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. Various medications have been evaluated for the treatment of horses with osteoarthritis, including NSAIDs, corticosteroids, and hyaluronan.

Drugs used to treat osteoarthritis can be considered as modifying the clinical signs of osteoarthritis or as modifying the disease itself. In general, the former alleviate pain and inflammation and lead to a positive clinical outcome but may not affect the progression of the underlying disease process. The latter slow or reverse the disease process but may not have any direct effect on the clinical signs of the disease. The optimal medication for osteoarthritis would be a drug that provides both effects in a safe and convenient manner.

Nonsteroidal anti-inflammatory drugs remain one of the frontline treatments for lameness, despite the well-known detrimental effects that can occur following prolonged systemic use. The NSAIDs are generally regarded as affecting the clinical signs without any disease-modifying activity.

A topically administered DLC is now approved in the United States for treatment of horses with osteoarthritis. This formulation provides locally enhanced topical delivery without achieving a clinically important blood concentration of DLC, thus alleviating the adverse effects associated with systemically administered NSAIDs. Studies in laboratory animals reveal that when diclofenac is incorporated into large multilamellar liposomes, it readily penetrates the skin but is eliminated more slowly from the subcutaneous tissues than the same drug in a gel formulation. This formulation was developed for treatment

**Evaluation of topically administered diclofenac liposomal cream for treatment of horses with experimentally induced osteoarthritis**

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

**Objective**—To assess the clinical, biochemical, and histologic effects of topically administered diclofenac liposomal cream (DLC) in the treatment of horses with experimentally induced osteoarthritis.

**Animals**—24 horses.

**Procedures**—Osteoarthritis was induced arthroscopically in 1 middle carpal joint of all horses. Eight horses treated with DLC were given 7.3 g twice daily via topical application. Eight horses treated with phenylbutazone were given 2 g orally once daily. Eight control horses received no treatment. Evaluations included clinical, radiographic, magnetic resonance imaging, synovial fluid, gross, and histologic examinations as well as histochemical and biochemical analyses.

**Results**—No adverse treatment-related events were detected. Horses that were treated with DLC or phenylbutazone had significant clinical improvement of lameness, unlike the control horses. Treatment with DLC induced significant improvement in staining and total articular glycosaminoglycan content, compared with no treatment. Treatment with phenylbutazone induced significant reduction in synovial fluid prostaglandin E\textsubscript{2} concentration, compared with DLC and no treatment. Treatment with DLC induced significantly less radial carpal bone sclerosis and overall gross cartilage erosion, compared with phenylbutazone.

**Conclusions and Clinical Relevance**—Results indicated that DLC had both clinical sign-modifying and disease-modifying effects. Only clinical sign-modifying effects were detected in association with phenylbutazone administration. Treatment with DLC had significant beneficial effects, compared with phenylbutazone, and no detrimental effects. Results suggested that DLC is a viable therapeutic option for horses with osteoarthritis. (Am J Vet Res 2009;70:210–215)

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DLC</td>
<td>Diclofenac liposomal cream</td>
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<tr>
<td>dpm</td>
<td>Disintegrations per minute</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>PGE\textsubscript{2}</td>
<td>Prostaglandin E\textsubscript{2}</td>
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of horses with osteoarthritis. A double-blind controlled clinical study \(^{10}\) conducted with 122 horses with naturally occurring osteoarthritis revealed a significant change in lameness at 3 days, indicating improvement in the clinical signs.

Since the approval of topically administered DLC in the United States, the manufacturer estimates that > 10 million doses have been applied to horses. Presently, only 28 adverse drug reactions (1/357,000 doses) have been reported by veterinarians and horse owners. Most consisted of a mild skin reaction at the site of application. These data support the concept that application of NSAIDs via locally enhanced topical delivery provides safe and effective relief of the clinical signs of osteoarthritis in horses.

