Evaluation of polysulfated glycosaminoglycan or sodium hyaluronan administered intra-articularly for treatment of horses with experimentally induced osteoarthritis

David D. Frisbie, DVM, PhD; Chris E. Kawcak, DVM, PhD; C. Wayne McIlwraith, BVSc, PhD; Natasha M. Werpy, DVM

**Objective**—To assess clinical, biochemical, and histologic effects of polysulfated glycosaminoglycan (PSGAG) or sodium hyaluronan administered intra-articularly in treatment of horses with experimentally induced osteoarthritis.

**Animals**—24 horses.

**Procedures**—Osteoarthritis was induced arthroscopically in 1 middle carpal joint of all horses. Eight horses received hyaluronan (20 mg) and amikacin (125 mg) intra-articularly on study days 14, 21, and 28. Eight horses received PSGAG (250 mg) and amikacin (125 mg) intra-articularly on study days 14, 21, and 28. Eight control horses received 2 mL of saline (0.9% NaCl) solution and amikacin (125 mg) intra-articularly on study days 14, 21, and 28. Clinical, radiographic, synovial fluid analysis, gross, histologic, histochemical, and biochemical findings were evaluated.

**Results**—No adverse treatment-related events were detected. Induced osteoarthritis caused a substantial change in lameness, response to flexion, joint effusion, and radiographic findings, and of these, synovial fluid effusion was reduced with PSGAG, compared with control horses. No changes in clinical signs were seen with PSGAG or hyaluronan, compared with control horses. Histologically, the degree of synovial membrane vascularity and subintimal fibrosis was significantly reduced with PSGAG treatment, compared with controls. Histologically, significantly less fibrillation was seen with hyaluronan treatment, compared with controls.

**Conclusions and Clinical Relevance**—Results indicated that PSGAG and hyaluronan had beneficial disease-modifying effects and are viable therapeutic options for osteoarthritis in horses. (Am J Vet Res 2009;70:203–209)
Materials and Methods

Experimental design and induction of osteoarthritis—The Colorado State University Animal Care and Use Committee approved the study protocol for this experiment, which included the use of 24 healthy 2- to 5-year-old horses. Prior to inclusion in the study, horses underwent a lameness examination, and body condition, radiographs of the carpal joints, range of motion (flexion) of the carpal joints, and evidence of joint effusion were assessed to ensure all variables were within reference limits. On day 7 (7 days after induction of osteoarthritis), horses were evaluated for degree of lameness. The lameness scores were used to rank the horses from most to least lame and this list was used to blindly assign horses to 1 of the 3 treatment groups: hyaluronan (n = 8), PSGAG (8), or control (8). All evaluators were unaware of treatment assignment.

As described, on day 0, following anesthesia and routine preparation for surgery, each horse underwent bilateral arthroscopic surgery of the middle carpal joints to ensure that there were no preexisting abnormalities. During this procedure, an osteochondral fragment was created in 1 randomly selected middle carpal joint. The fragment was generated by use of an 8-mm curved osteotome directed perpendicular to the articular cartilage surface of the radial carpal bone at the level of the medial synovial plica. The fragment was allowed to remain adhered to the joint capsule proximally. A motorized arthroburr was used to debride the exposed subchondral bone between the fragment and parent bone. A 15-mm-wide defect bed for the 8-mm-wide fragment was created and the debris was not actively flushed from the joint, thereby inducing osteoarthritis. This joint was designated as the osteoarthritis-affected joint; the sham-operated joint was used as the control joint. The arthroscopic portals were closed with 2-0 nonabsorbable nylon suture in a simple interrupted pattern, and cyanoacrylate was applied to the incision. The forelimbs were bandaged, and the horses were allowed to recover from anesthesia and surgery. All study horses received 1 dose of ceftriaxone (2.2 mg/kg) IM prior to surgery and 2 g of phenylbutazone orally once daily for 5 days after surgery. Bandages were changed every 3 to 5 days and maintained until suture removal at 10 days after surgery.

