Efficacy of intravenous administration of hyaluronan, sodium chondroitin sulfate, and N-acetyl-d-glucosamine for prevention or treatment of osteoarthritis in horses

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OBJECTIVE
To evaluate the efficacy of IV administration of a product containing hyaluronan, sodium chondroitin sulfate, and N-acetyl-d-glucosamine for prevention or treatment of osteoarthritis in horses.

ANIMALS
32 healthy 2- to 5-year-old horses.

PROCEDURES
The study involved 2 portions. To evaluate prophylactic efficacy of the test product, horses received 5 mL of the product (n = 8) or saline (0.9% NaCl) solution (8; placebo) IV every fifth day, starting on day 0 (when osteoarthritis was induced in the middle carpal joint of 1 forelimb) and ending on day 70. To evaluate treatment efficacy, horses received either the product or placebo (n = 8/treatment) on days 16, 23, 30, 37, and 44 after osteoarthritis induction. Clinical, diagnostic imaging, synovial fluid, gross anatomic, and histologic evaluations and other tests were performed. Results of each study portion were compared between treatment groups.

RESULTS
Limb flexion and radiographic findings were significantly worse for horses that received the test product in the prophylactic efficacy portion than for placebo-treated horses or product-treated horses in the treatment efficacy portion. In the prophylactic efficacy portion, significantly less articular cartilage erosion was identified in product-treated versus placebo-treated horses. In the treatment efficacy portion, joints of product-treated horses had a greater degree of bone edema identified via MRI than did joints of placebo-treated horses but fewer microscopic articular cartilage abnormalities.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that caution should be used when administering the evaluated product IV to horses, particularly when administering it prophylactically, as it may have no benefit or may even cause harm. (Am J Vet Res 2016;77:1064–1070)

Joint disease, specifically osteoarthritis, is one of the most prevalent and debilitating diseases affecting horses and has a considerable economic impact on the equine industry.1-4 Various medications have been evaluated or used for the treatment of osteoarthritis in horses, including hyaluronan and glucosamine as well as combinations of these products.5

In a recent survey of equine practitioners, 18% of respondents indicated that they had used a commercial product containing hyaluronan, sodium chondroitin sulfate, and N-acetyl-d-glucosamine for the treatment of horses with osteoarthritis. This formulation, which has not been approved by the US FDA for use in horses, is typically administered as a 5-mL dose that contains 25 mg of hyaluronic acid sodium salt, 500 mg of sodium chondroitin sulfate, and 500 mg of N-acetyl-d-glucosamine. Information on the product’s label suggests intra-articular use as a postsurgical joint lavage solution, and a prior study by our research group revealed beneficial effects when administered this way. Interestingly, 60% of practitioners in the aforementioned survey reported administering the product IV, 22% intra-articularly, and 18% IM, despite the label recommendations for intra-articular use. The purpose of the study reported here was to assess the effects of this product when administered IV before or after the onset of osteoarthritis on clinical signs or disease in horses with.
experimentally induced osteoarthritis and to monitor for any adverse effects as a result of this administration.

**Materials and Methods**

**Horses**

Thirty-two healthy 2- to 5-year-old horses were included in the study. The horses were of mixed breed and mares or geldings obtained from the university vendor. Results of musculoskeletal examination were unremarkable for all horses. The study protocol was approved by the Institutional Animal Care and Use Committee of Colorado State University (approval No. 09-120A-02).

**Experimental design**

Eight horses were randomly assigned to each of 4 treatment groups by drawing from a hat. The number of horses assigned to each group had been determined through a power calculation that incorporated data from previous research by our group. Specifically, an effect size of 1 (SD, 0.56) for lameness grade yielded a power of 0.94 when 8 horses were included in each group. This sample size also provided a power > 0.80 for detection of differences in other outcome variables such as synovial fluid total protein concentration, synovial membrane cellular infiltration and hyperplasia, or articular cartilage erosion.

