

Effects of carprofen and dexamethasone on canine chondrocytes in a three-dimensional culture model of osteoarthritis

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Objective—To determine effects of carprofen and dexamethasone on chondrocytes in a culture model of osteoarthritis (OA).

Sample Population—Chondrocytes isolated from articular cartilage of the humeral head of 5 adult dogs.

Procedure—Chondrocytes were harvested, cultured and subcultured in monolayer, and then cultured in a 3-dimensional (3-D) medium. Cells from each dog were distributed into 6 groups with differing content of liquid medium for each 3-D construct (agarose [AG], AG plus interleukin [IL]-1 β , AG plus carprofen [4 μ g/mL], AG plus dexamethasone [1 mg/mL], AG plus IL-1 β [20 ng/mL] plus carprofen [4 μ g/mL], and AG plus IL-1 β (20 ng/mL) plus dexamethasone (1 mg/mL). On days 3, 6, 12, and 20 of culture, samples from all groups were collected. Liquid media were assayed for glycosaminoglycan, prostaglandin (PGE)₂, matrix metalloproteinase (MMP)-3, and MMP-13 concentrations. All 3-D constructs were evaluated for viability, cell morphology, proteoglycan staining, and collagen type-II concentration. Total glycosaminoglycan content in each 3-D construct was quantitated by spectrophotometric assay.

Results—Addition of IL-1 β caused a significant loss of cell viability and matrix production. Addition of carprofen or dexamethasone caused significant decreases in PGE₂ in the liquid media, and each was minimally effective in protecting chondrocytes against negative effects of IL-1 β .

Conclusions and Clinical Relevance—Human recombinant IL-1 β resulted in loss of cell viability, alterations in extracellular matrix components, and production of PG and MMP. Carprofen and dexamethasone had little effect on cell and matrix variables but did decrease PGE₂ concentrations and primarily affected the inflammatory pathway of osteoarthritis. (*Am J Vet Res* 2002;63:1363–1369)

Osteoarthritis (OA) is a progressive, degenerative disease of diarthrodial joints resulting in high morbidity. Many species are affected by OA, and it has been estimated that more than 20% of dogs > 1 year old are affected by OA.¹ Osteoarthritis is characterized by deterioration of articular cartilage (through the action of degradative enzymes) and nonpurulent

inflammation.¹ Primary goals in palliative treatment of animals with OA are to decrease pain and increase function. Two groups of pharmaceuticals most commonly used for the treatment of animals with OA are **nonsteroidal anti-inflammatory drugs (NSAIDs)** and corticosteroids. In addition to the known anti-inflammatory effects of these 2 classes of drugs, these pharmaceuticals reportedly have direct effects on cartilage metabolism and may stimulate synthesis of **interleukin (IL)-1**.²⁻⁸

Carprofen, a carboxylic acid, belongs to the group of propionic acid-derived NSAIDs. Members of this group include ibuprofen, ketoprofen, naproxen, and fenoprofen.⁹ Carprofen is a reversible inhibitor of **cyclooxygenase (COX)**, with greater potency against the COX-2 isoenzyme than against the COX-1 isoenzyme. By blocking the production of **prostaglandin (PG)** and inhibiting leukocyte migration, carprofen provides analgesic, anti-inflammatory, and antipyretic effects.^{10,11} Pharmacodynamics and pharmacokinetics of carprofen are characterized by a small volume of distribution, high affinity for plasma protein binding sites, and elimination by hepatic biotransformation.¹² Carprofen can directly influence chondrocyte activity in monolayer culture, resulting in dose-related changes in the rate of production of cartilage matrix.^{13,14}

Dexamethasone is a synthetic, highly potent, long-acting corticosteroid that possesses glucocorticoid activity. Corticosteroids inhibit the activity of phospholipase A₂, inhibiting both the cyclooxygenase and lipoxygenase pathways, thereby diminishing the inflammatory response. Although potentially beneficial for addressing the inflammatory components of OA, the use of corticosteroids for treatment of patients with OA is controversial.¹⁵ In many studies,¹⁶⁻²⁰ inhibition of mitotic activity and cell growth and inhibition of collagen synthesis by chondrocytes exposed to corticosteroids have been documented. In contrast, corticosteroids reportedly have a protective effect in osteoarthritic joints by reducing the formation or size of osteophytes and reducing the synthesis of inflammatory mediators.^{21,22}

