Serial injections of 4% polyacrylamide hydrogel have no detrimental effects in equine joints following clinical, histologic, and synovial biomarker evaluation

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OBJECTIVE
Polyacrylamide hydrogel (4% PAHG) is an inert viscoelastic supplement used to manage osteoarthritis in horses. Even with a prolonged clinical effect, horses may be administered multiple doses during their performance career. The effect of the serial 4% PAHG treatments is not known. The objectives of this study were to evaluate the clinical, histologic, and synovial fluid biomarker effects following serial administration of 4% PAHG in normal equine fetlock joints.

ANIMALS
8 healthy horses.

METHODS
In a blinded, controlled in vivo study, horses received serial intra-articular injections of 4% PAHG (Noltrex Vet; Nucleus ProVets LLC) and contralateral 0.9% saline control on days 0, 45, 90, and 135. Treatment and control joints were randomly assigned. Synovial fluid was collected before administration of 4% PAHG or 0.9% saline on day 0 and at study completion for cellular and biomarker evaluation. Serial physical and lameness examinations were performed throughout the study. On day 240, gross examination and harvest of cartilage and synovial membrane for histology were completed.

RESULTS
There were no histologic changes in articular cartilage or synovial fluid biomarkers. The 4% PAHG was seen on the surface of the synovium in 5 of 8 treated joints 105 days after the last treatment. There are minimal effects following serial injections of 4% PAHG on normal joints in horses following administration at 0, 45, 90, and 135 days, with final evaluation on day 240.

CLINICAL RELEVANCE
Serial administration of intra-articular 4% PAHG in horses may provide long-term joint lubrication with no detrimental effects.

Keywords: osteoarthritis, equine, polyacrylamide hydrogel, joint, treatment

Polyacrylamide hydrogels (PAHG) are being utilized as a device for intra-articular lubrication of osteoarthritic joints in humans and horses. The PAHGs have been shown to decrease lameness for prolonged periods of time after treatment administration. This inert material has mechanical properties similar to normal synovial fluid and has recently been shown to effectively decrease friction following mechanical and IL-1β biochemical-induced surface damage to cartilage.

There are recognized differences between the PAHGs available for intra-articular use in the horse. The initial differences in polyacrylamide content (2.5% and 4.0%) indicate a basic difference of 1.5%, but they are likely different in physical structure. The 2.5% PAHG was designed as an aesthetic filler and...
then utilized for intra-articular therapy.4,5,12 This 2.5% PAHG has been shown to integrate into the synovial membrane and remain there for an extended period of time.13 The 4% PAHG (Noltrex Vet; Nucleus ProVets, LLC) was engineered to mimic the viscoelastic properties of normal synovial fluid.7,14 This material aggregates to the surface of damaged cartilage and decreases the coefficient of friction, effectively lubricating damaged cartilage surfaces.8 The 4% PAHG appears to be slowly phagocytosed from within the joint, resulting in minimal inflammatory response.15 When PAHGs are manufactured, differences in cross-linkage content, type of cross-linker, and processing factors, including temperature, can create large differences in their final physical structure and behavior in biomedical applications.16–18

Utilizing the 4% PAHG, data available following a single intra-articular treatment of the equine fetlock through 56 days following administration demonstrated no negative effects.15 Further information about the effect of this 4% PAHG on normal equine joints following serial injections with a longer follow-up time is needed. The objectives of this study were to evaluate the clinical, histologic, and synovial fluid biomarker effects following serial administration of 4% PAHG in normal equine fetlock joints. Our hypothesis is there will be no negative effects of serial injections of 4% PAHG on articular cartilage or the synovial membrane.

Methods

In a blinded, controlled in vivo study, 8 female horses were selected from an embryo-recipient herd. All of the horses were sound, 0/5 AAEP lameness score,19 based on a clinical examination and flexion tests, and metacarpal/metatarsal phalangeal (MCP/ MTP) joints were radiographically normal. The study was approved by an IACUC.