Experimentally induced osteoarthritis has been used in horses for > 10 years for assessment of the pathophysiologic process and the efficacy of therapeutic substances in a controlled environment.\(^{11–16}\) The purpose of the blinded controlled study reported here was to evaluate the clinical sign- and disease-modifying effects of topically administered DLC in horses, compared with effects in control horses and those treated with orally administered phenylbutazone, after experimental induction of osteoarthritis. Our hypothesis was that results in horses treated with DLC would be more favorable than results in control horses or those treated with phenylbutazone.

### Materials and Methods

#### Experimental design and induction of osteoarthritis—The Colorado State University Animal Care and Use Committee approved the study protocol for this experiment, which included the use of 24 healthy 2- to 5-year-old horses. Prior to inclusion in the study, horses underwent a lameness examination, body condition assessment, radiography of the carpal joints, range of motion (flexion) testing of the carpal joints, and examination for joint effusion; only horses without abnormal findings were permitted in the study.

As described,\(^{17}\) on day 0 following anesthesia and routine preparation for surgery, each horse underwent bilateral arthroscopic surgery of the middle carpal joint 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 articulating cartilage surface of the radial carpal bone at the level of the medial synovial plica. The fragment was allowed to remain adhered to the joint capsule proximally. A motorized arthroburr was used to debride the exposed subchondral bone between the fragment and parent bone. A 15-mm-wide defect bed for the 8-mm-wide fragment was created, and the debris was not actively flushed from the joint, thereby inducing osteoarthritis. This joint was designated as the osteoarthritis-affected joint; the sham-operated joint was used as the control joint. The arthroscopic portals were closed with 2-0 nylon suture in a simple interrupted pattern, and cyanoacrylate\(^{18}\) was applied to the incision. The forelimbs were bandaged, and the horses were allowed to recover from anesthesia and surgery. All study horses received 1 dose of cefiofur\(^ {19}\) (2.2 mg/kg) IM just prior to surgery and 2 g of phenylbutazone\(^ {20}\) orally once daily for 5 days prior to surgery. Bandages were changed every 3 to 5 days and maintained until suture removal at 10 days after surgery.

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

#### Treatment groups—One week after surgery (day 7), the horses were ranked by lameness score and randomly assigned to 1 of 3 groups with 8 horses in each group (DLC, phenylbutazone, and control). All evaluators were unaware of treatment assignment. Treatment began on day 14. Horses in the DLC group were treated topically with 7.3 g of DLC twice daily, which is the label dose. The cream was applied to the joint for 5 minutes or until the cream was absorbed, whichever occurred first. Horses in the phenylbutazone group were administered 2 g of phenylbutazone paste PO once daily. Control group horses received no further treatment.

#### Assessment of clinical outcomes—Animal-care personnel assessed horses daily for apparent comfort, movement, and respiratory character. Clinical examinations of both forelimbs were performed weekly from day 0 (baseline; before surgery) throughout the study period. Lameness was graded on a standardized 0 to 5 scale with the horse trotting on hard ground.\(^ {18}\) Horses were evaluated and videographed moving in a straight line both away from and toward the evaluator as well as from a point perpendicular to the evaluator, with each side of the horse being assessed. To account for variation in the degree of lameness between horses, a change in lameness score was calculated by use of day 14 (the last pretreatment evaluation) as the postosteoarthritis pretreatment baseline (a positive change in score indicated improvement). All other clinical outcomes were graded on a 0 to 4 scale (0 represented normal, and 4 represented severe change); a similar change in flexion and joint effusion score was calculated by use of day 14 as the postosteoarthritis pretreatment baseline. As an indication of joint pain, carpal flexion was performed after lameness grading. As an indication of increased synovial inflammation in the middle carpal joint, the extent of the effusion was graded after carpal flexion. All clinical outcomes were assessed by a board-certified large animal surgeon who focuses on equine lameness. To prevent evaluator bias, the carpal joints of all horses were washed followed by the application of a nonlabeled placebo cream\(^ {18}\) applied to all joints except for the DLC-treated joints. Immediately prior to clinical examination, all joints were wiped free of excess material, and the evaluator wore latex gloves.

