Clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum in horses with experimentally induced osteoarthritis

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Objective—To assess the clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum (ACS) in the treatment of experimentally induced osteoarthritis in horses.

Animals—16 horses.

Procedures—Osteoarthritis was induced arthroscopically in 1 middle carpal joint of all horses. In 8 placebo- and 8 ACS-treated horses, 6 mL of PBS solution or 6 mL of ACS was injected into the osteoarthritis-affected joint on days 14, 21, 28, and 35, respectively; PBS solution was administered in the other sham-operated joints. Evaluations included clinical assessment of lameness and synovial fluid analysis (performed biweekly); gross pathologic and histologic examinations of cartilage and synovial membrane samples were performed at necropsy.

Results—No adverse treatment-related events were detected. Horses that were treated with ACS had significant clinical improvement in lameness, unlike the placebo-treated horses. Among the osteoarthritis-affected joints, ACS treatment significantly decreased synovial membrane hyperplasia, compared with placebo-treated joints; although not significant, the ACS-treated joints also appeared to have less gross cartilage fibrillation and synovial membrane hemorrhage. The synovial fluid concentration of interleukin-1 receptor antagonist (assessed by use of mouse anti–interleukin-1 receptor antagonist antibody) was increased following treatment with ACS.

Conclusions and Clinical Relevance—Results of this controlled study indicated that there was significant clinical and histologic improvement in osteoarthritis-affected joints of horses following treatment with ACS, compared with placebo treatment. On the basis of these findings, further controlled clinical trials to assess this treatment are warranted, and investigation of the mechanisms of action of ACS should be pursued concurrently. (*Am J Vet Res* 2007;68:xxx–xxx)

Joint disease, specifically osteoarthritis, is one of the most prevalent and debilitating diseases affecting horses and has a notable economic impact on the equine industry.[1–4] Various medications have been evaluated for the treatment of osteoarthritis in horses,[5–7] including corticosteroids, hyaluronan, and, more recently, cytokine inhibitors.[5] An experimental model of osteoarthritis[8–12,a] has been used in horses for more than 10 years for assessment of the pathophysiologic process and biological markers of the disease, as well as evaluation of the efficacy of therapeutic substances in a controlled fashion. In humans, the use of ACS[b] has been reported in Europe to be beneficial in the treatment of osteoarthritis, rheumatoid arthritis, spinal disorders, and muscle injuries.[13,14] It has been used in more than 30,000 humans and 3,000 horses with osteoarthritis.[c]

	ABBREVIATIONS
ACS	Autologous conditioned serum
IL	Interleukin
IL-1Ra	IL-1 receptor antagonist
GAG	Glycosaminoglycan
PG	Prostaglandin
SOFG	Safranin-O fast green

However, to our knowledge, there are no reports of controlled studies of ACS as a treatment for osteoarthritis in either humans or horses.

Autologous conditioned serum is believed to have its main effect through upregulation of the expression of several beneficial cytokines including IL-1Ra. Specifically, it has been shown that incubation of human whole blood with medical-grade glass beads exposed to chromium sulfate stimulates the production of IL-4, IL-10, and IL-1Ra[13] as well as fibroblastic growth factor-1, hepatocyte growth factor, and transforming factor-β1, resulting in higher concentrations of those cytokines and factors in human ACS.[14] The increase in these anti-inflammatory mediators does not appear to be accompanied by an increase in the proinflammatory cyto-

kines IL-1β or tumor necrosis factor-α. Because IL-1Ra expression is as much as 140-fold greater than other anti-inflammatory proteins in ACS after such stimulation of whole blood, it has been assumed that IL-1Ra is one of the major mediators responsible for clinical improvement in patients with osteoarthritis.