Chondrocytes in **3-dimensional (3-D)** culture produce extracellular matrix with morphologic and functional characteristics similar to those of intact articular cartilage.²³⁻²⁵ An in vitro model of OA has been established by use of canine chondrocytes in 3-D culture with human recombinant IL-1 β .²⁶ This model reportedly provides at least a subset of the pathophysiologic events associated with naturally occurring OA and allows analysis of the effects of selected compounds on

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chondrocyte viability, extracellular matrix production, and biochemical events associated with OA. The purpose of the study reported here was to evaluate the effects of carprofen and dexamethasone on canine chondrocytes in a 3-D culture model of OA.

Materials and Methods

Sample population—Full-thickness articular cartilage was harvested from the humeral head of 5 adult dogs. The dogs were euthanized for reasons unrelated to orthopedic abnormalities, and all articular and periarticular surfaces of the shoulder joint were examined to ensure that they were free of gross abnormalities.

Chondrocyte culture—Canine chondrocytes were harvested, cultured and subcultured in monolayer to amplify cell numbers, and then transferred to a 3-D agarose-based medium, as described elsewhere.²⁵ Chondrocyte viability at the time of 3-D culture was determined on the basis of trypan blue exclusion; cells from each dog were > 95% viable. Equal volumes of 2% low-melting agarose (mean \pm SD gelling temperature, $25 \pm 5^\circ\text{C}$) in Dulbecco phosphate-buffered saline solution and double-strength RPMI containing 20% fetal calf serum were added to produce a cell concentration of 10^6 cells/mL. For each dog, 1 mL of cell suspension was added to wells of a 24-well tissue-culture plate. Five wells (1 well/dog) were filled with 1 mL of agarose-media suspension that did not contain cells. Plates were placed in a refrigerator at 4°C for 5 minutes.

Cells from each dog were distributed into 6 treatment groups, which were determined on the basis of the content of the liquid media that was added to each well. Treatment groups were agarose alone (AG), agarose plus 20 ng of IL-1 β /mL (IL), agarose plus 4 μg of carprofen/mL (C), agarose plus 1 mg of dexamethasone/mL (D), agarose plus 20 ng of IL-1 β /mL plus 4 μg of carprofen/mL (IL-C), and agarose plus 20 ng of IL-1 β /mL plus 1 mg of dexamethasone/mL (IL-D). One milliliter of the respective liquid media was added to each well containing the 3-D constructs. Plates were incubated at 37°C with 5% CO_2 and 95% humidity. First day of culture of 3-D constructs was designated as day 0. The liquid media in each well was replaced at 3-day intervals during culture of 3-D constructs.

Collection of media—On days 3, 6, 12, and 20, samples of media were collected from cells of each dog in all groups. Liquid medium from each well was collected and stored at -80°C for subsequent assay of glycosaminoglycan (GAG), PGE₂, matrix metalloproteinase (MMP)-3, and MMP-13 concentrations. Medium in each well of the 3-D constructs was collected and divided into 2 portions. One portion was placed in neutral-buffered 10% formalin for histologic processing, and the other portion was weighed, placed in distilled deionized water, and stored at -80°C for subsequent determination of GAG concentration.

Histologic and immunohistochemical evaluation—After routine processing for histologic examination, sections (thickness of 5 μm) were stained with H&E and toluidine blue. Sections were evaluated in triplicate by a single investigator (JLC) who was unaware of the treatment group and dog. Sections were evaluated for morphologic characteristics indicative of cell viability and to determine toluidine blue-stained proteoglycans.

Unstained sections (thickness of 5 μm) were deparaffinized. Endogenous peroxidase quenching was performed by use of 3% H_2O_2 in water. Pepsin digestion was performed during a 30-minute incubation. Immunohistochemical staining was performed by use of a commercially available avidin-biotin-peroxidase kit.⁴ Serum was blocked, the primary anti-

body^c (rabbit anti-bovine collagen type II at a dilution of 1:1,000) was applied, and samples were incubated for 18 hours at 4°C . Hematoxylin counterstain was applied. Sections were examined for evidence of type-II collagen staining.