Radiographic evaluation of both carpi was performed prior to inclusion in the study (day –7), following the induction of osteoarthritis (day 14), and at termination of the study (day 70). A board-certified radiologist assessed images (radiographic and MRI). The
radiographic images were evaluated for bony proliferation at the joint capsule attachment, subchondral bone lysis, and osteophyte formation on a 0 to 4 scale (0 represented normal, and 4 represented severe change). For each horse, bilateral carpal MRIs (sagittal, transverse, and frontal plane images made by use of proton density and short T1 inversion recovery sequences) were performed on all horses on days 13 and 56 and after euthanasia (day 70). The MRI images were evaluated for synovial fluid, synovial membrane proliferation, joint capsule thickness, joint capsule edema, joint capsule fibrosis, edema, and sclerosis of the radial carpal and third carpals bones on a 0 to 10 scale (0 represented normal, and 10 represented severe change). A total MRI score was also calculated on the basis of a cumulative value for the measured MRI variables.

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

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

Diclofenac concentrations were determined in synovial fluid from 4 horses in the DLC group at days 14, 28, and 70 by use of liquid chromatography–mass spectroscopy method. The limit of quantitation (limit at which 2 values can be distinguished) was 0.5 ng/mL, and the limit of detection was 0.2 ng/mL.

Gross pathologic evaluation of joints—At the end of the study, all horses were euthanatized via administration of pentobarbital sodium. For each horse, a necropsy examination was performed, during which both middle carpal joints were specifically examined for degree and location of articular cartilage fibrillation or erosion. A subjective grade (scale of 0 to 4) was assigned for partial- or 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 erosion score also was assigned on the basis of the degree of articular cartilage fibrillation and synovial membrane hemorrhage in the entire joint by use of a 0 to 4 scale.

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

Samples of articular cartilage (5 mm²) were obtained from each joint. Samples were stored in neutral-buffered 10% formalin for 7 days, decalcified, embedded in paraffin, sectioned at 5 μm, mounted on glass slides, and stained with H&E as well as safranin-O fast green. Sampling 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). All histologic examinations were performed by an evaluator who was unaware of treatment assignment.

The H&E-stained sections were evaluated for articular cartilage fibrillation, chondrocyte necrosis, chondrone formation (chondrocyte division within a lacuna), focal cell loss. Numeric values ranging from 0 to 4 were assigned to each variable (0 represented no abnormal change, and 4 represented the most severe change). A cumulative pathology score for each articular cartilage sample was also determined by addition of scores for all variables.

Articular cartilage sections stained with safranin-O fast green were evaluated for intensity of staining in the tangential, intermediate, radiate territorial, and radiate interterritorial zones of the third carpal, fourth carpal, and radial carpal bones. Numeric values ranging from 0 to 4 were assigned to each variable (0 indicated no stain uptake, and 4 indicated normal stain uptake), and a cumulative score for each articular cartilage sample was calculated by summation of the zonal scores.

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

For analysis of cartilage matrix metabolism, articular cartilage samples were aseptically collected from the weight-bearing surface that was remote from the osteochondral fragment within each joint (Figure 1), and incorporation of sulfur 35 radiolabeled SO₄ was measured, by use of reported methods. Samples were processed in duplicate, and the results were reported as counts per minute per milligram of dry weight.

Statistical analysis—Data for categoric variables measured multiple times during the study were evaluated by use of a multinomial ANOVA or multinomial ANCOVA framework with a software program, depending
on the presence or absence of a covariate, respectively. Data for categoric variables measured once were statistically evaluated by use of a generalized linear mixed model in a multinomial ANOVA framework with the same software program. Data for continuous variables measured multiple times over the course of the study were statistically evaluated by use of an ANOVA or ANCOVA framework with a software program, depending on the presence or absence of a covariate, respectively.

The ANOVA tables were used to determine significant main effects and interactions between main effect variables. When individual comparisons were made, least square means were used. The pivotal variables were considered lameness scores, MRI findings, synovial fluid PGE2 concentration, synovial fluid total protein concentration, total erosion score, and cumulative pathology score. A value of \( P \leq 0.05 \) was considered significant for all comparisons. Values are reported as mean ± SEM.