The beneficial role of IL-1Ra in the pathogenesis of human and equine osteoarthritis is well established in the literature.[5,15,16] In addition to a vast amount of in vitro data, investigations[5,17,18] in people and other species (including horses) have revealed that administration of IL-1Ra protein has beneficial effects in joint disease. It is also well accepted that IL-1 is a major mediator in joint disease, and it is believed to be at the top of the cytokine cascade in all species in which it has been studied.[19] The administration of IL-1Ra[5] through gene transfer results in greater improvement in clinical signs, articular cartilage morphology, and radiographic abnormalities associated with experimentally induced osteoarthritis in horses, compared with administration of corticosteroids, hyaluronan, polysulfated glycosaminoglycan, or pentosan polysulfate.[20]

The purpose of the blinded controlled study reported here was to assess the effects of ACS following intra-articular administration in joints of horses with experimentally induced osteoarthritis by evaluation of clinical (joint lameness, range of motion, response to flexion, and synovial effusion), radiographic, gross, biochemical, immunologic, and histologic outcome measures. Our hypotheses were that the concentration of IL-1Ra would be increased in the ACS, compared with unprocessed serum, and that significant clinical, biochemical, and histologic improvements in signs of osteoarthritis would be detectable following the administration of ACS in affected joints.

Materials and Methods

Experimental design and induction of osteoarthritis—Following approval of the Colorado State University Animal Care and Use Committee, 16 healthy 2- to 3-year-old horses that were owned by the University were used in the study. Prior to inclusion in the study, horses underwent a lameness examination, and body condition, radiographic views 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. The horses were randomly assigned to either an ACS-treated (n = 8) or placebo-treated group (8). Horses in the ACS-treated group were to receive ACS treatment of the osteoarthritis-affected joint and placebo treatment of the other sham-operated joint. Horses in the placebo-treated group were to receive placebo treatment of both the osteoarthritis-affected and sham-operated joints.

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 me-

dial 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.[5] 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 other sham-operated joint was used as the control joint. The arthroscopic portals were closed with 2-0 nylon suture in a simple interrupted or cruciate pattern. The forelimbs were bandaged, and the horses were allowed to recover from anesthesia and surgery. Each horse received 2 g of phenylbutazone orally once daily for 5 days after surgery. Bandages were changed every 3 to 5 days and maintained for 2 weeks. Sutures were removed 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 underwent trotting (16 to 19 km/h) for 2 minutes, galloping (approx 32 km/h) for 2 minutes, followed by trotting (16 to 19 km/h) for 2 minutes to simulate the strenuous exercise of race training.

Treatment groups—On day 7 of the study, blood was collected from a jugular vein of all horses. For each horse that was randomized into the placebo-treated group, blood was collected into serum tubes (stored at –80°C). For each horse that was randomized into the ACS-treated group, blood was collected into a 50-mL ACS syringe; the protocol for ACS preparation followed the kit manufacturer's directions.[b] Briefly, the protocol included incubation of the blood and syringe (containing chromium sulfate-etched medical-grade glass beads) at 37°C for 24 hours, followed by centrifugation of the blood to collect serum. After filtration by use of a 0.2-μm membrane, the serum was frozen at –20°C in 6-mL aliquots until used. An aliquot of unprocessed serum was also frozen to estimate the baseline concentration of IL-1Ra. On days 14, 21, 28, and 35, 6 mL of the prepared serum was administered into the osteoarthritis-affected joint of horses in the ACS-treated group, whereas the other sham-operated joint received 6 mL of PBS solution; both joints of horses in the placebo-treated group received 6 mL of PBS solution. The treatment protocol was based on anecdotal information from the manufacturer and from veterinarians in Europe who were experienced with the use of this product in clinical cases of joint disease. Treatment was initiated on day 14 of the study to mimic other intra-articular treatment protocols evaluated in horses with similarly induced osteoarthritis.[5,9,11]

Assessment of clinical outcomes—For each horse, clinical examinations of both forelimbs were performed biweekly from prior to day 0 (baseline) throughout the study period. Lameness was graded on a scale of 0 to 5 (0 represented normal gait, and 5 represented severe lameness).[1] After lameness grading, flexion of the carpal joint was performed followed by observation of the horse during trotting as an indication of increase in pain at the trot following manipulation and stress

concentrated around the joint of interest (middle carpal joint); the response to flexion was graded on a scale of 0 to 4 (0 represented no response, and 4 represented a severe response). Carpal joints were palpated to detect effusion (an indicator of increased volume of synovial fluid [ie, inflammation] in the middle carpal joint); the extent of the effusion was graded on a scale of 0 to 4 (0 represented a normal amount of fluid, and 4 represented a severe increase in fluid volume). All clinical outcome parameters were assessed by a board-certified large animal surgeon (CEK; a specialist in equine lameness) who was unaware of treatment assignments.