GAG assay—Total sulfated GAG content was quantitated by use of the dimethylmethylene blue (DMMB) assay.²⁷ Frozen samples were thawed and digested in 1.5 mL of a solution of 0.5 mg of papain (14 U/mg)/mL in distilled deionized water at 65°C for 4 hours. Two 100- μL aliquots of each digest solution were assayed for total GAG content by addition of 2.5 mL of DMMB solution and spectrophotometric determination of absorbance at 525 nm.

Known concentrations of bovine trachea chondroitin sulfate A were used to construct a standard curve for the DMMB assay. Results were standardized to correct for differences in sample weights. Correction for media differences was not necessary, as determined on the basis of values for the control samples. Total GAG content for samples was reported as micrograms per milliliter for liquid media samples and micrograms per milliliter per gram for digested 3-D samples.

PGE₂, MMP-3, and MMP-13 assays—Total PGE₂, MMP-3, and MMP-13 content in the liquid medium of each well was quantitated by use of enzyme immunoassay systems.^{4h} Stored media were thawed and then assayed for PGE₂, MMP-3, and MMP-13 content in accordance with manufacturer's instructions. All samples were assayed in duplicate. Sample concentrations determined from the standard curve were used for data analysis. Measured concentrations were reported as picograms per well for PGE₂ and as nanograms per milliliter for MMP-3 and MMP-13.

Statistical analysis—Statistical analyses were performed by use of a computer software program.¹ Data for cells of each of the 5 dogs in each treatment group were combined, and mean \pm SEM values were determined. Data were compared among treatment groups at each collection time and within each treatment group over time. A 1-way ANOVA and post hoc test were performed to determine differences within treatment groups for GAG content, GAG loss, PGE₂ production, MMP-3 production, and MMP-13 production. Differences among treatment groups for each variable were analyzed in the same manner for GAG, PGE₂, MMP-3, and MMP-13 content. Pearson product-moment correlation analysis was used to determine whether changes in GAG content, GAG loss, PGE₂ production, MMP-3 production, and MMP-13 production were correlated with each other. Significance was set at $P \leq 0.05$.

Results

Histologic examination—Canine chondrocytes in groups AG and C maintained characteristics of viability and differentiation throughout the study. Chondrocytes were uniformly distributed throughout the sections and appeared round to oval with round basophilic nuclei on H&E-stained sections in group AG and C at all sample points. Cells in sections from group D maintained characteristics of viability through day 12. However, on day 20, most of the cells from this group had histologic characteristics indicative of loss of viability. Cells in sections from the IL group had characteristics indicative of loss of viability, including nuclear pyknosis, karyorrhexis, and lysis, throughout the study. Most cells in groups IL-C and IL-D maintained characteristics of viability and differentiation throughout the study. However, subjectively more cells

with loss of viability were evident in sections from these groups than in cells from groups AG and C.

Proteoglycan production, as evidenced by toluidine blue staining, was detected for all treatment groups. In groups AG, C, IL-C, and IL-D, pericellular and territorial matrix production increased consistently throughout the study. Subjectively, there was little difference in matrix staining among these groups. Proteoglycan staining for group D was similar to these groups on days 3, 6, and 12. However, sections from group D on day 20 had pericellular and territorial matrix staining that was poorly defined or totally lacking. Matrix production was subjectively less in the IL group throughout the study. Little or no territorial matrix was evident in any section, and matrix staining was nearly nonexistent in sections obtained on day 20 (Fig 1).

GAG content—All treatment groups contained measurable amounts of GAG in the 3-D constructs at all sample times. Concentrations of GAG correlated well with histologic findings regarding matrix production. Concentrations of GAG were significantly lower in the IL group on days 3 ($P = 0.013$) and 6 ($P < 0.001$), compared with values for all other groups on those days. On day 6, GAG content was significantly higher