To evaluate the prophylactic efficacy of the product containing hyaluronan, sodium chondroitin sulfate, and N-acetyl-d-glucosamine (test product) for preventing osteoarthritis, horses in the treatment group received 5 mL of test product IV and horses in the placebo group received 5 mL of saline (0.9% NaCl) solution IV. Both groups received their assigned treatment every fifth day, starting at the time of surgical induction of osteoarthritis (day 0) and ending at study termination (day 70). This portion of the study was run concurrently with the study in which the intra-articular effect of the test product had been evaluated. Horses in the placebo group in this portion of the study were the same as those used in the other study. Horses also received intra-articular administration of 5 mL of saline solution as well as 125 mg of amikacin sulfate solution in both middle carpal joints on days 0, 7, 14, and 28.

To evaluate the efficacy of the test product for treatment of osteoarthritis, horses in the treatment group received 5 mL of test product IV and horses in the placebo group received 5 mL of saline solution IV on days 16, 23, 30, 37, and 44 after osteoarthritis induction (on day 0). Horses in this portion of the study received no intra-articular treatments. Dose and administration frequencies for the test product in both portions of the study were chosen on the basis of feedback provided to the manufacturer by practitioners.

**Experimental induction of osteoarthritis**

Phenylbutazone paste (2 g) was administered PO once daily for 5 days beginning just prior to experimental induction of osteoarthritis. Then, osteoarthritis was induced in each horse as described elsewhere. Briefly, on day 0, each horse received ceftiofur sodium (2.2 mg/kg, IM) and then underwent bilateral arthroscopic surgery of the middle carpal joints, with 1 randomly selected middle carpal joint serving as a sham-operated control joint. An osteochondral fragment was created on the radial carpal bone of the other (osteoarthritis-affected) middle carpal joint. A motorized arthroburr was used to debride the exposed subchondral bone between the created fragment and parent bone, leaving a 15-mm-wide defect bed; the debris was not actively flushed from the joint. The arthroscopic portals were closed, and both forelimbs were bandaged in a routine manner. Bandages were changed every 3 to 5 days and maintained until day 7; sutures were removed 10 days after surgery.

**Exercise regimen**

Horses were housed in stalls (3.65 X 3.65 m). Beginning on day 16, horses were exercised on a high-speed treadmill 5 days/wk for 8 weeks (until day 70). For each exercise session, horses were encouraged to trot (16 to 19 km/h) for 2 minutes, gallop (approx 32 km/h) for 2 minutes, and trot again (16 to 19 km/h) for 2 minutes to simulate the strenuous exercise of race training.

**Assessment of clinical outcomes**

For all subjective assessments of clinical outcomes, evaluators were unaware of (blinded to) treatment assignment and were experts (≥ 10 years' experience) in the subject matter of their evaluation. The same person performed all subjective lameness assessments (CEK), radiographic and MRI evaluations (NMW), and all other assessments (DDF).

Clinical lameness examinations of both forelimbs of each horse were performed by use of a 6-point grading system (0 = no lameness and 5 = non-weight bearing) every other week from day 0 (baseline) throughout the study period. After lameness severity was evaluated, signs of joint pain were assessed by performance of carpal flexion tests, and severity of synovial effusion in the middle carpal joint was also evaluated. For these 2 variables, a 5-point scale was used, by which 0 represented unremarkable and 4 represented severe changes.

**Diagnostic imaging of middle carpal joints**

Bilateral carpal radiographic evaluation was performed on day 0 (prior to osteoarthritis induction) and days 14 and 70. Radiographic images of each forelimb were subjectively evaluated for 4 variables: subchondral bone lysis, bone proliferation at the attachment of the joint capsule, trabecular bone sclerosis, and periaricular osteophyte formation. Changes relative to the previous assessment point were scored on a scale of 0 to 4, by which 0 represented no important changes, 1 represented slight changes, 2 represented mild changes, 3 represented moderate changes, and 4 represented severe changes.
ere changes (ie, day 14 vs day 0 or day 70 vs day 14). A total radiographic score was also calculated for each limb at all assessment points on the basis of combined values of the scores assigned for the 4 subjectively assessed variables, allowing for a total value of 0 to 16.