For each horse, radiographic evaluation of both carpi was performed prior to inclusion in the study and induction of osteoarthritis, following the induction of osteoarthritis (after osteochondral fragment creation), and at the termination of the study (day 70). A board-certified radiologist (RDP) and a board-qualified radiologist (NMW), who were unaware of treatment groups, assessed the radiographic views for boney proliferation at the joint capsule attachment, subchondral bone lysis, and osteophyte formation; each abnormality was graded on a scale of 0 to 3 (0 represented no detectable abnormality, and 3 represented the most severe change).

Synovial fluid—Beginning on day 0 until the end of the study (day 70), a synovial fluid sample was aseptically aspirated once per week from both middle carpal joints of each horse in both groups. 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 color, clarity, total protein concentration, WBC count and differential, and mucin clot formation. Synovial fluid color, clarity, mucin content, and blood contamination were evaluated subjectively. Color of synovial fluid was graded on a scale of 1 to 5 (1 = yellow, 2 = colorless, 3 = straw colored, 4 = orange, and 5 = red). Clarity was graded on a scale of 1 to 3 (1 = clear, 2 = hazy, and 3 = cloudy). Mucin content was graded on a scale of 1 to 3 (1 = good, 2 = fair, and 3 = poor). Blood contamination of synovial fluid samples was graded as 1 or 2 (1 = samples with no blood contamination, and 2 = samples with blood contamination).[5] Total protein and WBC concentrations were determined via refractometry and use of an automated cell counter, respectively. Smears of synovial fluid were examined cytologically to determine the differential WBC count.

Three biomarker protein assays were performed on synovial fluid collected weekly (samples that had been stored frozen at –80°C). A modified 1,9-dimethyl-methylene blue dye-binding assay was used to determine GAG concentration.[21] Concentration of PGE_2 was assessed in 2 major steps: PGE_2 extraction and PGE_2 evaluation. Extraction of PGE_2 from synovial fluid was performed by use of C2 columns[d] as suggested by the manufacturer's instructions. The concentration of PGE_2 was estimated by use of a commercially available, competitive, high-sensitivity enzyme immunoassay kit[e] as directed by the manufacturer's instructions. The synovial fluid concentration of IL-1Ra was estimated by use of commercially available kits with either human or mouse antibody against IL-1Ra as directed by the manufacturer's instructions.[f]

Gross observation of joints—At the end of the study, all horses were euthanatized by administration of an overdose 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 cumulative pathology score was also calculated by summation of the erosion and synovial membrane hemorrhage scores.

Histologic examinations—At necropsy, samples of synovial membrane and joint capsule were collected 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 (DDF), 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.[5] 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 mm²) 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. Half of the 5-µm sections were stained with H&E, and the remainder was stained with SOFG. 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).

Sections stained with H&E were evaluated blindly for articular cartilage fibrillation, chondrocyte necrosis, chondrone formation (chondrocyte division within a lacuna), and focal loss of cells.[5] 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 for each articular cartilage sample was also determined, and location of sample collection was taken into account.

Articular cartilage sections stained with SOFG were evaluated blindly for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones.[5] 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.