**Results**

**Clinical outcomes**—All horses had a significant (\( P < 0.001 \)) increase in lameness in the affected limb (score, 2.52 ± 0.83), compared with the sham-operated limb (score, 0.40 ± 0.57) for days 7 and 14. A significant (\( P < 0.001 \)) treatment effect was observed in the change in lameness scores independent of limb (ie, osteoarthritic; DLC, 0.13 ± 0.05; phenylbutazone, 0.27 ± 0.05; no treatment, 0.12 ± 0.05). Although not significant, there was an increase in lameness for both limbs in the control group (control joint, –0.16 ± 0.13; osteoarthritic joint, –0.06 ± 0.13), a decrease in lameness in both limbs of the phenylbutazone group (control joint, 0.20 ± 0.13; osteoarthritic joint, 0.34 ± 0.13), and, as expected, a decrease in lameness only in the osteoarthritic limb (0.27 ± 0.13) in the DLC group. There was no significant difference between the DLC and phenylbutazone osteoarthritic limbs with respect to decreases in lameness (\( P = 0.668 \)).

All horses had a significant (\( P < 0.001 \)) increase in flexion score in the osteoarthritic-affected limb (0.72 ± 0.10), compared with that in the sham-operated limb (0.31 ± 0.09), for days 7 and 14. There were no significant treatment effects.

All horses had a significant (\( P < 0.001 \)) increase in effusion score in the osteoarthritic-affected limb (2.38 ± 0.10), compared with that in the sham-operated limb (1.17 ± 0.09), for days 7 and 14. There were no significant treatment effects.

**Radiographic evaluation**—A significant increase in radiographically detected joint lesions was induced for each radiographic outcome variable after surgery. Total radiographic scores before treatment for sham-operated joints (0.36 ± 0.22), compared with those for osteoarthritic-affected joints (4.22 ± 0.22), were significantly (\( P < 0.001 \)) different. No significant treatment effects were detected. MRI scores—A significant (\( P < 0.001 \)) increase was detected in the total MRI score for osteoarthritic-affected (19.69 ± 0.34) versus sham-operated joints (14.15 ± 0.56) after surgery in all 3 groups. There were no significant effects in the total MRI score seen with phenylbutazone or DLC treatment. However, a significant (\( P = 0.006 \)) treatment effect was observed in the degree of sclerosis in the radial carpal bone. The phenylbutazone-treated (2.46 ± 0.10) horses had a significantly greater degree of sclerosis than the DLC (2.04 ± 0.10) or control (2.02 ± 0.10) horses. No other significant treatment effects were detected for any other MRI variable.

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**Figure 1**—Schematic drawing of the middle carpal joint (dorsal surface) of a horse, indicating the area of osteochondral fragmentation (darkly shaded region) on the radial carpal bone (CR) and sample collection sites (outlined boxes). The outlined boxes include the cartilage collection site for a proteoglycan synthesis assay (a), the collection site for articular cartilage glycosaminoglycan content assay (b), and the collection site for histologic and histochemical analyses (c). C1 = Intermediate carpal bone, C2 = Sectioned carpal bone, C3 = Third carpal bone, C4 = Fourth carpal bone. (Adapted from McIlwraith CW, Wright I, Nixon AJ, et al. Chapter 4. In: Diagnostic and surgical arthroscopy in the horse. 3rd ed. St. Louis: WB Saunders Co, 2005:47–127. Reprinted with permission.)

**Figure 2**—Photomicrographs of sections of articular cartilage of osteoarthritic carpal joints of horses that received no treatment (control), phenylbutazone, or DLC. Safranin-O fast green stain; bar = 200 μm.
Synovial fluid—Results of routine synovial fluid analysis indicated that, as expected, the total protein concentration increased significantly (P < 0.001) with induction of osteoarthritis throughout the study period between sham-operated joints (2.44 ± 0.14 g/dL) and osteoarthritis-affected joints (3.55 ± 0.10 g/dL). Synovial fluid WBC counts were not significantly increased (P = 0.454) by induction of osteoarthritis. There were no significant treatment effects in synovial fluid total protein concentration or WBC count.

