Evaluation of avocado and soybean unsaponifiable extracts for treatment of horses with experimentally induced osteoarthritis

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Objective—To evaluate the use of a combination of avocado and soybean unsaponifiable (ASU) extracts for the treatment of experimentally induced osteoarthritis in horses.

Animals—16 horses.

Procedures—Osteoarthritis was induced via osteochondral fragmentation in 1 middle carpal joint of each horse; the other joint underwent a sham operation. Horses were randomly allocated to receive oral treatment with ASU extracts (1:2 [avocado-to-soybean] ratio mixed in 6 mL of molasses; n = 8) or molasses (6 mL) alone (placebo treatment; 8) once daily from days 0 to 70. Lameness, response to joint flexion, synovial effusion, gross and histologic joint assessments, and serum and synovial fluid biochemical data were compared between treatment groups to identify effects of treatment.

Results—Osteochondral fragmentation induced significant increases in various variables indicative of joint pain and disease. Treatment with ASU extracts did not have an effect on signs of pain or lameness; however, there was a significant reduction in severity of articular cartilage erosion and synovial hemorrhage (assessed grossly) and significant increase in articular cartilage glycosaminoglycan synthesis, compared with placebo-treated horses.

Conclusions and Clinical Relevance—Although treatment with ASU extracts did not decrease clinical signs of pain in horses with experimentally induced osteoarthritis, there did appear to be a disease-modifying effect of treatment, compared with findings in placebo-treated horses. These objective data support the use of ASU extracts as a disease-modifying treatment for management of osteoarthritis in horses. (*Am J Vet Res* 2007;68:598–604)

Lameness is a common cause of reduced work and early retirement of horses, and the most common cause of lameness is osteoarthritis; approximately 60% of lameness problems in horses is related to osteoarthritis.[1] Several intra-articular and systemic methods have been used to manage horses with osteoarthritis. Intra-articular methods commonly rely on reducing inflammation through the use of medications such as corticosteroids, hyaluronic acid, and polysulfated GAG. Newer medications have been developed that target specific biological pathways and can be administered intra-articularly as protein or gene expression products.[2] Systemic treatments include administration of anti-inflammatory medications and products that are intended to supplement existing cartilage matrix components. Unlike anti-inflammatory medications that suppress clinical signs of osteoarthritis, certain supplements (commonly referred to as nutraceuticals) are, in theory, able to modify the actual disease process by influencing matrical component biosynthesis and degradation.[3] This theoretically leads to stronger articular cartilage matrix components that are less prone to degradation. However, objective, in vivo evaluation of nutraceutical-type products in horses is lacking.

In reference to equine musculoskeletal disease, the term nutraceutical encompasses a wide array of products such as those containing chondroitin sulfate, glucosamine, and hyaluronic acid. Efficacy of these products is contentious, and research continues into their use for management of osteoarthritis.[3] In addition, there is clinical and in vitro evidence from humans to support the use of ASU extracts for the management of osteoarthritis.[4-8] The unsaponifiable portions of avocado and soybean oils are extracted via hydrolysis, and the extracts have been shown to effectively treat several connective tissue diseases such as scleroderma, pyorrhea, and periodontal diseases.[9] Results of an in vitro study[9] have indicated that ASU extracts reduce production of stromelysin, interleukin-6, interleukin-8, and PGE_2 by human articular chondrocytes exposed to interleukin-1β. The metalloproteinase inhibition may be a result

Abbreviations

GAG	Glycosaminoglycan
ASU	Avocado and soybean unsaponifiable
PGE_2	Prostaglandin E_2
DMMB	1,9-dimethylmethylene blue
CPII	C-terminal propeptide of type II collagen
SOFG	Safranin-O fast green

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of stimulation of plasminogen activator inhibitor-1 synthesis induced by ASU extracts, as evident in chondrocyte culture.[7] Anabolic effects in vitro have also been determined. Specifically, chondrocytes cultured with ASU extracts increase their production of transforming growth factors β1 and β2,[7] and ASU extracts stimulate aggrecan production, promote aggrecan recovery, and reduce matrix metalloproteinase-3 and tissue inhibitor of metalloproteinase-1 concentrations after interleukin-1β conditioning of chondrocytes.[8] Therefore, ASU extracts appear to have a positive effect on both the inflammatory cascade and structural components of articular cartilage matrix.