Exercise—Horses were housed in stalls (3.65 × 3.65 m each). Beginning on day 15, horses were exercised on a high-speed treadmill 5 days each week until the end of the study. Each day, the horses were trotted (16 to 19 km/h) for 2 minutes, galloped (approx 32 km/h) for 2 minutes, and trotted again (16 to 19 km/h) for 2 minutes to simulate the strenuous exercise of race training.

Treatment groups—Treatment began on day 14. The 8 horses treated with hyaluronan were administered 20 mg and 125 mg of amikacin intra-articularly on study days 14, 21, and 28. The 8 horses treated with PSGAG were administered 250 mg and 125 mg of amikacin intra-articularly on study days 14, 21, and 28. The 8 placebo horses received 2 mL of saline (0.9% NaCl) solution and 125 mg of amikacin intra-articularly on study days 14, 21, and 28.

Assessment of clinical outcomes—Animal care personnel assessed horses daily for comfort, movement, and respiratory character. Clinical examinations of both forelimbs were performed weekly from day 0 (baseline) throughout the study period. Lameness was graded on a standardized scale of 0 to 5. All other clinical outcomes were graded on a scale of 0 to 4 (0 represented normal, and 4 represented severe change). As an indication of joint pain, carpal flexion was performed after lameness grading. As an indication of increased volume of synovial inflammation in the middle carpal joint, the extent of the effusion was graded after carpal flexion. All clinical outcome variables were assessed by a board-certified large animal surgeon who focuses on equine lameness.

Radiographic evaluation of both carpi was performed prior to inclusion in the study (day –7), following the induction of osteoarthritis (day 14), and at termination of the study (day 70). A board-certified radiologist assessed images. The radiographic images were evaluated for bony proliferation at the joint capsule attachment, subchondral bone lysis, and osteophyte formation on a scale of 0 to 4 (0 represented normal, and 4 represented severe change).

Synovial fluid—Beginning on day 0 until the end of the study (day 70), synovial fluid was aseptically aspirated once per week from both middle carpal joints of each horse. Synovial fluid (2 to 4 mL) was directly aspirated from the joints by use of a 20-gauge needle and syringe. Samples were placed in tubes containing EDTA for routine synovial fluid analysis (total protein concentration, cytologic evaluation, and total WBC count) or stored at –80°C for biochemical protein analysis.

The conventional analysis of synovial fluid included assessment of total protein concentration, WBC count, and differential count. Total protein and WBC concentrations were determined via refractometry and use of an automated cell counter, respectively. Smears of synovial fluid were examined microscopically to determine the differential WBC count.

Two biomarker protein assays were performed on synovial fluid. A modified 1,9-dimethyl-methylene blue dye–binding assay was used to determine glycosaminoglycan concentration. Synovial fluid concentration of PGE 2 was assessed by use of a commercially available, competitive, high-sensitivity enzyme immunoassay kit as directed by the manufacturer’s instructions. This included extraction by use of C2 columns as suggested by the manufacturer. Samples were processed in duplicate, and results were expressed in picograms per milliliter.

Gross pathologic observations of joints—At the end of the study, all horses were euthanatized by administration of pentobarbital sodium. For each horse, a necropsy examination was performed during which both middle carpal joints were specifically examined for degree and location of articular cartilage fibrillation or erosion. A subjective grade (scale of 0 to 4) was assigned for partial- and full-thickness cartilage erosion as well as synovial membrane hemorrhage; for each of the 3 variables, grade 0 represented no pathologic change...
and 4 represented a severe change. A total erosion score was assigned on the basis of overall joint health, also with a scale of 0 to 4.

**Histologic examinations**—At necropsy, samples of synovial membrane and joint capsule were harvested from the region dorsal to the osteochondral fragment and placed in neutral-buffered 10% formalin for H&E staining. Five-micron sections of the tissue samples were prepared. An evaluator who was unaware of treatment assignments assessed the sections of synovial membrane and joint capsule for cellular infiltration, synovial intimal hyperplasia, subintimal edema, subintimal fibrosis, and subintimal vascularity. Each variable was graded on a scale of 0 to 4 (0 represented no abnormal change, and 4 represented the most severe change). A cumulative pathology score was also calculated for synovial membrane samples.