Bilateral MRI of carpal joints (sagittal, transverse, and frontal plane images from proton density and short tau inversion recovery sequences) was performed for horses in the treatment efficacy portion of the study on days 13 to 15 and day 70. The MRI images were subjectively evaluated for 7 variables: synovial fluid, synovial proliferation, joint capsule thickness, joint capsule edema, joint capsule fibrosis, edema, and sclerosis of the radial carpal and third carpal bones. An 11-point scale was used to score each variable, by which 0 represented no change and 10 represented severe changes, compared with findings on days 13 to 15. A total MRI score was also calculated for each forelimb at all assessment points on the basis of the combined value for the 7 MRI outcome variables, allowing for a total value of 0 to 70.

**Assessment of synovial fluid**

Beginning on day 0, synovial fluid samples were aseptically collected once per week from both middle carpal joints of each horse. Collected samples were analyzed for total protein concentration, WBC content, and differential WBC counts. Total protein and WBC concentrations were determined via refractometry and an automated cell counter, respectively. Smear preparations made from synovial fluid samples were examined microscopically to determine the differential WBC count. An aliquot of each synovial fluid sample was also stored at –80°C for biochemical analysis.

Two biomarker protein assays were performed on synovial fluid. A modified 1,9-dimethyl-methylene blue dye-binding assay was used to measure GAG concentration. Synovial fluid concentration of prostaglandin E₂ was assessed by use of a competitive, high-sensitivity enzyme immunoassay kit as directed by the manufacturer. This included extraction by use of C2 columns as suggested by the manufacturer. All samples were processed in duplicate.

**Gross pathological observations of joints**

At the end of the study on day 70, all horses were euthanized by administration of an overdose of pentobarbital sodium solution. Both forelimbs were harvested, and middle carpal joints were examined for degree and location of articular cartilage fibrillation or erosion. A subjective grade (scale of 0 to 4) was assigned to each joint for 3 variables: partial and full thickness cartilage erosion and synovial membrane hemorrhage. For each of the 3 variables, 0 represented no pathological change, 1 represented slight change, 2 represented mild change, 3 represented moderate change, and 4 represented severe change. A total erosion score was assigned to each joint to reflect overall joint health by means of the same grading scheme.

**Histologic examination of joint tissues**

Samples of synovial membrane and articular cartilage were harvested from specific locations (Figure 1) in both middle carpal joints and placed in neutral-buffered 10% formalin. Sections (5 µm thick) were prepared from the samples and stained with H&E; cartilage sections were also stained with SOFG. Synovial membrane sections were graded for degrees of cellular infiltration, synovial intimal hyperplasia, subintimal edema, subintimal fibrosis, and subintimal vascularity. Articular cartilage sections were graded for cartilage fibrillation, chondrocyte necrosis, cluster formation (chondrocyte division within a lacuna), and focal cell loss. For all variables, scores ranged from 0 to 4, with 0 representing no abnormal change and 4 representing most severe change. A cumulative synovial membrane or cartilage morphology score (modified Mankin score) was calculated. Articular cartilage sections stained with SOFG were also evaluated for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones of the third carpal, fourth carpal, and radial carpal bones. Numeric scores ranging from 0 to 4 were assigned to each variable (0 = no stain uptake, 1 = slight stain uptake, 2 = mild stain uptake, 3 = moderate stain uptake, and 4 = normal stain uptake), and a cumulative score (total SOFG score) for articular cartilage sections in each location was calculated by summation of the zonal scores, allowing for a total value of 0 to 20 for each sample.

**Articular cartilage matrix evaluation**

Total articular cartilage GAG content was measured by use of a previously reported technique. Articular cartilage pieces were obtained from the area directly adjacent to the osteochondral fragment and a remote site within each joint or from a similar location in control joints (Figure 1). Each piece was stored at –80°C prior to further processing and analysis. For analysis, cartilage was digested at a ratio of 10 mg of tissue (wt weight)/μL of papain digest, and GAG concentration was measured for each location in each joint.