In addition to the data provided by the in vitro study of ASU extracts efficacy, there is clinical evidence in human medicine that ASU extracts may have a positive effect in the management of osteoarthritis. When compared with placebo treatment of humans with symptomatic osteoarthritis of the knee or hip joint, ASU extracts were significantly more effective as indicated by reduced nonsteroidal anti-inflammatory drug intake and increased functional use score.[6,10] In addition, ASU extracts had significant symptom-modifying effects in humans with knee osteoarthritis alone.[4] However, in a study[11] of humans with osteoarthritis of the hip joints, there was no significant effect of ASU extracts on radiographic progression of the disease, although the investigators did determine that in a subgroup that had the most severe initial narrowing of joint space, radiographic progression of the disease after treatment with ASU extracts was slower than that achieved after placebo treatment. It was concluded that ASU extracts could have structural effects. However, the investigators cautioned that a larger study[11] was needed to confirm the findings. Therefore, on the basis of the results of these aforementioned studies, it appears that ASU extracts may have clinical benefits in relieving clinical signs of osteoarthritis in humans and, perhaps, in other animals.

In addition to in an vitro study[12] and clinical trials, the efficacy of ASU extracts in the treatment of joint disease has been investigated in animals with experimentally induced osteoarthritis. In sheep with meniscectomy-induced osteoarthritis, ASU extracts were ineffective in preventing development of focal articular cartilage lesions, although the animals treated with ASU extracts had a higher articular cartilage proteoglycan index (as measured histologically) and greater articular cartilage thickness than nontreated animals.[12] In addition, ASU extracts–treated sheep had comparatively less subchondral bone sclerosis. A shortcoming of that study, however, was that clinical signs of pain were not reported. Therefore, it appears that ASU extracts had a subtle effect on articular cartilage morphology, suggesting that the combination may have an effect on structural components of osteochondral tissues, although experimental data are lacking to support its efficacy in pain relief.[12]

In a systematic review[13] of the human clinical trials in which ASU extracts were evaluated to date, those investigators concluded that the data support the effectiveness of ASU extracts for treatment of osteoarthritis, but that more research is needed. With this in mind, and because of the lack of objective data available for assessment of the efficacy of nutraceutical-type products in horses, the purpose of the study reported here was to use a well-established procedure to experimentally induce osteoarthritis in horses and assess the clinical, biochemical, and histologic effects of ASU extracts in those horses. We hypothesized that the combination of ASU extracts would have a positive disease-modifying effect on both clinical signs and structural effects of osteoarthritis in horses.

Materials and Methods

Osteochondral fragment model—Following approval of the Colorado State University Animal Care and Use Committee, 16 healthy 2- to 5-year-old horses were used in the study. Prior to being admitted into the study, body condition scores, radiography of the carpal regions, lameness examination, assessment of the response to flexion of the carpal joints, and analysis of synovial effusion were performed to ensure that all variables were within reference limits. The authors have had considerable experience with the method of induction of osteoarthritis used in the study to evaluate the efficacy of ASU extracts.[14-18]

The horses were randomly assigned to either the ASU extracts–treated (ASU extracts in molasses[a]; n = 8) or placebo-treated (molasses only; 8) group. Unsaponifiable residues of avocado and soybean oils were prepared in a 1:2 (avocado-to-soybean) ratio and mixed in 6 mL of molasses. Placebo treatment consisted of 6 mL of molasses. Palatability was suitable for effective oral administration, and all horses were treated once daily with their respective treatments starting on day 0 and ending on day 70.

On day 0, following routine preparation for anesthesia and surgery, all horses underwent bilateral arthroscopic surgery of the middle carpal joints to ensure that preexisting abnormalities did not exist. During this procedure, an osteochondral fragment was created in 1 randomly selected middle carpal joint of each horse. This was accomplished by use of an 8-mm curved osteotome that was directed perpendicular to the articular cartilage surface of the distal aspect 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 of the 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 promoting induction of osteoarthritis.[14-18] The joint with the fragment was designated as the osteoarthritis-affected joint; the other sham-operated joint was designated as the control joint. The arthroscopic portals were closed with 2-0 nylon suture in a simple interrupted pattern. The limbs were bandaged, and the horses were allowed to recover from anesthesia and surgery. Each horse received phenylbutazone (4.4 mg/kg, PO) for 5 days. Bandages were changed every 3 to 5 days and maintained for 2 weeks. Sutures were removed 10 days after surgery.

