log (base 10) transformed before analysis because data were not distributed normally. Multivariate linear regression analysis<sup>6</sup> was used to determine the relationship between the dependent variable of interest (log<sub>10</sub> triamcinolone acetonide measurements) and treatment group. Horse was included in the model to control for individual variation. Contrast analysis was performed to determine differences among the 3 treatment groups (group 1 vs systemic control, group 2 vs systemic control, and group 1 vs group 2), with a value of <i>P</i> < 0.05 considered significant.

**Results**

In the preliminary phase, 9 nondiluted navicular bursa synovial fluid samples were obtained from 14 forelimbs of 7 horses. Two samples each at time periods 0.5, 3, and 6 hours and 1 sample at 9 hours were analyzed. The second 9-hour sample was omitted as an outlier because the triamcinolone acetonide value recorded for this observation greatly exceeded measurements for the group (> 3 SDs greater than the mean value). The highest concentration of triamcinolone acetonide was detected 6 hours after initial injection into the DIPJ. Therefore, bursal samples from the 11 horses in the primary phase of the study were collected 6 hours after injection into the DIPJ.

In the primary phase of the study, 6 left forelimb and 5 right forelimb navicular bursae were used in group 1, and 5 left forelimb and 6 right forelimb navicular bursae were used in group 2. The systemic control group included 5 left hind limb and 5 right hind limb navicular bursae. One observation from each of the triamcinolone acetonide–hyaluronan and systemic control groups was omitted as an outlier from the final analysis because the triamcinolone acetonide value recorded for these observations greatly exceeded measurements for the group (> 3 SDs greater than the mean value).

No detectable triamcinolone acetonide concentrations were identified in pooled preinjection DIPJ samples from primary-phase horses. There were detectable concentrations of triamcinolone acetonide in navicular bursa synovial fluid of all groups after injection. The triamcinolone acetonide log<sub>10</sub> concentration (mean ± SE) for group 1 was 3.90 ± 0.37, for group 2 was 4.35 ± 0.32, and for the systemic control group was 1.59 ± 0.40. Groups 1 and group 2 had significantly (<i>P</i> < 0.001) greater concentrations of triamcinolone acetonide than did the systemic control group. There was no significant (<i>P</i> = 0.385) difference between group 1 and group 2. There was no effect of horse in the model. Postsampling navicular bursography was performed in 19 of 22 forelimbs, and no direct communication between the navicular bursa and the DIPJ was identified.

**Discussion**

Triamcinolone acetonide was found in the corresponding navicular bursa of all horses after injection.
of 10 mg of triamcinolone acetonide into the DIPJ regardless of whether hyaluronan was combined with triamcinolone acetonide. Because no direct communication was observed between the DIPJ and navicular bursa, movement of triamcinolone acetonide from the DIPJ into the navicular bursa was caused by either direct or systemic diffusion. Triamcinolone acetonide was detected in all systemic control bursae in the study, which revealed systemic diffusion of corticosteroid into remote sites. However, the triamcinolone acetonide concentrations in forelimb navicular bursae were significantly higher than the concentration in hind limb navicular bursae, indicating that systemic diffusion played a minimal role.

Previous studies have revealed direct communication between the DIPJ and the navicular bursa in 1 of 122 limbs by use of Evan blue dye, 4 of 50 limbs by use of iodinated contrast medium, 6 and of 20 limbs by use of arthrography. 20 In fresh cadaver limb DIPJs that received an injection of Evan blue or luxol fast blue dye, 17 of 26 navicular bursae had a blue tinge in the synovial fluid, suggesting an indirect method of communication between the 2 synovial structures. 6 Smaller molecular weight agents, such as mepivacaine and triamcinolone, diffuse from the DIPJ into the navicular bursa. 21,22 The synovial joint lining is not a continuous cellular layer and lacks tight junctions characteristic of other membranes, such as endothelia and epithelia. The 1-µm-wide gaps between synovial lining cells allow size-selective molecular sieving by synovium. 21,22 Thus, it is likely that triamcinolone acetonide (434.5 Da) is able to passively diffuse through the synovial lining into the interstitial space and then into the adjacent bursa. In the interstitial space, it is likely that additional properties of the corticosteroid and tissue could promote or inhibit diffusion as well, but the tissue miscibility of triamcinolone acetonide in the interstitial space is unclear. In addition, there is a possibility that there is direct lymphatic drainage between the DIPJ and navicular bursa, but to our knowledge, there are no reports to support this pathway of diffusion.