For analysis of cartilage matrix metabolism, articular cartilage samples were collected from a specific location in each joint (Figure 1) and radiolabeled. Radiolabeled SO₄ incorporation was measured by use of methods described elsewhere. Samples were processed in duplicate, and results were reported as counts per minute per mg of tissue (dry weight) for each joint.

**Statistical analysis**

Data from the prophylactic and treatment efficacy portions of the study were considered separately. Results are reported as mean ± SEM. Within-horse comparisons were made between osteoarthritis-affected and control joints for all measured variables by means of a mixed-model ANOVA with horse as a random effect. The ANOVA tables were used to identify
significant main effects and 2- and 3-way interactions between main effect variables when appropriate. All comparisons were taken into account and the interactions of highest significance reported. When individual comparisons were indicated by results of a protected \( F \) test, least squares means were calculated. Values of \( P \leq 0.05 \) were considered significant for all tests.

**Results**

**Clinical outcomes**

All horses had a significant \( (P < 0.001) \) increase in lameness grade in the osteoarthritis-affected limb (mean \( \pm \) SEM grade, 2.3 \( \pm \) 0.1), compared with grades for sham-operated control joints (0.0 \( \pm \) 0.1) at each assessment point throughout the 71-day study period. This result was achieved both for horses in the prophylactic efficacy portion of the study and for horses in the treatment efficacy portion, independent of whether horses received the test product or the placebo IV. No significant improvement in degree of lameness was achieved with test product administration, compared with placebo administration, in either portion of the study at any assessment point.

All horses had a significant \( (P < 0.001) \) increase in synovial effusion score for the middle carpal joint of the osteoarthritis-affected limb (mean score, 2.4 \( \pm \) 0.2), compared with the score for the control limb (0.4 \( \pm \) 0.2) at each assessment point throughout the study period, independent of treatment received. No significant effects on synovial effusion scores were achieved with test product administration, compared with placebo administration, in either portion of the study at any assessment point.

**Diagnostic imaging**

Radiographic scores for all measured variables were significantly greater than those before disease induction (day 0) for osteoarthritis-affected joints, independent of treatment received. The final (day 70) total radiographic score for osteoarthritis-affected joints at each assessment point was also significantly \( (P < 0.001) \) greater than that for control joints. In the prophylactic efficacy portion of the study, the degree of pathological change for the osteoarthritis-affected middle carpal joint of product-treated hors-
es was significantly greater than that of placebo-treated horses (Figures 1 and 2). When the same comparison was made between the 2 groups in the treatment efficacy portion, a similar pattern existed but the difference was not significantly different. Scores for bone proliferation, sclerosis, and osteophyte explained the significant increase in total radiographic score associated with test product administration in the prophylactic efficacy portion of the study (Table 1).

Results of MRI evaluations indicated that osteoarthritis-affected joints had a significantly \((P < 0.001)\) greater degree of pathological change as represented by cumulative MRI scores (mean score, \(17.7 \pm 0.4\)) than did control joints (\(12.5 \pm 0.4\)), independent of time and treatment received. The only significant \((P = 0.03)\) difference identified was a greater score for edema around the radial carpal bone on day 70 for the osteoarthritis-affected limb of horses that received the test product (\(1.3 \pm 0.19\)) than for the affected limb of horses that received the placebo (\(0.7 \pm 0.2\)).

**Synovial fluid**

Total protein concentration in synovial fluid samples was significantly \((P < 0.02)\) greater for osteoarthritis-affected joints (\(2.55 \pm 0.06 \text{ g/dL}\)) than for control joints (\(2.19 \pm 0.06 \text{ g/dL}\)) at all assessment points, independent of treatment received. Synovial fluid GAG concentration was also significantly \((P < 0.02)\) greater for osteoarthritis-affected joints (\(4.09 \pm 0.03 \text{ mg/dL}\)) than for control joints (\(3.98 \pm 0.03\)) at all assessment points, independent of treatment received. No significant effect of treatment with test product on synovial total protein or GAG concentration was identified in either portion of the study, compared with effects of the placebo.