Articular cartilage matrix evaluation—To estimate articular cartilage proteoglycan content, the total articular cartilage GAG content was measured by use of a previously reported 1,9-dimethyl-methylene blue technique.[5] Articular cartilage pieces were obtained aseptically from
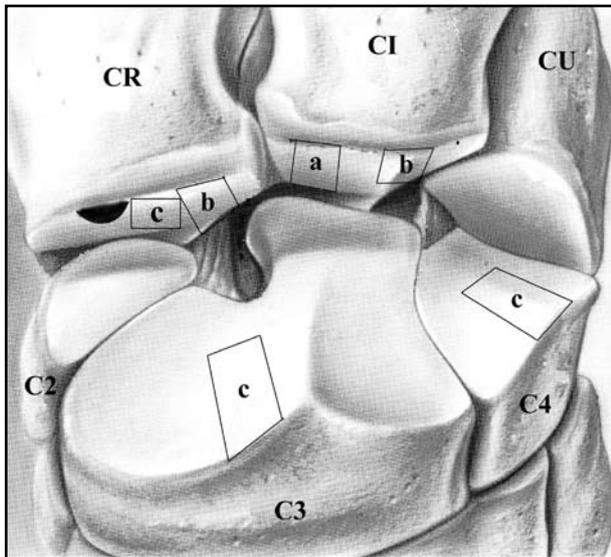
Figure 1—Schematic drawing of the equine middle carpal joint (dorsal surface) indicating the area of osteochondral fragmentation (darkly shaded region) on the radial carpal bone (CR) and sample collection sites (outline boxes). The outlined areas include the cartilage collection site for the proteoglycan synthesis assay (a), the collection site for the articular cartilage GAG content assay (b), and the collection site for the histologic and histochemical analyses (c). CI = Intermediate carpal bone. CU = Ulnar carpal bone. C2 = Second carpal bone. C3 = Third carpal bone. C4 = Fourth carpal bone. (Reprinted from *Diagnostic and surgical arthroscopy in the horse*, 3rd ed, McIlwraith CW, Wright I, Nixon AJ, Boening KJ, Chapter 4, 47–127, 2005, with permission from Elsevier.)
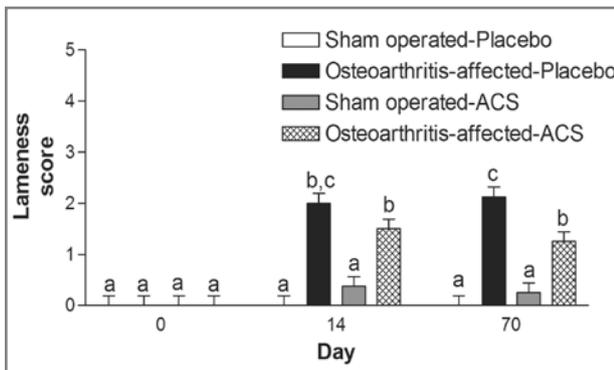

Figure 2—Lameness scores* assigned to the middle carpal joints of 16 horses involved in a study of the clinical and histologic effects of intra-articular administration of ACS in the treatment of experimentally induced osteoarthritis. In all horses, osteoarthritis was induced in 1 middle carpal joint (osteoarthritis-affected joints) and a sham operation was performed on the other (sham-operated joints); horses received either ACS treatment of the osteoarthritis-affected joint and placebo (PBS solution) treatment of the sham-operated joint (n = 8) or placebo treatment of both joints (8). Scores were assigned at baseline (day 0; prior to induction of osteoarthritis), after osteochondral fragment creation and osteoarthritis induction but prior to treatment (day 14), and at termination of the study 35 days after the last treatment (day 70). *Scores assigned from 0 to 5 (0 represented normal gait and 5 represented severe lameness).[1] a–c: Different letters indicate a significant ($P < 0.05$) difference between bars.

the area directly adjacent to the osteochondral fragment and a remote site within each joint (**Figure 2**), and each piece was stored at –80°C prior to further processing and analysis. Samples were processed in duplicate.

For analysis of cartilage matrix metabolism, articular cartilage samples were also aseptically collected from the weight-bearing surface that was remote from the osteochondral fragment within each joint (Figure 2), and radiolabeled SO_4 ($^{35}SO_4$) incorporation was measured by use of previously reported methods.[5] Samples were processed in duplicate, and the results were reported as counts per minute (cpm) per milligram of dry weight.