Each horse was housed in a 3.65 × 3.65-m stall. Beginning on day 14, all horses were exercised on a high-speed treadmill 5 d/wk until the end of the study (day

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70). To simulate the strenuous exercise of race training, horses underwent trotting (16 to 19 km/h) for 2 minutes and galloping (32 km/h) for 2 minutes, followed by trotting (16 to 19 km/h) for 2 minutes each day.[14-17]

Clinical variables—For each horse, clinical examinations of both forelimbs were performed biweekly from day 0 (baseline) through day 70 by an investigator (CEK) who was unaware of the treatment. As an indicator of pain in the osteoarthritis-affected and sham-operated joints during trotting (> 3 m/s), lameness was scored for each forelimb on a scale of 0 to 5 (0 = normal gait and 5 = severe lameness[19]). Flexion of both carpal joints of each horse was performed. Following manipulation and concentration of stress around each carpus, lameness during trotting was scored as an indicator of increased pain; scores were assigned on a scale of 0 to 4 (0 = no response, 1 = slight response, 2 = mild response, 3 = moderate response, and 4 = severe response). Carpal joints were palpated to detect effusion as an indicator of increased synovial fluid volume in the middle carpal joint; the extent of the effusion was scored on a scale of 0 to 4 (0 = an apparently normal amount of fluid, 1 = slight increase in fluid volume, 2 = mild increase in fluid volume, 3 = moderate increase in fluid volume, and 4 = a severe increase in fluid volume).

Radiography of both carpi of each horse was performed at the time of inclusion in the study (baseline), following the induction of osteoarthritis (ie, after osteochondral fragment creation), and at the termination of the study (day 70). A board-certified radiologist (NMW) who was unaware of treatment groups evaluated the radiographic images for boney proliferation at the joint capsule attachment, subchondral bone lysis, and osteophyte formation and assigned scores on a scale of 0 to 3 (0 = no abnormal findings, 1 = mild abnormal change, 2 = moderate abnormal change, and 3 = the most severe change[14]).

Serum analysis—To obtain serum, a blood sample (20 mL) was collected prior to surgery and weekly after surgery; among the latter samples, analyses were performed and results reported only for those collected on days 14, 56, and 70. All samples were frozen at –80°C until analysis. A modified DMMB dye-binding assay was used to determine serum GAG concentration as an indicator of GAG release into the bloodstream.[20] For assessment of collagen synthesis, the CPII concentration was also measured in serum by use of a commercial ELISA kit[b] and data were recorded as micrograms per milliliter.[21] As a measure of newly synthesized aggrecan, the concentration of chondroitin sulfate 846 epitope (CS846) was determined in serum by use of a commercial ELISA kit[c] and data were recorded as nanograms per milliliter.[21] An inhibition ELISA was used to measure type II collagen breakdown neoepitope (234CEQ) as an assessment of articular cartilage resorption, and data were recorded as micrograms per milliliter.[22]

Synovial fluid analysis—Synovial fluid (approx 4 mL) was aseptically aspirated from both middle carpal joints of each horse prior to surgery and once a week for the duration of the study; among the latter samples, analyses were performed and results reported for only those collected on days 14, 56, and 70. A portion of the collected fluid was placed into a tube containing EDTA, and the remainder was stored at –80°C for biochemical analysis.

The conventional analysis of synovial fluid included assessment of color, clarity, total protein concentration, WBC count and differential assessment, and mucin clot formation. Synovial fluid color, clarity, mucin content, and blood contamination were evaluated subjectively. Color of synovial fluid was scored on a scale of 1 to 5 (1 = yellow, 2 = colorless, 3 = straw colored, 4 = orange, and 5 = red). Clarity was scored on a scale of 1 to 3 (1 = clear, 2 = hazy, and 3 = cloudy). Mucin content was scored on a scale of 1 to 3 (1 = good, 2 = fair, and 3 = poor). Blood contamination was reported as present or absent, but was not statistically analyzed or reported. Total protein and WBC concentrations were determined by use of refractometry and an automated cell counter, respectively. An automated cell counter was also used to determine the differential WBC count.[14]