**Gross pathological scores**

A significant increase in full- \((P < 0.01)\) and partial- \((P < 0.05)\) thickness cartilage erosions as well as synovial membrane hemorrhage \((P < 0.03)\) was identified in osteoarthritis-affected versus control joints, independent of treatment received. Mean total erosion score was \(1.3 \pm 1.8\) for osteoarthritis-affected joints and \(0.5 \pm 1.8\) for control joints, and mean total hemorrhage scores were \(1.4 \pm 2\) and \(0.6 \pm 0.2\), respectively. Significantly less full-thickness articular cartilage erosion in the osteoarthritis-affected joint was identified for horses that prophylactically received the test product (mean score, \(0.6 \pm 0.2\)) than for those that prophylactically received the placebo (\(1.4 \pm 0.2\)). A similarly significant difference was not identified in the treatment efficacy portion of the study.

**Histologic evaluation**

Cumulative histologic scores for middle carpal joint tissues were greater, albeit not significantly (F test, \(P = 0.059\)), for horses that received the test product (mean score, \(6.75 \pm 0.73\)) than for those that received the placebo (\(4.50 \pm 0.73\)) in both portions of the study. No other significant differences in cumulative histologic scores were identified.

Histologic evaluation of the articular cartilage revealed significantly \((P < 0.03)\) greater scores for all histologic variables as well as the cumulative modified Mankin score for osteoarthritis-affected joints versus control joints, independent of treatment received. For osteoarthritis-affected cartilage on the radial carpal bone specifically, a significantly \((P = 0.001)\) lower cumulative pathological score was identified for horses in the treatment efficacy portion of the study that received the test product (mean score, \(2.4 \pm 0.7\)) versus those that received the placebo (\(4.6\))

Table 1—Mean ± SEM combined scores for specific radiographic variables assigned on days 14 and 70 and total radiographic score assigned on day 70 for middle carpal joints of horses that received 5 mL of a product containing hyaluronic, sodium chondroitin sulfate, and N-acetyl-D-glucosamine, IV, before (B) or at various points after (C) experimental induction of osteoarthritis, beginning on day 14. In panel A, no evidence of osseous proliferation or osteophyte formation is visible at the site of the surgically induced osteochondral fragment. In contrast, in panels B and C, osseous proliferation or osteophyte formation is evident at the site of the surgically induced osteochondral fragment and is characterized by an irregular dorsodistal medial margin and a sharply marginated, abnormally shaped periarticular margin, which is more pronounced in panel C.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Product</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone proliferation at the attachment of the joint capsule</td>
<td>(1.4 \pm 0.2^a)</td>
<td>(0.9 \pm 0.7^b)</td>
</tr>
<tr>
<td>Subchondral bone sclerosis</td>
<td>(2.9 \pm 0.2^a)</td>
<td>(2.4 \pm 0.2)</td>
</tr>
<tr>
<td>Osteophyte formation</td>
<td>(1.1 \pm 0.2^a)</td>
<td>(0.5 \pm 0.2)</td>
</tr>
<tr>
<td>Subchondral bone lysis</td>
<td>(2.1 \pm 0.2^a)</td>
<td>(2.0 \pm 0.2)</td>
</tr>
<tr>
<td>Total radiographic score</td>
<td>(7.5 \pm 0.3^a)</td>
<td>(5.6 \pm 0.3)</td>
</tr>
</tbody>
</table>