Each horse had a pair of forelimb or rear limb MCP/MTP joints randomly selected. One joint was then randomly selected to serve as the treated group, while the contralateral joint acted as the saline control group. Randomization resulted in 3 MTP joint pairs and 5 MCP joint pairs being included. A volume of at least 2 mL of synovial fluid was aspirated before administration of 4% PAHG (4.0% 3-D polyacrylamide, 96% purified water, and 0.0001% to 0.0025% silver ions) or 0.9% saline on day 0 for clinicopathologic analysis. All samples were frozen at –70 °C for biomarker analysis. Commercially available competitive ELISA tests were used to measure type 2 collagen synthesis biomarker (CPII-type II collagen synthesis; IBEX Diagnostics), type II collagen cleavage (C2C-cartilage degradation; IBEX Diagnostics), type I and II collagen cleavage (C1,2C-type I and II collagen degradation; IBEX Diagnostics), and chondroitin sulfate degradation (CS846-aggrekan synthesis; IBEX Diagnostics). The samples were run in duplicate at appropriate dilutions (C1,2C and C2C assay no dilution, CPII1:2 dilution, and CS846 1:20 dilution) using the protocol provided by the ELISA manufacturer. Plates were read with an automated plate reader (Power Wave 340 & KC Junior; Bio-Tek Instruments) at an optical density of 450 nm. Software was used to calculate the concentration of the epitope in the sample, compared with a standard curve. The coefficients of variation were calculated on the raw data. All of these assays have been previously validated for use in equine synovial fluid.22–26

Injection protocol

On days 0, 45, 90, and 135, the assigned joints were treated with either 2.5 mL of 4% PAHG or 2.5 mL of 0.9% saline solution by an unblinded investigator who was not involved in any data collection. The viscosity of the 4% PAHG was clearly different from the saline, preventing blinding of all study participants. Horses were sedated with detomidine hydrochloride before each arthrocentesis, and standard aseptic procedures were utilized. Using a lateral approach with the fetlock joint flexed, a 19-gauge 1-inch needle was inserted through the lateral collateral sesamoidean ligament.20 After aspiration, 2.5 mL of 4.0% PAHG or 2.5 mL of 0.9% sterile saline solution was injected.

Physical observations

Observations were recorded before treatment, daily for 4 days after each arthrocentesis, and at weekly intervals between treatments, until 1 week after the final treatment and before the end of the study. Any abnormalities noted outside of these observation times would trigger additional examinations. The general attitude, body temperature, heart rate, and mucus membranes were recorded as normal or abnormal. Lameness was scored as 0 to 5 based on the AAEP lameness scale.19 Fetlock flexion tests were done holding the limb for 45 seconds and scored as follows: 0 = no change, 1 = slight change, 2 = mild change, 3 = moderate change, and 4 = severe change, and heat and effusion were scored 0 to 4: 0 = normal, 1 = slight, 2 = mild, 3 = moderate, and 4 = severe.21

Synovial fluid analysis

Clinicopathologic evaluation was done on the day of collection with a 2-mL minimal sample at a commercial laboratory (IDEXX), and 2-mL samples were frozen at –70 °C for biomarker analysis. Clinicopathologic evaluation included total protein, total WBC count, and percent lymphocytes, neutrophils, large monocytes, and eosinophils. Commercially available competitive ELISA tests were used to measure type 2 collagen synthesis biomarker (CPII-type II collagen synthesis; IBEX Diagnostics), type II collagen cleavage (C2C-cartilage degradation; IBEX Diagnostics), type I and II collagen cleavage (C1,2C-type I and II collagen degradation; IBEX Diagnostics), and chondroitin sulfate degradation (CS846-aggrekan synthesis; IBEX Diagnostics). The samples were run in duplicate at appropriate dilutions (C1,2C and C2C assay no dilution, CPII1:2 dilution, and CS846 1:20 dilution) using the protocol provided by the ELISA manufacturer. Plates were read with an automated plate reader (Power Wave 340 & KC Junior; Bio-Tek Instruments) at an optical density of 450 nm. Software was used to calculate the concentration of the epitope in the sample, compared with a standard curve. The coefficients of variation were calculated on the raw data. All of these assays have been previously validated for use in equine synovial fluid.22–26