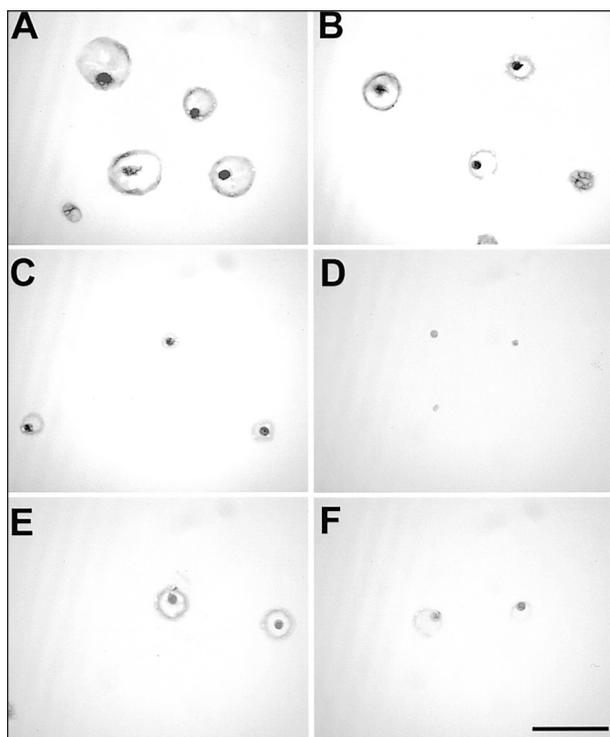


Figure 1—Photomicrograph of a section of a 3-dimensional (3-D) gel medium containing canine chondrocytes cultured for 20 days. Chondrocytes were cultured in medium (agarose) alone (A) or in agarose containing 4 μg of carprofen/mL (B), 1 mg of dexamethasone/mL (C), 20 ng of interleukin (IL)-1 β /mL (D), 20 ng of IL-1 β /mL plus 4 μg of carprofen/mL (E), or 20 ng of IL-1 β /mL plus 1 mg of dexamethasone/mL (F). Notice that chondrocytes that were not exposed to IL-1 β or dexamethasone (A, B, E) appear viable and have abundant pericellular and territorial matrix, whereas chondrocytes exposed to dexamethasone or IL-1 β (C, D, F) appear nonviable (pyknosis, karyorrhexis, lysis) and do not have appreciable matrix. Toluidine blue stain; bar = 50 μm .

in the AG ($P < 0.001$) and IL-D ($P = 0.012$) groups, compared with GAG content in groups C, D, IL, and IL-C. The GAG concentrations did not differ significantly between groups AG and IL-D on day 6. Concentration of GAG on day 12 was significantly lower for the IL group, compared with GAG concentrations in groups AG, C, IL-C, and IL-D; however, it was not significantly lower than GAG content for group D. On day 20, the IL group had significantly less GAG content than groups AG and C (Fig 2).

GAG loss—Concentrations of GAG in the liquid media were measurable at all collection times. Loss of GAG into the media was significantly ($P < 0.001$) higher in the IL group than all other groups on day 3. Also on day 3, group IL-C had significantly ($P = 0.02$) more GAG loss, compared with values for groups AG, C, and D, and group IL-D had significantly ($P = 0.003$) more GAG loss, compared with values for groups AG and C. On day 6, loss of GAG into the media was significantly ($P < 0.001$) higher in the IL group than all other groups. Also on day 6, IL-D had significantly ($P = 0.027$) higher GAG loss than groups AG, C, D, and IL-C, and IL-C had significantly ($P = 0.023$) higher GAG loss than groups AG and C. On day 12, there was significantly higher GAG loss into the liquid media for groups D ($P = 0.045$) and IL-D ($P = 0.015$) than for groups AG and C. On day 20, there was not a significant difference in GAG loss between groups (Fig 3). We did not detect a significant correlation between GAG content and GAG loss.

PGE₂ concentrations—Concentrations of PGE₂ were measurable in all treatment groups at all collection times. Concentrations of PGE₂ were significantly higher on days 3 and 6 ($P < 0.05$) and on days 12 and 20 ($P < 0.001$) in the IL group, compared with concentrations in the other groups. The highest PGE₂ concentrations were detected during the first 6 days of 3-