Numerous studies 20,21,22,23 have detected systemic absorption of triamcinolone acetonide after intra-articular injection. Chen et al. 23 found that serum triamcinolone acetonide concentrations peaked 4 hours after intra-articular administration of 18 mg of triamcinolone acetonide (6 mg into 3 joints) and were undetectable at 3 days. In another study, 30 30 mg of triamcinolone acetonide was injected intra-articularly and mean peak serum concentrations were detected at 12 hours. Although a previous study 27 did not detect corticosteroids in the contralateral joint after an intra-articular injection, remote-site effects of corticosteroids have been observed on articular cartilage glycosaminoglycan content (with triamcinolone acetonide) 28 and on type II collagen anabolism, 29 suggesting a systemic effect of the intra-articularly injected drugs. 30 Our study corroborated those findings; triamcinolone acetonide was detected in all hind limb control navicular bursae. However, because the concentrations of triamcinolone acetonide in the hind limb navicular bursae were significantly less than the triamcinolone acetonide concentrations in the forelimbs, we believe that systemic absorption played a minor role in concentrations measured in the forelimb bursae.

Synovial fluid hyaluronan plays an important role in regulating synovial fluid flow rates and drainage. The effect of hyaluronan on synovial membrane permeability is dependent on the chain length (molecular weight) and concentration; as hyaluronan molecular weight is reduced in the joint, outflow buffering by the hyaluronan is lost and fluid drains away more rapidly. 31 However, increasing the concentration of hyaluronan in the joint (by addition of exogenous hyaluronan) attenuates fluid drainage. 21,22 In the present study, the addition of hyaluronan did not increase or decrease movement of triamcinolone acetonide into the navicular bursa, compared with the triamcinolone acetonide-LRS mixture. The molecular weight of hyaluronan used in this study (292 kDa) is the lowest of the presently available hyaluronan preparations for use in injection in equine joints. It is not known whether the higher molecular weight hyaluronans will have a different effect on triamcinolone acetonide diffusion. Post hoc power analysis at 80% indicated that a sample size of 16 horses/group was required to detect a significant difference.

The combination of HPLC and MS/MS was used to detect and quantify triamcinolone acetonide in the navicular bursa because this method for extraction and quantitation is more sensitive and specific than other reported methods 22,31 and because only a small sample size (10 µL) is required for sample assay. In the present study, the mass spectrometer was able to detect a concentration of synovial hyaluronan as low as 0.02 pg. The use of deuterium-labeled triamcinolone acetonide as an internal standard has been validated for development of a triamcinolone acetonide standard curve by use of serum and urine samples. 32 We chose to measure triamcinolone acetonide instead of triamcinolone because of reported extraction difficulty and high variability in recovery of triamcinolone in serum and plasma 34 and because of the unknown rate of hydrolysis of triamcinolone acetonide to triamcinolone in equine joints.

A wide range in synovial fluid triamcinolone acetonide concentrations in the bursae was observed in both phases of the study. Inconsistent diffusion between noncommunicating joints has been reported in other studies with injection of methylprednisolone acetate into equine tarsocrural joints 35 and triamcinolone acetonide into middle carpal and tarsocrural joints. 23 Erratic diffusion of methylprednisolone acetate and triamcinolone acetonide between DIPJs and navicular bursae was observed in the study by Pauwels et al. 13 The cause was unknown but is possibly attributable to individual animal variation in synovial structure or to variable rates of hydrolysis of triamcinolone acetonide in synovial fluid. Intra-articular methylprednisolone acetate hydrolysis peaks between 2 and 10 hours after administration. 33 The rate of triamcinolone acetonide breakdown in equine synovial structures is not well established. In human studies, 24 the pharmacokinetics of triamcinolone acetonide after intra-articular injection indicate that the mean elimination half-life in knee joints is 3.2 to 4.3 days, suggesting that hydrolysis is relatively slow.
In comparison to the study by Pauwels et al.,\textsuperscript{13} the mean concentration of triamcinolone acetonide in the navicular bursae detected in the present study was substantially larger. This may be explained by the measurement of different substances (triamcinolone acetonide vs triamcinolone), different measuring techniques, different sampling techniques, or different sampling times. Results of studies\textsuperscript{26,37} evaluating the biotransformation of triamcinolone acetonide after oral and IV administration in humans indicate that triamcinolone is not a major metabolite of triamcinolone acetonide. The mechanism of triamcinolone acetonide hydrolysis in the synovial fluid is not well known, and it is possible that triamcinolone is not a major metabolite of triamcinolone acetonide in equine joints. Thus, it may be found in small quantities in the joint. Navicular bursa synovial fluid samples were obtained at 3 hours in the Pauwels et al\textsuperscript{13} study, and it is possible that hydrolysis of triamcinolone acetonide to triamcinolone occurs to a greater degree after that point. The mechanism of diffusion of triamcinolone acetonide from the DIPJ to the navicular bursa is unclear, and it is likely that there are interhorse variations in diffusion as well as metabolism of the corticosteroid.