The glycosaminoglycan concentration in synovial fluid did not significantly (P = 0.069) increase after induction of osteoarthritis. There were no significant treatment effects in synovial fluid glycosaminoglycan concentrations.

Synovial fluid PGE\textsubscript{2} concentration significantly (P < 0.001) increased after induction of osteoarthritis (217.00 ± 29.59 pg/mL), compared with findings in the sham-operated joints (33.00 ± 3.80 pg/mL). There was a significant (P < 0.001) treatment effect for synovial fluid PGE\textsubscript{2} concentration. The phenylbutazone group (711 ± 1.22 pg/mL) had significantly lower PGE\textsubscript{2} concentrations, compared with the DLC (2,314 ± 1.22 pg/mL) and control (2,081 ± 1.22 pg/mL) groups for the sham-operated and osteoarthritic joints.

On day 14 of the study, diclofenac concentrations were less than the limit of detection in all synovial fluid samples tested. On days 28 and 70, the concentration of diclofenac in synovial fluid samples ranged from 0.8 ng/mL to less than the limit of detection.

Gross pathologic findings—At necropsy, hemorrhage within the synovial membrane was significantly (P = 0.002) increased in osteoarthritis-affected joints (score, 1.29 ± 0.12), compared with that in sham-operated joints (score, 0.63 ± 0.12). Similarly, the articular cartilage total erosion score was significantly (P < 0.001) increased in osteoarthritis-affected joints (1.79 ± 0.14), compared with that in sham-operated joints (1.03 ± 0.14). The total score for erosion was significantly (P = 0.019) decreased in osteoarthritis-affected joints treated with DLC (1.37 ± 0.25), compared with osteoarthritis-affected joints treated with phenylbutazone (2.25 ± 0.25), but not significantly (P = 0.301) different, compared with the osteoarthritic-affected control joints (1.75 ± 0.25). No other significant treatment effects were detected for other variables evaluated at necropsy in the osteoarthritis-affected joints.

Histologic examinations—The individual and cumulative scores for synovial membrane lesions were not significantly different between the osteoarthritis-affected joints and the sham-operated joints. Likewise, there were no significant differences in cumulative scores between the horses that received phenylbutazone or DLC treatment.

Histologic evaluation of H&E-stained articular cartilage revealed a significant (P < 0.001) increase in the cumulative pathology score in osteoarthritis-affected (3.53 ± 0.26) versus sham-operated (2.30 ± 0.26) joints, when all locations were considered. There were no significant differences between horses that received phenylbutazone or DLC treatment.

Evaluation of articular cartilage for safranin-O fast green staining revealed a significant (P = 0.005) decrease in osteoarthritis-affected (score, 4.38 ± 0.27) versus sham-operated (5.46 ± 0.27) joints, for the cumulative score in all locations. An increase (P = 0.053) in the cumulative score was observed in the DLC (5.36 ± 0.33) versus the phenylbutazone (4.48 ± 0.33) and the control (4.71 ± 0.33) group, independent of limb (Figure 2). When the same comparison was made considering only the radial carpal bone location, a significant (P = 0.011) association was also detected.

Articular cartilage matrix evaluation—Evaluation of total articular cartilage glycosaminoglycan content over all locations did not reveal significant differences between osteoarthritis-affected and sham-operated joints. However, articular cartilage from the intermediate carpal bone had a significant (P = 0.024) treatment effect. Both the DLC (416 ± 8.3 dpm/mg of dry weight cartilage) and phenylbutazone (400 ± 8.3 dpm/mg of dry weight cartilage) groups had significantly higher articular cartilage glycosaminoglycan content, compared with that of the control group (383 ± 8.3 dpm/mg of dry weight cartilage), independent of limb. No significant differences were detected in matrix metabolism with induction of osteoarthritis or treatment.