Several biomarker assays were performed on synovial fluid samples. The DMMB dye-binding assay was used to determine GAG concentration,[20] and PGE_2 concentrations were assessed in 2 major steps: PGE_2 extraction and PGE_2 evaluation.[14] Extraction of PGE_2 from synovial fluid was performed by use of anion-exchange, reversed-phase C2 columns,[d] as suggested by the manufacturer's directions. The concentration of PGE_2 was estimated by use of a commercially available, competitive, high-sensitivity enzyme immunoassay kit, as directed by the manufacturer's instructions.[e] The CPII concentration was also measured in synovial fluid by use of a commercial ELISA kit,[b] and data were recorded as micrograms per milliliter. The concentration of chondroitin sulfate 846 epitope (CS846) was determined in synovial fluid by use of a commercial ELISA kit,[c] and data were recorded as nanograms per milliliter. An inhibition ELISA was used to measure synovial fluid type II collagen breakdown neoepitope (234 CEQ) concentration, and data were recorded as micrograms per milliliter.[22]

Gross examination—On day 70, horses were euthanized with an overdose of pentobarbital sodium (88 mg/kg, IV) according to animal care and use committee protocols. A necropsy examination of each horse was performed by an evaluator (CEK) who was unaware of treatment assignments, and the middle carpal joints were specifically examined for degree and location of articular cartilage fibrillation and erosion. A subjective score was assigned for partial- and full-thickness cartilage erosions as well as for synovial membrane hemorrhage; a scale of 0 to 4 was used (0 = apparently normal findings, 1 = slight abnormality, 2 = mild abnormal change, 3 = moderate abnormal change, and 4 = severe abnormal change). A total score was also calculated by summing the scores for those variables (possible total score of 12).

Histologic examination—Samples of synovial membrane and joint capsule in the area dorsal to the osteochondral fragment were collected and placed in neutral-buffered 10% formalin for H&E staining. Five-micron sections were evaluated and scored for cellular infiltration, synovial intimal hyperplasia, subintimal

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edema, subintimal fibrosis, and vascularity by an evaluator (DDF) who was unaware of treatment assignments. Each variable was scored on a scale of 0 to 4 (0 = normal, 1 = slight change, 2 = mild change, 3 = moderate change, and 4 = severe change). Total scores were determined and reported for each section with a possible total score of 20.

Articular cartilage pieces (5 mm^2) were obtained from the radial carpal, third carpal, and fourth carpal bones of each joint and stored in neutral-buffered 10% formalin for 7 days before undergoing routine histologic processing. One half of the 5-µm sections were stained with H&E, and the remainder was stained with SOFG. Sections stained with H&E were evaluated blindly for articular cartilage fibrillation, chondrocyte necrosis, chondrone formation, and focal loss of cells; a score on a scale of 0 to 4 was assigned to each variable (0 = no abnormal change, and higher values represented progressively more pathologic change). Total scores were determined (possible total score of 16)[14] and reported for each section. Articular cartilage sections stained with SOFG were evaluated blindly for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones; a score on a scale of 0 to 4 was assigned to each variable (0 = no stain uptake and 4 = apparently normal stain uptake). Total scores were determined (possible total score of 16)[14] and reported for each section.

Articular cartilage matrix evaluation—To estimate articular cartilage proteoglycan content, the total articular cartilage GAG concentration was measured with the previously reported DMMB dye-binding technique.[23] Articular cartilage pieces were obtained aseptically from the distal aspects of the radial carpal and intermediate carpal bones within each joint, and each piece was stored at –80°C prior to further processing and analysis. Samples were analyzed in duplicate, and findings were recorded as micrograms of GAG per milligram of cartilage dry weight.[14] For estimation of proteoglycan synthesis, articular cartilage samples were also aseptically collected from the weight-bearing surfaces of the distal aspect of the radial carpal bone and the proximal aspects of the third and fourth carpal bones within each joint; radiolabeled sulfate ($^{35}SO_4$) incorporation was quantified by use of previously reported methods[24] as a measure of GAG synthesis. Samples were analyzed in duplicate, and results were recorded as counts per minute per milligram of cartilage dry weight.

Statistical analysis—Results were analyzed by use of a mixed-model ANOVA[f] unless otherwise noted. Residual plots were constructed to test for fulfillment of model assumptions; if model assumptions were not met, data transformation was performed and reported. The only data requiring transformation were GAG concentration (µg of GAG/mg of cartilage dry weight), which were log transformed for analysis. The following independent variables were considered: presence or absence of osteoarthritis, treatment (ASU extracts or placebo), day of sample collection, and all interactions between main effect variables. Dependent variables measured at ≤ 2 time points were analyzed similarly, with the exclusion of day of sample collection in the model. The subject (horse) was considered a random variable. When individual comparisons were made, a least square means procedure was used and a value of $P < 0.05$ was considered significant.