Articular cartilage pieces (5 mm³) were obtained from each joint (Figure 1). Samples were stored in neutral-buffered 10% formalin for 7 days and then processed routinely for histologic examination by an evaluator who was unaware of treatment assignment. The 5-μm sections were stained with both H&E and safranin O fast green. Collection locations were chosen to represent an area directly adjacent to the osteochondral fragment, a portion of the opposing articulating surface (third carpal bone), and a remote location (fourth carpal bone).

The H&E-stained sections were evaluated for articular cartilage fibrillation, chondrocyte necrosis, chondrone formation (chondrocyte division within a lacuna), and focal cell loss. Numeric values ranging from 0 to 4 were assigned to each variable (0 represented no abnormal change, and 4 represented the most severe change). A cumulative pathology score (modified Mankin score) for each articular cartilage sample was also determined. The analysis of the histologic outcome parameters also considered location of sample collection.

![Image](Figure 1—Illustration of cartilage collection sites (a) in the middle carpal joint of horses. Articular cartilage was collected for histologic evaluation from the intermediate carpal bone (C1). CR = Radial carpal bone. CU = Ulnar carpal bone. C2 = Second carpal bone. C3 = Third carpal bone. C4 = Fourth carpal bone.)

![Image](Figure 2—Plot of the improvement in lameness score in control horses and horses with induced osteoarthritis treated with hyaluronic acid, no treatment, and PSGAG. A positive score represents improvement in lameness following treatment. Different letters indicate significant (P < 0.05) differences among groups. PCB = Placebo. HA = Hyaluronic. No OA = No osteoarthritis (control). OA = Osteoarthritis.)

Without knowledge of treatment assignments, articular cartilage sections stained with safranin O, fast green were evaluated for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones of the third carpal bone, fourth carpal, and radial carpal bones. Numeric values ranging from 0 to 4 were assigned to each variable (0 indicated no stain uptake, and 4 indicated normal stain uptake), and a cumulative score for each articular cartilage sample was calculated by summation of the zonal scores.

**Articular cartilage matrix evaluation**—To estimate articular cartilage proteoglycan content, the total articular cartilage glycosaminoglycan content was measured by use of a 1,9-dimethyl-methylene blue technique. Articular cartilage pieces were obtained from the area directly adjacent to the osteochondral fragment and a remote site within each joint. Each piece was stored at –80°C prior to further processing and analysis. Samples were processed in duplicate, and results were reported in micrograms of glycosaminoglycan per milliliter (cartilage was digested prior to analysis at a ratio of 10 mg of wet weight/mL of papain digest).

For analysis of cartilage matrix metabolism, articular cartilage samples were aseptically collected from the weight-bearing surface that was remote from the osteochondral fragment within each joint, and incorporation of sulfur 35 radiolabeled SO₄ was measured by use of reported methods. Samples were processed in duplicate, and the results were reported as counts per minute per milligram of dry weight.

**Statistical analysis**—Data were evaluated by use of an ANOVA framework with a software program with the horse as a random variable. The ANOVA tables were used to determine significant (P < 0.05) main effects and interactions between main effect variables. When individual comparisons were made, least square means was used and P < 0.05 was considered significant. Values are reported as mean ± SEM.
Results

Musculoskeletal variables—All horses had a significant ($P < 0.001$) increase in lameness score in the osteoarthritis-affected (mean ± SEM, 2.25 ± 0.13) limb, compared with that in the control limb (0.38 ± 0.13) for days 7 and 14. Change in lameness was calculated by use of day 14 (the last pretreatment evaluation) as the postosteoarthritis pretreatment baseline (a positive change score indicates improvement). There was no significant improvement in lameness score with respect to treatment (Figure 2).

Flexion—All horses had a significant ($P < 0.001$) increase in flexion score in the osteoarthritis-affected (1.80 ± 0.11) limb, compared with that in the control limb (0.29 ± 0.11) for days 7 and 14. Change in flexion was calculated by use of day 14 as the postosteoarthritis pretreatment baseline. No significant treatment effects were observed.

Joint effusion—All horses had a significant ($P < 0.001$) increase in synovial effusion score in the osteoarthritis-affected joints, compared with that of control joints (1.13 ± 0.13) for day 14. Change in joint effusion was calculated by use of day 14 as the postosteoarthritis pretreatment baseline. There was a significant ($P < 0.001$) improvement in joint effusion for osteoarthritis-affected joints in horses treated with PSGAG, compared with effusion in control or hyaluronan-treated horses (Figure 3).