Postmortem examination

Horses were euthanized with a barbiturate overdose. Following euthanasia, each joint was clipped and aseptically prepared. Synovial fluid was aspirated before the joint was opened and when opened any remaining fluid was aspirated so all available fluid was obtained. All joints yielded at least 4 mL
with this method. Any articular cartilage wear lines, surface erosion, palmar/plantar arthroses, and synovial membrane hyperemia were scored 0 to 3 and also summed for a gross pathology score.27,28

**Synovial membrane histopathology**

Samples of the synovial membrane and joint capsule were collected from the dorsal aspect of the joint in the mid-medial condyle region. Cellular infiltration, vascularity, intimal hyperplasia, subintimal edema, and subintimal fibrosis, were all scored from 0 to 4 based on a previously published grading system28 and summed for a synovial membrane histopathology score. A scoring system (0 = normal, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe) was applied to basophilic staining on the surface of the synovium, basophilic material associated with hyperplastic synoviocytes, subsynovial basophilic material, and cellular infiltrate associated with subsynovial basophilic material similar in appearance to what was seen in a previous 4% PAHG study,15 and the categories were summed for statistical evaluation.

**Cartilage histology**

A 1-cm-wide section of the medial third MCP and MTP condyle and proximal first phalanx were removed with a band saw and fixed in 10% buffered neutral formalin for 7 days, then demineralized by formic acid, and cut into 5-μm sections for histopathologic evaluation. Each section was stained with both H&E and safranin O/fast green stain. A previously described scoring system28 was used to grade the articular cartilage. A score of 0 to 4 was assigned to chondrocyte necrosis, chondrone formation, focal loss of cells, and articular cartilage fibrillation on the H&E staining and on the safranin O/fast green stain with 0 being normal and 4 being the most severe for each category. Each parameter was evaluated individually, and a sum score for cartilage histology was obtained for evaluation.

**Statistical analysis**

Treated and control data at each collection time point were compared with a 2-sided Wilcoxon signed-rank test (R Core Team. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2000; https://www.R-project.org/). The physical examination data were evaluated graphically over time. For the clinicalopathologic data, time (pre and post) and treatment (control and treated) were dependent variables. To assess the change in clinicalopathologic data, the pretreatment data were subtracted from the post-treatment, and the groups were compared with the Wilcoxon signed-rank test.

All data were obtained in a blinded fashion. Because multiple comparisons were being made in an exploratory study, a P value of less than .05 was considered suggestive of differences between groups.

**Results**

The study included one 5, 6, and 7-year-old; two 8-year-old; two 9-year-old; and one 10-year-old (median, 8 years) mares with a median weight of 412.5 kg (range, 418 to 609 kg). All horses had normal behavior, temperature, and mucous membranes, with no palpable increase in heat in the investigation joints throughout the study. One horse was lame in the treated limb on day 7, and another treated horse was lame in the treated limb on days 126 and 240. One control limb was lame on day 49. All were grade 1 lame and transient, and no further localization was done. The only limb that was lame and had a positive flexion test was grade 1 lame with a score of 2 on flexion in a treated limb on day 139. Again, this was a transient finding with no further localization.

There were 12 recordings of positive flexion tests of treatment and control joints that were sporadic.

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**Figure 1**—The mean (SD) for the treated and control groups at each time point evaluated. Graphically, the data suggest that following administration of 4% polyacrylamide hydrogel (PAHG), there is an increase in effusion score. *Days the joints were administered the saline (control) or 4% PAHG (treatment).
and not related to the treatment group. The observed mean effusion score for the treatment group was larger than that for the control group in the period immediately following the treatment. That difference was not statistically significant (Figure 1). Except for the presence of effusion in some fetlocks, there were no other abnormal clinical signs associated with arthrocentesis and administration of the 4% PAHG or saline throughout the study period.