Results

Clinical examination—Osteochondral fragmentation induced a significant increase in lameness score ($P < 0.001$), response to joint flexion ($P < 0.001$), and

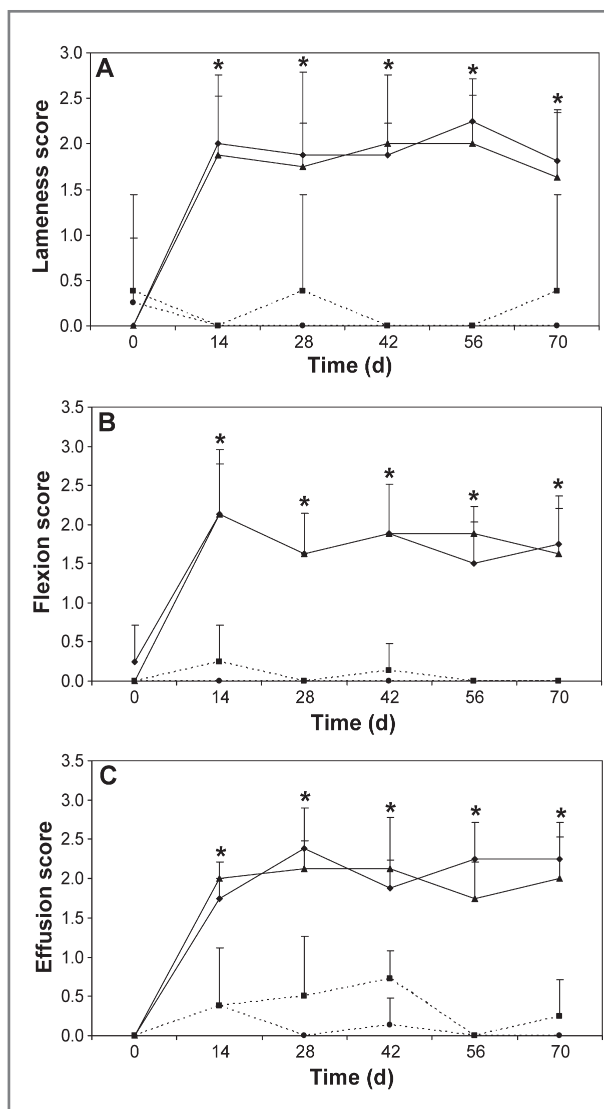

Figure 1—Mean ± SD scores for lameness (A), joint flexion (B), and joint effusion (C) in horses that had undergone experimental induction of osteoarthritis in 1 middle carpal joint and a sham operation in the other middle carpal joint, followed by oral treatment with ASU extracts (1:2 [avocado-to-soybean] ratio mixed in 6 mL of molasses) or molasses (6 mL) alone (placebo treatment) once daily from days 0 to 70. Data represent mean values from 8 horses that received ASU extracts (osteoarthritis-affected joints, triangles; sham-operated joints, squares) and 8 horses that received placebo (osteoarthritis-affected joints, diamonds; sham-operated joints, circles). There was a significant increase in lameness, response to joint flexion, and synovial effusion attributable to osteochondral fragmentation, specifically at times denoted by an asterisk. Note the lack of treatment effects on the scores.

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joint effusion score ($P < 0.001$; **Figure 1**). However, treatment did not have an effect on those 3 variables. In addition, the osteochondral fragment induced a significant ($P < 0.001$) increase in mean ± SD radiographic score at the end of the study (osteoarthritis-affected joints, 1.44 ± 1.86; sham-operated control joints, 0.06 ± 0.25); there was no significant effect of treatment with ASU extracts, compared with placebo treatment.

Serum analysis—There was a significant ($P < 0.001$) increase in total serum GAG concentration associated with osteochondral fragmentation on days 56 and 70, but no effect of treatment. There was a significant ($P < 0.001$) increase in serum CS846 concentration associated with osteochondral fragmentation on day 14 of the study; this value returned to baseline on day 56, but there was no effect of treatment. In addition, there was no effect of osteoarthritis, time, or treatment on CPII or 234CEQ.