The variability in diffusion concentrations in the present study may have been caused by low sample volume, blood contamination, or unequal distribution of triamcinolone acetonide in the bursae. In 8 of 22 forelimb navicular bursa fluid samples, < 0.3 mL of fluid was obtained. It is possible that this amount was not a true representation of bursal fluid. Dilutional techniques such as the urea model of dilution\textsuperscript{44} have been used to increase the amount of fluid sample; however, we felt that the potential problems related to dilution, such as inaccuracy at greater dilution or possible re-equilibration between serum concentration and diluted synovial concentration of urea, would result in less precise results. There was blood contamination in 18\% (4/22) of the forelimb navicular bursa fluid samples. Although we did not measure blood serum concentrations of triamcinolone acetonide at time of sample collection, the volume dilution from systemic circulation should have minimized the effect of blood contamination in the sample. The synovial samples were obtained from the region of bursa directly palmar to the navicular bone; triamcinolone acetonide concentration could also be affected by a potential inequality in distribution between the high-pressure area palmar to the navicular bone and the large proximal bulbous portion of the bursa. Also, the horses were walked after injection but were then placed in a stall until sample collection. Lack of movement in the stall may have affected corticosteroid distribution.

It was not within the scope of this study to determine whether the concentration of triamcinolone acetonide in the navicular bursa was at therapeutic concentrations. To our knowledge, there is no published intra-articular therapeutic dose of triamcinolone acetonide. In vitro work has detected anti-inflammatory or supportive effects of triamcinolone acetonide on cartilage explants,\textsuperscript{30,41} but those values are not easily translated to in vivo concentrations. In vivo studies have revealed improvement in lameness\textsuperscript{42} and beneficial effects of triamcinolone acetonide on articular cartilage metabolism in osteochondral fragment models.\textsuperscript{28,42} Previous studies\textsuperscript{33,39,40} have extrapolated a therapeutic dose for methylprednisolone (37 ng/mL) on the basis of a study by Kantrowitz et al\textsuperscript{28} in which dexamethasone- and hydrocortisone-induced inhibition of prostaglandin E\textsubscript{2} in rheumatoid synovia explants was observed at concentrations of 10\textsuperscript{+} to 10\textsuperscript{8}M. The median concentrations of triamcinolone acetonide in group 1, group 2, and the systemic control group in the present study were 469.9, 918.3, and 4.1 ng/mL, respectively. Given that the potency of triamcinolone acetonide is between that of dexamethasone and hydrocortisone, similar to methylprednisolone acetate, the median concentrations of triamcinolone acetonide in the navicular bursae of the present study would be considered to be therapeutic according to these guidelines. However, because of the wide variability observed in this and other studies, some horses may respond better than others.

The horses used in the present study did not have navicular syndrome. Pathological changes associated with navicular syndrome, such as bursitis, capsulitis, deep digital flexor tendonitis, or adhesions of the deep digital flexor tendon to the navicular bursa, could affect diffusion of triamcinolone acetonide directly or indirectly. Inflammation increases synovial membrane permeability to small molecules\textsuperscript{44} and decreases hyaluronan chain length and concentration,\textsuperscript{21} which may increase diffusion. Conversely, chronic inflammation can lead to synovial hypertrophy and joint capsule thickening, which may interfere with diffusion. Metabolism of triamcinolone acetonide may be altered in an inflamed joint, and triamcinolone acetonide could be hydrolyzed at a different rate than in healthy joints.

The present study revealed that triamcinolone acetonide diffused directly from the DIPJ into the navicular bursa in clinically normal horses. Addition of hyaluronan to the corticosteroid did not significantly affect diffusion. Further studies with horses with navicular syndrome are needed to determine whether diffusion occurs similarly in clinically affected horses.

\begin{itemize}
  \item a. Legend, 10 mg/mL, Bayer, Shawnee Mission, Kan.
  \item b. Kenalog-40, 40 mg/mL, Bristol-Meyers Squibb Co, Princeton, NJ.
  \item c. Anased, 100 mg/mL, Lloyd Laboratories, Shenandoah, Iowa.
  \item d. Dormosedan, 10 mg/mL, Pfizer Animal Health, Exton, Pa.
  \item e. Carbocaine, 20 mg/mL, Pharmacia & Upjohn Co, Kalamazoo, Mich.
  \item f. Omnipaque, GE Healthcare Inc, Princeton, NJ.
  \item g. C/DIN Isotopes Inc, Pointe-Claire, QC, Canada.
  \item h. Agilent Technologies, New Castle, Del.
  \item i. Applied Biosystems, Foster City, Calif.
  \item j. Dionex Co, Sunnyvale, Calif.
  \item k. Michrom Bioresources, Auburn, Calif.
  \item l. New Objective, Woburn, Mass.
  \item m. Microsoft Office 2003, SP3, Microsoft Corp, Redmond, Wash.
  \item n. Proc MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.
\end{itemize}

References


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