Synovial fluid volume on day 0 did not yield an adequate volume of sample material for both clinicopathologic and biomarker evaluation; therefore, the biomarkers were only available at the completion of the study. The mean WBC count increased for both the treated (pre = 386; post = 786 cells/µL) and control groups (pre = 267; post = 697 cells/µL) (Figure 2). The increase was predominantly from large mononuclear cells, and it was similar between treated (pre = 45%; post = 74%) and control (pre = 44%; post = 78%) groups. Statistical comparisons between treatment and control groups all resulted in a P value greater than .05.

On day 240 of the study, there were no significant differences in CPII between the treated group (684.5 ± 68.8; median ± SE) and the control group (776.5 ± 223.3) (Figure 3). There were also no significant findings between treated and control joints for C2C (T = 168.7 ± 16.0; C = 153.6 ± 30.3), C1,2C (T = 0.19 ± 0.05; C = 0.2 ± 0.02), and CS846 (T = 2861 ± 228; C = 3,117 ± 1,240).

Figure 2—The mean (SEM) WBC count was similar between treated and control groups at both time points with both groups having an increased total WBC count at the end of the study. The increase in WBCs was predominantly the result of increased number of mononuclear cells.

Figure 3—The mean (SEM) for the biomarkers evaluated on day 240 of the study are shown. The type 2 collagen synthesis biomarker (CPII), type II collagen cleavage (C2C), type I and II collagen cleavage (C1,2C), and chondroitin sulfate degradation (CS846) were not significantly different between treatment and control groups at the completion of the study.

Figure 4—The 4% polyacrylamide hydrogel (PAHG) was histologically evident in 5 of the 8 treated joints. The basophilic staining on the surface of the synovium (Baso Stain Syn), basophilic material associated with hyperplastic synoviocytes (Baso Syn), subsynovial basophilic material (Subsyn Baso), and cellular infiltrate (CI) associated with subsynovial basophilic material indicated there was PAHG in the joint 105 days after the final administration. There was no indication that PAHG was present in the saline-treated control joints. The mean (SEM) of histological findings in treated joints are shown, with individual data points.
The gross postmortem evaluation did not show any significant findings for the individual categories nor the sum score of the categories. One horse included in the study had bilateral erosions in the MCP joints. This horse did not show lameness and was not positive to flexion tests, and the clinicopathologic data were similar to the remainder of the horses.

There were no statistical differences in cellular infiltration, vascularity, intimal hyperplasia, subintimal edema, or subintimal fibrosis between the treated and control groups. At the end of the study, on day 240, PAHG was seen histologically in 5 of 8 treated joints and in none of the control joints. The PAHG on the surface of the synovium (median, 1; range, 0 to 2; \( P = .0533 \)) and on the subsynovial layer (1.0; 0 to 2; \( P = .0477 \)) (Figures 4 and 5), were all suggestive of differences between groups.

All of the cartilage scores for chondrocyte necrosis, cluster formation, fibrillation, and focal loss were 0, and all safranin O/fast green uptake scores were 0 or 1, and the \( P \) value for all comparisons was greater than .05.

**Discussion**

In this study, there were few differences noted between joints with serial administration of 4% PAHG and saline. Viscosupplementation with PAHG is a relatively new treatment for osteoarthritis in the horse, and there is a relative paucity of information. The mechanism of action of the 4% PAHG is surface and boundary lubrication.\(^8\) Initial investigations with this PAHG show that the material decreases friction and is present and even concentrated in areas of cartilage damage. With an inert material providing surface lubrication, it would be expected that there would be few differences between treated and saline control groups other than those simply related to the presence of the PAHG. The differences seen between groups were related to the presence of the PAHG. The effusion seen following injection was similar to that previously reported.\(^1\) PAHG is very hydrophilic,\(^2\) so when dispersed over the synovial membrane, it acts to hold fluid with it, resulting in the visual and palpable thickening of the joint. This did not result in any pathologic changes in the cartilage, synovial fluid, or synovial membrane. Histologically, the overall scores for cellular infiltration, vascularity, intimal hyperplasia, subintimal edema, and subintimal fibrosis were similar between the treated and control joints. The differences that were noted between groups were associated with the presence of the PAHG in and on the synovial membrane. It has previously been shown that there is ultimately phagocytosis of the PAHG by macrophagic cells in the synovial membrane, which is consistent with more reactive synoviocytes being present in the treated joints.\(^1\)