Synovial fluid analysis—Osteochondral fragmentation did not induce significant changes in counts of WBCs, lymphocytes, neutrophils, or eosinophils or in concentrations of total GAG, CS846, or 234CEQ within the synovial fluid. However, osteochondral fragmentation did induce a significant increase in synovial fluid total protein concentration ($P = 0.001$), monocyte count ($P = 0.03$), PGE_2 concentration ($P < 0.001$), and CPII concentration ($P = 0.003$). Treatment with ASU extracts did not affect any of the assessed synovial fluid variables.

Gross examination—Compared with findings for the sham-operated joints, osteochondral fragmentation induced a significant increase in total articular cartilage erosion score ($P < 0.001$), combined erosion and hemorrhage score ($P = 0.006$), and full-thickness erosion score ($P = 0.009$) in placebo-treated horses **(Figure 2)**. Treatment with ASU extracts induced a significant ($P = 0.04$) reduction in combined erosion and hemorrhage score for osteoarthritis-affected joints, compared with the combined score for osteoarthritis-affected joints in the placebo group. Treatment with ASU extracts induced a reduction in articular cartilage erosion score for osteoarthritis-affected joints, compared with the score for osteoarthritis-affected joints in the placebo group, but this difference was not significant ($P = 0.054$).

Histologic examination—There was no significant effect of osteochondral fragmentation or treatment on synovial membrane score. However, there was a significant ($P = 0.012$) increase in focal cell loss score in articular cartilage from the radial carpal bone of osteoarthritis-affected joints, compared with sham-operated joints (mean ± SD focal cell loss score, 1.5 ± 0.7 and 0.8 ± 0.7, respectively). In addition, there was a significant ($P = 0.023$) decrease in SOFG score in articular cartilage from the fourth carpal bone of osteoarthritis-affected joints, compared with sham-operated joints (mean ± SD SOFG score, 0.9 ± 0.6 and 1.4 ± 0.7, respectively). There was no significant effect of treatment on articular cartilage histologic or SOFG scores.

Articular cartilage matrix evaluation—There was no effect of osteochondral fragmentation or treatment on total GAG content in articular cartilage. However, there was a significant ($P = 0.02$) effect of treatment with ASU extracts on total GAG synthesis. Glycosaminoglycan synthesis was 140.75 ± 42.3 cpm/mg of cartilage dry weight in osteoarthritis-affected joints treated with ASU extracts, compared with 109.49 ± 34.9 cpm/mg of cartilage dry weight in sham-operated joints treated with ASU extracts. In placebo-treated, osteoarthritis-af-