Statistical analysis—Results were analyzed by use of a mixed-model ANOVA unless otherwise stated.[g] Residual plots were constructed to test for fulfillment of model assumptions; if model assumptions were not met, data transformation was performed and reported. The following fixed effects were considered: presence or absence of osteoarthritis, treatment (ACS or placebo), day of sample collection, and all interactions between main effect variables. The subject (horse) was considered a random effect. When individual comparisons were made, a least squares mean procedure was used. When synovial fluid IL-1Ra values were assessed to determine whether they were significantly different than zero, a t test was used. For statistical analyses, a value of $P < 0.05$ was considered significant.

Results

Assay for IL-1Ra content in ACS—Preparation of ACS from each horse was accomplished as outlined by the kit manufacturer; an aliquot of each preparation was frozen at –80°C for later quantification of IL-1Ra by use of the human and mouse antibodies against IL-1Ra. Historically, the authors have successfully used a human IL-1Ra ELISA to detect equine IL-1Ra (the equine IL-1Ra concentrations were an order of magnitude higher than those detected with the mouse antibody in the present study); however, no IL-1Ra was detected in any ACS preparation by use of human anti-IL-1Ra antibody. The range of IL-1Ra concentrations was 0 to 23 pg/mL (mean ± SEM, 5.2 ± 3.3 pg/mL), which was not significantly ($P = 0.16$) different from zero as determined by use of a probability t test. The same ACS samples were evaluated by use of a mouse IL-1Ra ELISA; the range of IL-1Ra concentrations was 106 to 389 pg/mL (mean value, 234 ± 30.6 pg/mL), which was significantly ($P < 0.001$) greater than zero as determined by use of a probability t test. For each of the horses, a sample of serum that was collected on day 7 was not processed via the ACS technique. Among all horses, mean IL-1Ra concentration in these serum samples was 343 ± 212 pg/mL as estimated by use of the mouse IL-1Ra ELISA. Inspection of the data revealed that 1 horse was a significant outlier with a serum IL-1Ra concentration of 2,430 pg/mL. Because this value was more than 3 SDs greater than the mean value, this horse was removed and the data were reanalyzed. Reanalysis revealed that the mean serum IL-1Ra concentration among all horses was 45 ± 29 pg/mL, which was not significantly ($P = 0.143$) different from zero as determined by use of a probability t test. Furthermore, with this outlier data removed, the mean IL-1Ra concentration in ACS was significantly ($P < 0.001$) greater than that in unprocessed serum (234 ± 27 pg/mL and 45 ± 29 pg/mL, respectively).

Clinical assessments—All horses entered the study with acceptable body condition scores. Clinical

examinations revealed that a mild degree of lameness (significantly [$P < 0.001$] different from the prestudy baseline assessment) was induced in the osteoarthritis-affected joints (of all horses) by day 14 of the study, and this persisted throughout the study period in the placebo-treated group. Significant ($P = 0.001$) improvement in the degree of lameness was detected on day 70 in the osteoarthritis-affected joints treated with ACS, compared with the osteoarthritis-affected joints treated with placebo (Figure 2). Although the amount of joint effusion and response to flexion and joint manipulation were significantly ($P < 0.001$) greater than prestudy baseline assessments by day 7 after induction of osteoarthritis, no significant improvement in either variable was detected in response to treatment at any time point.

Compared with the sham-operated control joints, surgery to induce osteoarthritis resulted in significant radiographic joint changes (ie, proliferation or mineralization at the joint capsule attachment [$P = 0.001$], subchondral bone lysis [$P < 0.001$], and osteophyte formation [$P = 0.011$]); however, no significant treatment effects were detectable.