*Within a row, values with different superscript letters differ significantly \((P < 0.05)\).*
Radiographic score (product-treated horses having the treatment efficacy portion with respect to total lactic efficacy portion of the study was identified in the outcomes observed in the present study. Where otherwise beneficial versus detrimental effect of the crease in proliferation at the joint capsule attachment ion may have been associated with the significant increase in adverse response to carpal flex administration. However, interaction between the tested product and amikacin could not be ruled out. It also should be pointed out that the experimental model of osteoarthritis used in the present study did not represent all degrees of osteoarthritis severity in clinically affected horses, and this should be considered in interpretation of the results. When the product containing hyaluronan, sodium chondroitin sulfate, and N-acetyl-D-glucosamine was administered prophylactically in the present study, a decrease in gross degree of articular cartilage erosion in osteoarthritis-affected middle carpal joints was observed; however, joint flexion test and radiographic outcomes in that portion of the study were clinically worse in product-versus placebo-treated horses. Differences were not significant. Magnetic resonance imaging was performed to obtain a more in-depth evaluation of osteoarthritis-affected joints, revealing more bone edema in product-treated versus placebo-treated horses in the treatment efficacy portion of the study. We presume that the difference in the results of product treatment in that portion versus the prophylactic efficacy portion was attributable to the timing of administration. We therefore conclude that caution should be used when administering the evaluated product to horses IV, particularly when more beneficial in vivo results were obtained with intra-articular administration of the product in the previous study.

Discussion

In a recent survey, equine practitioners reported that the most common reason for IV administration of a product containing hyaluronan, sodium chondroitin sulfate, and N-acetyl-D-glucosamine to horses was the treatment or prevention (prophylactic) of joint problems. When such use was evaluated in horses with experimentally induced osteoarthritis in the present study, surprising results were observed. Specifically, there was no indication of modifying effects on clinical signs (ie, no decrease in lameness severity). In fact, horses prophylactically treated with the product (5 mL, IV) were more adversely responsive to carpal flexion tests than were horses prophylactically treated with a placebo, and those product-treated horses also had more severe radiographic changes in the osteoarthritis-affected joint than did the placebo-treated horses. Specifically, bone proliferation at the joint capsule attachment and osteophyte formation were greater in product-treated horses than in placebo-treated horses in the present study and in horses that received the product intra-articularly in a previous study. The degree of radiographic subchondral bone sclerosis in osteoarthritis-affected joints was also greater in product-treated horses than in placebo-treated horses in the prophylactic efficacy portion of the study. Also important was the finding that the total radiographic score at the end of the assessment period in the present study was 29% greater for product-treated versus placebo-treated horses in the prophylactic efficacy portion, which would be considered clinically relevant in horses with clinical disease. No beneficial effect of IV product administration was identified radiographically.

For osteoarthritis-affected joints, the gross degree of full-thickness articular cartilage erosions was less for horses that received the product prophylactically than in horses that received the placebo prophylactically in the present study or the product intra-articularly in the previous study. We presumed that the increase in adverse response to carpal flexion may have been associated with the significant increase in proliferation at the joint capsule attachment identified via radiography. However, the conflicting findings regarding erosion severity suggested some otherwise beneficial versus detrimental effect of the test product.

The timing of treatment administration changed the outcomes observed in the present study. Whereas a pattern similar to that obtained in the prophylactic efficacy portion of the study was identified in the treatment efficacy portion with respect to total radiographic score (product-treated horses having larger scores for osteoarthritis-affected joints than placebo-treated horses), differences were not significant. Magnetic resonance imaging was performed to obtain a more in-depth evaluation of osteoarthritis-affected joints, revealing more bone edema in product-treated versus placebo-treated horses in the treatment efficacy portion of the study. We presume that the difference in the results of product treatment in that portion versus the prophylactic efficacy portion was attributable to the timing of administration. We therefore conclude that caution should be used when administering the evaluated product to horses IV, particularly when more beneficial in vivo results were obtained with intra-articular administration of the product in the previous study.
treated horses. When the product was administered after onset of disease (day 14), these changes were in a similar direction but fewer were significant.

Acknowledgments

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Preliminary results presented in abstract form at the American Association of Equine Practitioners Annual Convention, Las Vegas, December 2009.

Footnotes

c. Equi-Phar, Schering-Plough Animal Health, Summit, NJ.
e. PGE2 kit, Assay Designs, Farmingdale, NY.
g. PROC GLIMMIX, SAS, version 9.2, SAS Institute Inc, Cary, NC.

References

3. NAHMS. Lameness and laminitis in US horses.