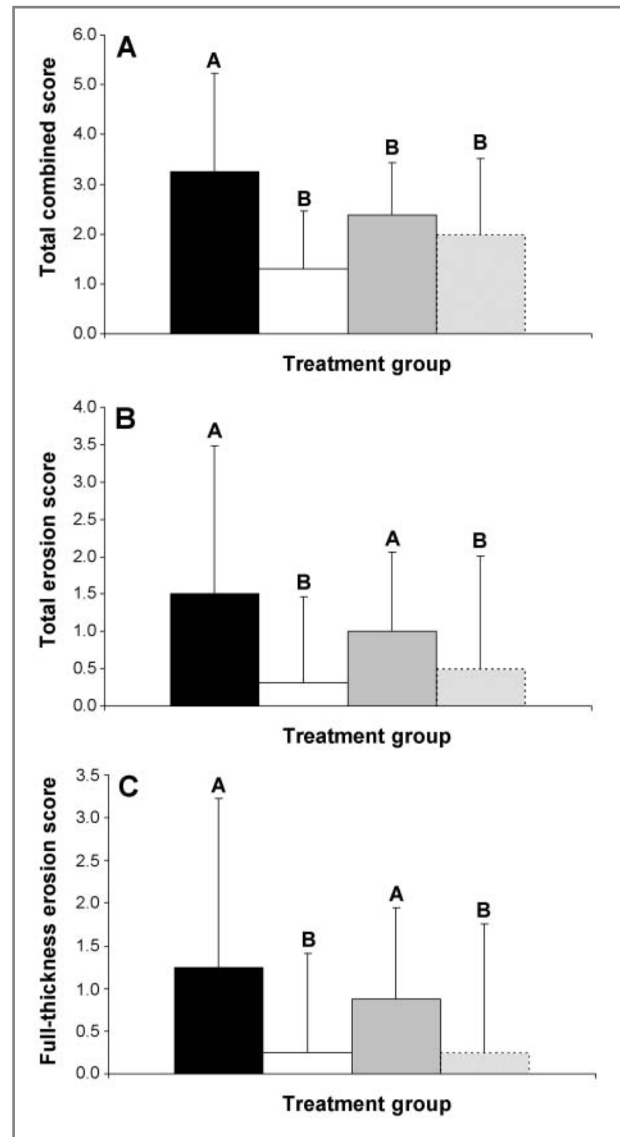

Figure 2—Mean ± SD scores for total combined synovial hemorrhage and articular erosion (A), total articular erosion (B), and full-thickness cartilage erosion (C) in horses that had undergone experimental induction of osteoarthritis in 1 middle carpal joint and a sham operation in the other middle carpal joint followed by oral treatment with ASU extracts (1:2 [avocado-to-soybean] ratio mixed in 6 mL of molasses) or molasses (6 mL) alone (placebo treatment) once daily from days 0 to 70. Data represent mean values from 8 horses that received ASU extracts (osteoarthritis-affected joints, light gray bars; sham-operated joints, dark gray bars) and 8 horses that received placebo (osteoarthritis-affected joints, black bars; sham-operated joints, white bars). Osteochondral fragmentation induced significant increases in all 3 scores; treatment with ASU reduced the combined erosion and synovial hemorrhage score, had no influence on full-thickness erosion score, and slightly decreased (albeit not significantly) the total erosion score. [A,B]Values with different letters are significantly ($P < 0.05$) different.

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fected, and sham-operated joints, GAG synthesis was 115.08 ± 34.9 cpm/mg and 136.61 ± 31.86 cpm/mg of cartilage dry weight, respectively; osteoarthritis-affected joints treated with ASU extracts had significantly higher GAG synthesis than osteoarthritis joints that were placebo treated.

Discussion

In horses with osteoarthritis of the middle carpal joint that had been experimentally induced via osteochondral fragmentation, treatment with ASU extracts failed to induce any modifying effects on clinical signs. However, the combination of ASU extracts was associated with reduced articular cartilage erosion and synovial membrane hemorrhage and increased articular cartilage GAG synthesis in joints with osteochondral fragmentation. Results of the present study suggest that the ASU extracts can induce structural modification of the joint surface, supporting our second hypothesis.

The method of osteochondral fragmentation used in our study has been shown to induce clinical signs and gross, histologic, and biochemical changes consistent with osteoarthritis.[14-18] These changes have been ameliorated to some extent by medications in previous studies,[14-18] and osteochondral fragmentation has proven to be a good experimental technique with which to evaluate medications intended to treat osteoarthritis. In the present study, the presence of an osteochondral fragment in a joint similarly induced notable increases in clinical, radiographic, and biochemical signs of joint disease and gross changes consistent with osteoarthritis.

Among the study horses, the ASU extracts failed to ameliorate the increased lameness, response to joint flexion, and synovial effusion induced by the presence of osteochondral fragments in joints. These data are in contrast to clinical reports[4,10] of the use of ASU extracts for treatment of knee and hip arthritis in humans, in which symptom-modifying effects were detected. Although the exact causes of clinical responses to the osteochondral fragment in joints are unknown, there has consistently been an increase in total protein and PGE_2 concentrations in synovial fluid in previous studies,[16,18] implying that a consistent degree of synovitis develops as a result of the fragmentation. However, the combination of ASU extracts did not have an effect on synovial fluid total protein and PGE_2 concentrations; this was in contrast to the reports of in vitro studies[8,9] in which ASU extracts inhibited the effects of inflammatory enzymes (such as PGE_2 and metalloproteinases) and aggrecanase on articular cartilage. An association between the degree of inflammatory response in our in vivo study and the previous in vitro studies cannot be made, but it appears that the levels of persistent synovitis and joint damage in the horses of the present study were severe enough to inhibit any possible anti-inflammatory effects of the ASU extracts. Consequently, clinical sign modification attributable to treatment with ASU extracts did not occur in our study. Additionally, the duration of the clinical studies in humans in which symptom-modifying effects following treatment with ASU extracts were detected was 3 to 24 months, whereas in the present study, the duration was 70 days. Therefore, the relatively shorter duration of the present study may have prevented development of the maximum effects of ASU extracts treatment for relief of osteoarthritis-associated clinical signs in horses.