Synovial fluid—Results of routine synovial fluid analysis indicated that, as expected, the WBC count and total protein concentration increased significantly ($P < 0.001$) with induction of osteoarthritis throughout the study period. Compared with the placebo-treated osteoarthritis-affected joints, a slight increase in the percentage of neutrophils in the total WBC count was evident in osteoarthritis-affected joints treated with ACS, although this difference was not significant ($P = 0.069$). On calculation of the mean percentages of neutrophils in the total WBC counts in joints with and without osteoarthritis within each treatment group, the percentage in the osteoarthritis-affected joints that were treated with ACS (11.4%) was significantly ($P < 0.001$) greater than the percentages in the sham-operated joints of the ACS-treated group (6.8%) and both the osteoarthritis-affected and sham-operated joints of the placebo-treated group (6.2% and 5.2%, respectively). The percentages of neutrophils in the synovial fluid of the osteoarthritis-affected and sham-operated joints of the placebo-treated group were not significantly different.

There was also a significant ($P < 0.001$) interaction between treatment and induction of osteoarthritis on total protein concentration of synovial fluid. Mean synovial fluid protein concentration was significantly decreased in the sham-operated joints of ACS-treated horses (1.58 ± 0.06 g/dL), compared with the sham-operated joints of placebo-treated horses (1.97 ± 0.06 g/dL); however, the protein concentration in synovial fluid from osteoarthritis-affected joints treated with ACS (2.53 ± 0.06 g/dL) was not significantly different from that of the osteoarthritis-affected joints treated with placebo (2.63 ± 0.06 g/dL). The color, clarity, and mucin clot formation were not significantly affected by ACS treatment.

The GAG and PGE_2 concentrations in synovial fluid were significantly ($P > 0.001$) increased with induction of osteoarthritis, compared with findings in the sham-operated joints. Although the mean concentrations of GAG and PGE_2 were lower in the osteoarthritis-affected joints treated with ACS, compared with the osteoarthri-

tis-affected joints treated with placebo, the difference was not significant for either variable.

The synovial fluid concentration of IL-1Ra (assessed by use of human anti-IL-1Ra antibody) was unchanged by induction of osteoarthritis or treatment with ACS throughout the study. When the IL-1Ra concentration in synovial fluid was estimated by use of mouse anti-IL-1Ra antibody, mean values calculated from both joints of the ACS-treated horses at days 35 and 70 were significantly ($P = 0.005$) increased, compared with mean values calculated from both joints of the placebo-treated horses (**Figure 3**). Although the synovial fluid IL-1Ra concentrations were typically higher in the osteoarthritis-affected joints of ACS-treated horses (compared with their sham-operated joints), there was no significant difference in this variable between the osteoarthritis-affected and sham-operated joints of the ACS-treated group at any time point (including the largest difference that was detected on day 70 [71 ± 13 pg/mL vs 44 ± 13 pg/mL, respectively]). The synovial fluid data were checked for outliers, and no values were > 3 SDs from the mean; thus, all horses were included in the analysis.

Gross pathologic observations—At necropsy examination, articular cartilage fibrillation (both full [$P = 0.002$] and partial thickness [$P < 0.001$]) as well as hemorrhage within the synovial membrane ($P < 0.001$) was significantly increased in osteoarthritis-affected joints, compared with sham-operated joints. Similarly, the total score for erosion and synovial membrane hemorrhage in the osteoarthritis-affected joints was significantly greater than the value in sham-operated joints. Compared with osteoarthritis-affected joints treated with placebo, the total score for erosion and synovial membrane hemorrhage in osteoarthritis-affected joints treated with ACS was improved (ie, decreased from 4.2 ± 0.6 to 2.9 ± 0.6), but this difference was not significant ($P = 0.093$). The total score for erosion and synovial membrane hemorrhage in sham-operated joints treated
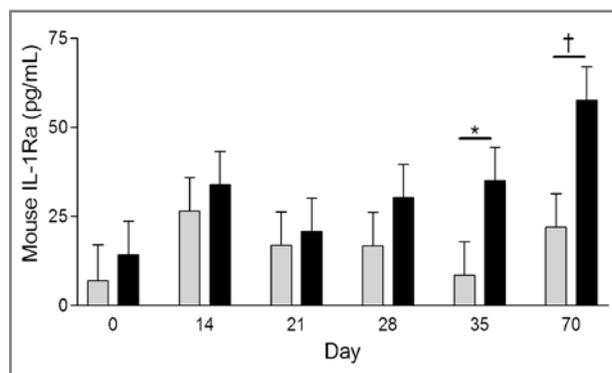