Radiographic evaluation—A significant increase in radiographic joint changes was detected for each radiographic outcome variable after surgery. Total radiographic scores before treatment for control (0.40 ± 0.35) versus osteoarthritis-affected (4.08 ± 0.26) joints were significantly ($P < 0.001$) different. No significant treatment effects were detected.

Synovial fluid—Results of routine synovial fluid analysis indicated that, as expected, the total protein concentration increased significantly ($P < 0.001$) with induction of osteoarthritis (2.37 ± 0.08 g/dL) throughout the study period, compared with that in control joints (2.04 ± 0.08 g/dL). Synovial fluid WBC count was significantly ($P < 0.001$) increased in osteoarthritic versus control joints (437 ± 30 cells/dL vs 308 ± 30 cells/dL, respectively). There were no significant treatment effects in synovial total protein concentration or WBC counts.

The glycosaminoglycan concentration in synovial fluid was significantly ($P < 0.001$) increased (3.85 ± 0.05 ln µg of glycosaminoglycan/mL) versus control (3.36 ± 0.05 ln µg of glycosaminoglycan/mL) joints. There were no significant treatment effects.

Synovial fluid PGE$_2$ concentrations were significantly ($P < 0.001$) increased with induction of osteoarthritis (5.19 ± 0.10 ln pg/mL), compared with control (4.22 ± 0.10 ln pg/mL). There were no significant treatment effects.

Gross pathologic observations of joints—At necropsy, hemorrhage score within the synovial...
membrane was significantly ($P < 0.001$) increased in osteoarthritis-affected ($1.83 \pm 0.15$) versus control ($0.63 \pm 0.15$) joints. Similarly, the articular cartilage total erosion score was significantly ($P < 0.001$) increased in osteoarthritis-affected ($1.67 \pm 0.16$) versus control ($0.42 \pm 0.16$) joints. No significant treatment effects were seen for any of the gross pathologic observations.

**Synovial membrane**—Induction of osteoarthritis did not result in significant effects on degree of synovial membrane cellular infiltration, subintimal edema, or intimal hyperplasia, and no significant treatment effects were observed. For the effect of treatment on synovial membrane vascularity, the $P$ value (0.085) approached significance. Specifically, when individual comparisons with control values were made, the $P$ value for joints treated with hyaluronan was 0.061 and for joints treated with PSGAG was 0.019 (Figure 4). Similarly, for synovial membrane fibrosis, the $P$ value (0.057) for treatment effects approached significance. Specifically, when individual comparisons with control values were made, the $P$ value for joints treated with hyaluronan was 0.077 and for joints treated with PSGAG was 0.022 (Figures 5 and 6).

**Articular cartilage**—Histologic evaluation of the articular cartilage via H&E staining revealed a significant ($P$...
< 0.001) increase in the modified Mankin score for osteoarthritis-affected joints (3.12 ± 0.32), compared with the score for control joints (1.04 ± 0.32), when all locations were considered. There were no significant treatment effects observed on the basis of the total modified Mankin score; however, fibrillation was significantly (P = 0.018) less with treatment by location from which cartilage samples were obtained, compared with fibrillation in osteoarthritis-affected joints in control horses. Specifically, when individual comparisons were made, treatment with hyaluronan in osteoarthritis-affected joints significantly (P = 0.007) improved fibrillation score and the P value for treatment with PSGAG (0.083) approached significance, compared with the value for control horses (Figures 7 and 8).

Articular cartilage stained with safranin O, fast green —Evaluation of articular cartilage for safranin O fast green staining score revealed a significant (P = 0.012) decrease in staining of osteoarthritis-affected joints (4.89 ± 0.47), compared with staining of control joints (6.33 ± 0.46) for the cumulative score on the third carpal bone. No other comparisons were significantly different, including treatment effects.