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 was detected in horses treated with DLC or phenylbutazone, compared with control horses. Lameness improved in both forelimbs in the phenylbutazone group and only 1 limb in the DLC group. This was an expected finding because phenylbutazone is administered systemically, whereas DLC is administered topically and was not expected to induce systemic effects. Supporting a lack of systemic absorption through uptake within the joint space was the detectable but not quantifiable concentrations of diclofenac in the synovial fluid, which would be the location expected to have one of the highest concentrations. It is clear that the tissue cage model used by Caldwell et al did not accurately predict the penetration of DLC into the synovial space; the difference between results of the present study and those of the Caldwell study was most likely caused by morphologic and physiologic differences between the 2 models. There were no significant (P = 0.068) differences between the DLC and phenylbutazone groups with regard to clinical signs in the osteoarthritis-affected limbs.

Regarding disease-modifying effects of treatment, significant differences were found between the DLC and control groups for safranin-O fast green staining and glycosaminoglycan concentrations in the articular cartilage. The cartilage concentration of glycosaminoglycan was also higher in the DLC and phenylbutazone groups when compared with the control group, but unlike the safranin-O fast green staining, significant differences between DLC and phenylbutazone groups were not observed. This disparity most likely stemmed from the zonal-spatial evaluation of the histologic sections...
and the smaller sample size, compared with the much larger sample size used for glycosaminoglycan analysis and independence of zonal-spatial orientation. Treatment with DCL also resulted in significantly less radial carpal bone sclerosis, gross articular cartilage erosion, and cartilage safranin-O fast green staining, compared with phenylbutazone. When the phenylbutazone group was compared with the control group for these variables, the numeric scores were higher, although not significantly.

Concentrations of PGE$_2$ in the synovial fluid were significantly lower in joints treated with phenylbutazone, compared with the control group. The PGE$_2$ concentrations in the synovial fluid were not significantly altered by treatment with DCL. This finding was surprising given the previous report$^5$ of an equine tissue cage study that revealed a decrease in PGE$_2$ concentration after topical application of DCL. However, in the previous study, diclofenac concentrations in the inflammatory exudates were approximately 80 ng/mL; in the present study, they were approximately 0.8 ng/mL, so it is likely that the diclofenac concentration was less than the threshold value necessary to inhibit the cyclooxygenase enzyme in the synovial fluid. The PGE$_2$ and diclofenac concentrations in the synovial fluid raise an important question. Because treatment with phenylbutazone induced a decrease in PGE$_2$ concentration but no disease-modifying effects other than increased glycosaminoglycan concentration, is it possible that a decrease in PGE$_2$ concentration in synovial fluid may not be a true indicator of overall joint health? Positive effects on the health of the joint were found in horses treated with DCL in the present study without changes in PGE$_2$ concentrations. This effect could have been mediated by the presence of diclofenac in the periarticular tissues, which was not determined in this study. Whether diclofenac had a positive effect on the osteoarthritic joint through some unrecognized mechanism, separate from its action as a cyclooxygenase enzyme inhibitor, will require further study.

Substantial modification of clinical signs by use of topically administered DCL has been reported in horses with osteoarthritis$^6$ and was confirmed again in the present study. To our knowledge, this is the first controlled study that revealed significant disease-modifying and clinical sign–modifying effects in osteoarthritis–affected joints of horses after treatment with diclofenac or any other NSAID. The precise mechanism by which disease-modifying effects were induced is unclear. Nevertheless, results of this study indicated that DCL is a viable therapeutic option in managing osteoarthritis in horses.

a. Surpass (1% diclofenac sodium) topical anti-inflammatory cream, IDEXX Pharmaceuticals Inc, Greensboro, NC.

b. Super Glue (cyanoacrylate adhesive), Pacer Technology, Rancho Cucamonga, Calif.

c. Nascel (celitofuran sodium), Pfizer Inc, New York, NY.

d. Equi-Phar (phenylbutazone paste), Schering-Plough Animal Health Corp, Union, NJ.

e. Cetaphil moisturizing cream, Galderma Laboratories Inc, Fort Worth, Tex.

f. OrthOne (1.0 T MRI), Oni Medical Systems Inc, Wilmington, Mass.

g. PGE, Kit, Assay Designs, Ann Arbor, Mich.

h. Amprep Mini-columns Ethyl C2 columns, GE Healthcare, Piscataway, NJ.

i. PROC GLIMMIX, SAS Institute Inc, Cary, NC.

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