Figure 3—Synovial fluid concentrations of IL-1Ra (estimated by use of mouse anti-IL-1Ra antibody) in the same horses described in Figure 1 at intervals during a 70-day study of the clinical and histologic effects of intra-articular administration of ACS in the treatment of experimentally induced osteoarthritis. Values represent the mean IL-1Ra concentration of both osteoarthritis-affected and sham-operated joints in horses treated with ACS (black bars) or those treated with placebo (light gray bars). *Value significantly ($P < 0.05$) different from value for placebo-treated joints at this time point. †Value significantly ($P < 0.05$) different from value for placebo-treated joints at this time point and from values for ACS-treated joints at days 0 through 28.

with placebo and with ACS was 0.8 ± 0.6 and 0.4 ± 0.6, respectively; this difference was not significant.

Histologic examinations—The histologic evaluation of the synovial membrane revealed a significant increase in cellular infiltration ($P < 0.001$), intimal hyperplasia ($P = 0.001$), and subintimal edema ($P = 0.009$) in joints with induced osteoarthritis, compared with findings in sham-operated joints. The degree of intimal hyperplasia was significantly ($P = 0.033$) less in osteoarthritis-affected joints treated with ACS (score, 0.4 ± 0.3), compared with osteoarthritis-affected joints treated with placebo (score, 1.3 ± 0.3). No other significant differences among joints or treatment groups were detected. No significant differences were noted for subintimal fibrosis or subintimal vascularity for any comparison.

Histologic evaluation of the H&E-stained articular cartilage sections from the third carpal bone revealed an increase in the cumulative score of osteoarthritis-induced joints from either treatment group, compared with their sham-operated counterparts (2.5 ± 0.6 and 4.4 ± 0.6, respectively), although the difference was not significant ($P = 0.059$). This increase was the result of changes mainly in the fibrillation and chondrone formation on the third carpal bone. No significant improvements in articular cartilage morphologic variables (fibrillation, chondrocyte necrosis, chondrone formation, or focal loss of cells) following ACS treatment were detected. Histochemical evaluation of SOFG-stained sections of articular cartilage revealed significant ($P = 0.004$) loss of stain deposition in all 4 evaluated regions of osteoarthritis-affected joints, but no significant improvements were detected following ACS treatment. No significant difference in cumulative SOFG staining scores between osteoarthritis-affected and sham-operated joints was detected in either group.

Articular cartilage matrix evaluation—The total articular cartilage GAG content was significantly ($P = 0.008$) decreased in osteoarthritis-affected joints, compared with sham-operated joints. Compared with osteoarthritis-affected joints that were treated with placebo, cartilage GAG content and $^{35}SO_4$ incorporation in osteoarthritis-affected joints that were treated with ACS were not significantly different.

Discussion

In the present study involving induction of osteoarthritis (via creation of a chip fracture) in the middle carpal joint of horses, significant improvement in clinical lameness and decreased pathologic changes within the synovial membrane of the osteoarthritis-affected joints after ACS treatment were detected, compared with findings in osteoarthritis-affected joints treated with placebo. Furthermore, other variables including total cartilage erosion and synovial membrane hemorrhage score and synovial fluid components appeared to indicate improvement in the ACS-treated joints, although these changes were not significant. There were no known negative effects (based on outcome measures) associated with the short-term duration of the present study, with the exception of an increase in the relative neutrophil percentage detected cytologically in synovial fluid collected from osteoarthritis-affected joints. There was a slight increase (albeit not significant) in the percentage of neutrophils in osteoarthritis-affected joints treated with ACS, compared with osteoarthritis-affected joints treated with placebo, and it is the authors' belief that this change represented an effect of the ACS treatment but was not clinically important.