Treatment with ASU extracts, however, reduced the severity of articular cartilage erosion and synovial hemorrhage, which commonly develop after osteochondral fragmentation in joints of horses. This differs from the results of an experimental study[12] in which treatment with ASU extracts in meniscectomized sheep did not decrease pathologic changes in articular cartilage. Care must be taken in interpreting the results from that previous study because lameness was not assessed and it is impossible to relate the gross changes to limb use. In our study, lameness scores indicated that lameness in osteoarthritis-affected joints treated with placebo and ASU extracts was the same, leading one to conclude that the reduced gross score in the ASU extracts–treated group was not simply a result of reduced weight bearing. In addition, in the study[12] of meniscectomized sheep, articular cartilage from ASU extracts–treated sheep had a higher proteoglycan index (as determined histologically), providing some evidence that the combination of ASU extracts stimulated proteoglycan synthesis or helped to maintain existing proteoglycan content.

In horses in the present study, GAG synthesis in the articular cartilage in ASU extracts–treated, osteoarthritis-affected joints was increased, compared with placebo-treated, osteoarthritis-affected joints. However, there was no effect of osteoarthritis or treatment on GAG content in the articular cartilage. The reason for the increase in GAG synthesis may be that the combination of ASU extracts stimulates production of transforming growth factors β1 and β2 in chondrocytes, as determined in vitro.[7] On the basis of those findings and results of our study, it appears that the effect of ASU extracts on GAG synthesis is more likely to be a direct effect on chondrocytes, as opposed to an indirect effect of reducing inflammatory mediator impact on GAG synthesis. This is in contrast to results of an in vitro study,[8] which indicated that ASU extracts reduce the effects of inflammatory mediators on chondrocytes. Data from the present study support previous findings that suggest that ASU extracts may have an anabolic effect directly on chondrocytes to increase GAG synthesis and, hence, prevent articular cartilage damage by enhancing the articular cartilage matrix structure.

Further investigations are needed to determine the cause of the positive effect that the combination of ASU extracts had on gross lesions and GAG synthesis in joints of the horses of the present study. Investigation is also needed to determine whether there is a protective mechanism with which ASU extracts exert increased collagen and proteoglycan material properties. Although biochemical analyses of collagen formation in serum and synovial fluid revealed no effect of treatment on collagen synthetic rates, the same was true of GAG synthetic rate systemically and in synovial fluid. However, increased GAG synthesis was detected at the tissue level. Therefore, in-depth analysis of collagen properties at the tissue level would be needed.

In our investigation, no adverse effects of treatment with ASU extracts were evident in the study horses; the

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product appeared to be easy to administer to the horses. In horses, treatment of osteoarthritis (induced via osteochondral fragmentation) via oral administration of ASU extracts resulted in no change in clinical signs of osteoarthritis, but reduced the severity of joint damage and increased the synthesis of GAG in articular cartilage of the osteoarthritis-affected joints. These effects can be beneficial in the management of osteoarthritis by reducing the progression of gross articular cartilage damage, although other methods for improving clinical signs would need to be used. In addition, it is impossible to know whether the ASU extracts would work with other medications that are targeted to relieve clinical signs and whether beneficial effects of ASU extracts on articular cartilage would be evident if increased weight bearing (resulting from relief of clinical signs) were to occur. Regardless, the results of our study have indicated that oral administration of the combination of ASU extracts would be clinically useful for the management of osteoarthritis in horses.

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a. Avocado SOYA unsaponifiable, Vetoquinol USA/Evsco Pharmaceuticals, Buena, NJ.
b. Procollagen II C-Propeptide ELISA, IBEX Diagnostics Inc, Montreal, QC, Canada.
c. Aggrecan chondroitin sulfate 846 epitope ELISA, IBEX Diagnostics Inc, Montreal, QC, Canada.
d. C2 ethyl Amprep mini columns, GE Healthcare, Piscataway, NJ.
e. Prostaglandin E_2 high-sensitivity enzyme immunoassay (EIA) kit, Assay Designs Inc, Ann Arbor, Mich.
f. SAS, version 8e, SAS Institute Inc, Cary, NC.

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