Articular cartilage matrix evaluation—No significant difference was detected regarding cartilage glycosaminoglycan content with respect to induction of disease or treatment group. The glycosaminoglycan synthesis was significantly (P = 0.013) higher in cartilage from osteoarthritis-affected joints (4,236 ± 262 disintegrations/min per µg of glycosaminoglycan), compared with that in control joints (3,379 ± 262 disintegrations/min per µg of glycosaminoglycan). A significant (P = 0.021) difference was also detected in glycosaminoglycan synthesis on the basis of treatment group in osteoarthritis-affected joints, compared with that in control joints. Specifically, osteoarthritis-affected joints treated with PSGAG had lower glycosaminoglycan synthesis, compared with control joints or hyaluronan-treated osteoarthritis-affected joints (Figure 9).

Discussion

In the present study, experimentally induced osteoarthritis effectively resulted in clinical, gross, histologic, and biochemical changes indicative of osteoarthritis. The lesions are thought to represent both acute synovitis and traditional osteoarthritis that includes full-thickness cartilage erosion. During this study, no adverse events were recorded with any of the treatments and a mild degree of lameness was induced (typically grade 2 on a scale published by the American College of Equine Practitioners).

There was no significant improvement in clinical lameness in any treatment group. This was in contrast to results of a study by Auer et al, in which harsher carpal osteochondral fragmentation but a similar hyaluronan product was used, and a study of clinical cases by Gaustad et al with a similar PSGAG and different hyaluronan. In the study by Auer et al, intra-articular treatment with hyaluronan improved force plate values in treated limbs. One explanation may be in the difference between use of arthroscopy versus arthrotomy to create articular fragments, which may affect the degree of soft tissue involvement. Other variables such as exercise protocol and bilateral fragments versus unilateral fragments also could explain differences. The variability of the clinical metacarpophalangeal (fetlock) joint cases reported by Gaustad et al makes comparison between results of that study and those of the present study difficult. It is interesting that IV administration of hyaluronan did reduce lameness in a previous study that used a similar technique. However, at that time, the technique induced more acute inflammatory changes in the synovial membrane and more acute synovitis with less articular cartilage change. Specifically, greater improvements in synovial membrane histologic scores and synovial fluid total protein concentrations including PGE₂, were detected, compared with improvements in the present study. In the present study, although beneficial improvements were detected in disease-modifying variables, no changes in clinical signs were detected.

Significant improvement in synovial fluid effusion scores was seen with PSGAG, suggesting a beneficial effect on joint soft tissues and joint inflammation that was superior to treatment with hyaluronan or saline solution. This was confirmed by improvements in synovial membrane histologic variables and was similar to previous observations in synovial fluid effusion after chemically induced synovitis. It was interesting that no change in synovial fluid PGE₂ concentration was observed, especially because that would be expected with a decrease in inflammation and that has been seen with PSGAG treatment in vitro. This was not a unique finding and may suggest that a reduction in PGE₂ concentration is not necessary for decreased synovial effusion and improvement in synovial membrane variables. When administered systemically, use of hyaluronan induced a significant reduction in synovial fluid PGE₂ concentration as well as a more profound effect on synovial membrane variables. Although not significant, scores for vascularity and subintimal fibrosis in the synovial membrane in treated versus control horses in this study suggested improvement but, compared with previous results, were of lesser magnitude. Conversely, the reduction in articular cartilage fibrillation was greater in the present study and suggested a more potent effect of locally administered hyaluronan to improve cartilage health in a diseased joint.

A significant reduction in glycosaminoglycan synthesis was observed in osteoarthritis-affected joints treated with PSGAG, compared with those treated with hyaluronan or saline solution. This finding suggested a beneficial effect of PSGAG. The authors have also reported an increase in glycosaminoglycan synthesis in association with early osteoarthritis changes.

Clinically, reports suggest that hyaluronan may work better in acute synovitis and PSGAG in osteoarthritis, but these findings were not supported by the present study. Both medications yielded similar improvements in various outcome variables, suggesting good efficacy especially in cases of synovitis. Hyaluronan appeared to have a greater effect on decreasing the degree of articular cartilage fibrillation, compared with PSGAG, and the magnitude of positive effects on the synovial membrane was greater with PSGAG, compared with hyaluronan. Both PSGAG and hyaluronan had disease-modifying osteoarthritis drug actions in the present study.
References