In a previous report[13] of ACS preparation from human blood, the method used in that investigation (and used in the present study) significantly increased the concentration of IL-1Ra and presumably other anti-inflammatory factors in the serum samples. Although an increase in serum IL-1RA concentration in ACS samples, compared with unprocessed serum samples, was not supported by results obtained by use of the human ELISA in our study, it was evident when the mouse ELISA was used. Stimulated by our study, results of a recent evaluation by our group of equine synovial fluid and serum samples spiked with recombinant equine IL-1Ra have suggested that the use of the mouse anti-IL-1Ra antibody is more appropriate than the human anti-IL-1Ra antibody for estimation of IL-1Ra concentrations in those samples.

In a previous study,[5] the authors assessed the administration of equine IL-1Ra via gene therapy. By use of the human ELISA (involving human anti-IL-1Ra antibody), synovial fluid concentration of IL-1Ra increased (compared with the sham-operated joints of the treated horses) and peaked at 40 ng/mL; the value remained significantly high for more than 21 days. Thus, the concentration of IL-1Ra was an order of magnitude greater than that detected in the present study. Also, although similar changes were identified, the outcome measures were more profoundly affected in the gene therapy study, compared with findings in the present study. Specifically, in the gene therapy study, there was a significant improvement in the horses' clinical signs of pain without significant decrease in total protein or PGE_2 concentrations in synovial fluid. Administration of IL-1Ra via gene therapy significantly decreased or improved synovial effusion, gross articular cartilage erosion, and synovial membrane vascularity, compared with osteoarthritis-affected placebo-treated joints; in addition, improvements in morphologic parameters in articular cartilage were detected via histochemical staining when ACS-treated and placebo-treated osteoarthritis-affected joints were compared. Although similar changes were detected in osteoarthritis-affected joints after ACS treatment in the present study, those treatment-associated differences were not significant. On comparison of the gene therapy study and the study of this report, there was no indication that a different mechanism was responsible for the beneficial effects associated with ACS treatment. Findings in other species and other models of osteoarthritis confirm that improvements (such as those identified previously and in our study) develop following the administration of IL-1Ra.[22,23]

An interesting finding in the present study was that mean synovial fluid IL-1Ra concentration in all joints of horses in the ACS-treated group significantly increased with time, compared with the mean value in all joints of horses in the placebo-treated group. This

difference became apparent at day 35 of the study and was still evident at study termination 35 days later (day 70). Although the IL-1Ra concentration was numerically highest in the osteoarthritis-affected joints in which ACS was directly administered, it appears that the increased synovial fluid IL-1Ra concentration was a systemic event. A similar finding was also apparent after the gene transfer of equine IL-1Ra. These data may suggest that the administration of IL-1Ra stimulates endogenous production of IL-1Ra.

Results of our study indicated that the concentration of IL-1Ra in ACS was modestly higher than the concentration in unprocessed serum. The outcome measures after ACS administration in osteoarthritis-affected joints have similar changes, albeit to a lesser extent, to those detected when IL-1Ra was administered via gene therapy; thus, the authors cannot rule out IL-1Ra as a major factor responsible for those changes. It is well accepted that a multitude of proteins are differentially regulated during the 24-hour culture period in the ACS protocol, and the exact composition of this souplike mixture is still unknown.

To our knowledge, this is the first controlled study that has identified significant clinical and histologic improvement in osteoarthritis-affected joints of horses after ACS treatment, compared with effects of a placebo treatment. Future controlled clinical trials to assess the effects and duration of action of ACS treatment in horses with naturally occurring osteoarthritis are warranted. Additionally, the mechanisms of action and timing of administration of ACS treatment should be investigated.

Author, please provide the missing information for footnote c.

a. Frisbie DD, Al-Sobayil F, Billinghurst RC, et al. Serum biomarkers distinguish exercise from osteoarthritic pathology (abstr). *Osteoarthritis Cartilage* 2002;10(suppl A):S53.
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c. Reinecke J, Weinberger T, **Department (if applicable)**, **School/College (if applicable)**, **University or Organization**, **City**, **State**: Personal communication, 2005.
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e. PGE2 ELISA, Assay Design, Ann Arbor, Mich.
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