It is important to note that the findings in this study are only applicable to the 4% PAHG evaluated.
The 4% PAHG utilized in this study was created to have viscoelastic properties similar to normal synovial fluid. These findings are different from those reported by Christensen et al using 2.5% PAHG. Christensen identified a thick layer of PAHG within the synovium with mononuclear inflammatory cells present 24 months after administration in osteoarthritic joints. The difference between these PAHGs is not simply a difference in concentration. Polyacrylamides are custom-made synthetic molecules designed for specific applications, including ocular fillers, cosmetic fillers, and intra-articular injections. Differences in PAHGs are not evident by typical means of comparison, such as concentration and molecular weight, but are determined by their manufacturing process and molecular structure. The gels are specifically manufactured differently with varying cross-linkages and monomer concentrations, making them different materials. Changes in total monomer, total cross-linker, type of cross-linker, and temperature, among other factors, can create materials with a similar percentage of polyacrylamide that are structurally and mechanically different. It is frequently misunderstood that data obtained with one PAHG is relevant with a different PAHG. These data suggest that the commercially available 2.5% and 4% PAHGs react differently in the joint. In this study, after 4 serial injections and 105 days after the final administration, there is no large deposition of PAHG within the synovial membrane and no overall increase in inflammation and cellular infiltration in the joint.

The selection of the time points for administration of the PAHG and the termination of the study was somewhat arbitrary for this exploratory study. The decision to treat at 45-day intervals was based on how it has been used by some veterinarians, 2 treatments at 45-day intervals. The number of doses was doubled to increase the likelihood of any adverse outcomes being identified. The selection of 105 days after the last administration to the study termination was to allow time for any potential cartilage degradation and initiation of degenerative/inflammatory cycles to result in pathology that would be identified. It is recognized that a single administration of PAHG may result in decreased lameness for a minimum of 90 days. However, the objective of the accumulation study performed here was to identify if there were any potential adverse effects of serial administration. PAHG can potentially provide long-term disease-modifying effects because there are no degradative enzymes to digest PAHG as there are for hyaluronate. The presence of PAHG on the surface of the cartilage 105 days after administration is a positive finding. The recognition of surface lubrication and adherence of the PAHG in areas of cartilage degradation would suggest the PAHG can provide long-term surface lubrication.

The limitations of this study include that it was conducted as an exploratory study. There were no long-term data to establish outcomes that need further investigation and no specific timelines to utilize for study design. The outcomes were not unexpected based on previous knowledge; however, statistically significant outcomes where numerous outcomes are evaluated can be affected by type I error inflation and overstated. Therefore, to address the numerous outcomes that were statistically tested, a P value of less than .05 is conservatively considered suggestive of significant differences between groups. Another limitation of the study is that no synovial fluid was analyzed shortly after treatment, which would show any acute changes seen following the administration of 4.0% PAHG. There were minimal changes seen in a previous study at 7 days with 4% PAHG.

These data suggest that there are no detrimental effects of concentrated serial administration of 4% PAHG in normal equine fetlock joints.

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None reported.

Disclosures

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References

7. Zar VV, Troitsky VM, Stepanov AI, Lopatin VV. Future of viscoelastic behavior of 3-D structure of artificial and natural samples of articular liquids at the pressure 0.1–100 MPa. In: The 16th International Conference on Chemical Thermodynamics. Suzdal, 2007;16.
8. Vishwanath K, McClure SR, Bonassar LJ. Polyacrylamide hydrogel lubricates cartilage after biochemical degradation