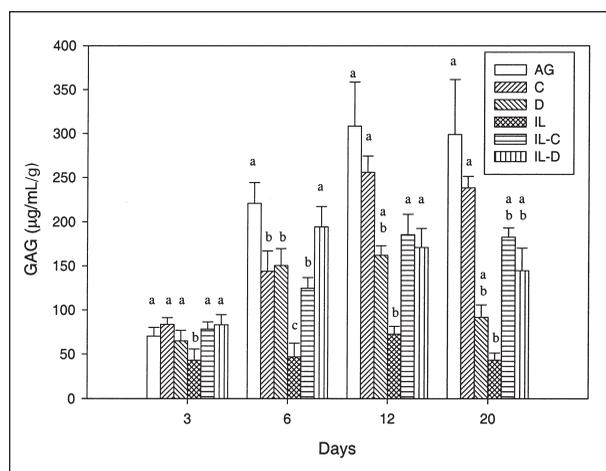


Figure 2—Mean \pm SEM glycosaminoglycan (GAG) content in gel medium of a 3-dimensional construct of cultured canine chondrocytes. Concentrations of GAG were measured on days 3, 6, 12, and 20 after initiation of culture. a-d Within each specific day, treatments with different letters differ significantly ($P < 0.05$). AG = Agarose alone (ie, culture medium). C = Agarose plus 4 μg of carprofen/mL. D = Agarose plus 1 mg of dexamethasone/mL. IL = Agarose plus 20 ng of IL-1 β /mL. IL-C = Agarose plus 20 ng of IL-1 β /mL plus 4 μg of carprofen/mL. IL-D = Agarose plus 20 ng of IL-1 β /mL plus 1 mg of dexamethasone/mL.

D culture in the IL group. On day 3, the PGE₂ concentration was significantly higher in the IL-C group, compared with concentrations in the AG group (Fig 4). We detected a significant negative correlation (r^2 , -0.504; P = 0.012) between GAG contents and PGE₂ concentrations. There was a significant positive correlation (r^2 , 0.779; P < 0.001) between GAG loss and PGE₂ concentrations. The PGE₂ concentrations were not significantly correlated with MMP-3 or MMP-13 concentrations when compared among treatment groups over time.

MMP-3 concentrations—Concentrations of MMP-3 were measurable at all sample times. On day 3, there was not a significant difference in MMP-3 concentrations among treatment groups. On day 6, MMP-3 concentrations were significantly (P = 0.04) lower in group C, compared with concentrations in groups IL, D, IL-C, and IL-D. There was not a significant difference in MMP-3 concentrations among groups IL, D, IL-C, and IL-D on day 6 or between groups AG and C. Concentrations of MMP-3 on day 12 were significantly

higher in the IL (P = 0.033), IL-C (P < 0.001), and IL-D (P < 0.001) groups, compared with concentrations in the AG group. The MMP-3 concentration for the IL group was also significantly (P = 0.033) higher than the concentrations for groups C and D on day 12. On day 20, groups AG, C, and D had significantly (P < 0.001) lower MMP-3 concentrations, compared with concentrations for groups IL, IL-C, and IL-D (Fig 5). We detected a significant positive correlation (r^2 , 0.671; P < 0.001) between concentrations of MMP-3 and MMP-13. There was not a significant correlation between MMP-3 concentrations and GAG content, GAG loss, or PGE₂ concentrations when compared among treatment groups over time.

MMP-13 concentrations—Concentrations of MMP-13 were measurable at all collection times. In general, MMP-13 concentrations were consistently higher in the IL group, compared with concentrations in all other groups. On day 3, MMP-13 concentrations were significantly (P < 0.001) higher in the IL and IL-C groups, compared with concentrations in groups

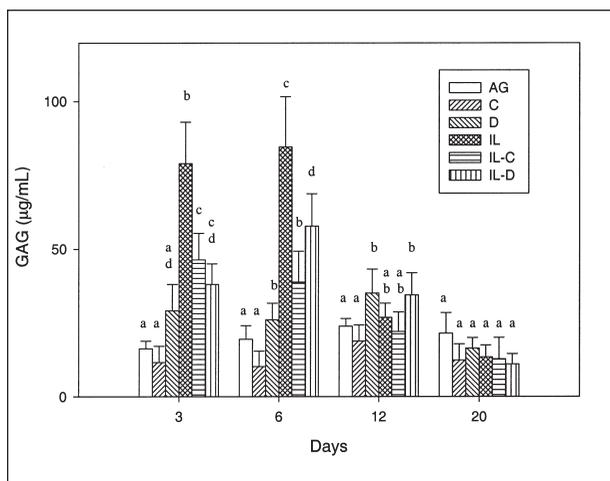


Figure 3—Mean ± SEM GAG loss into liquid medium for canine chondrocytes in 3-D culture. See Figure 2 for key.

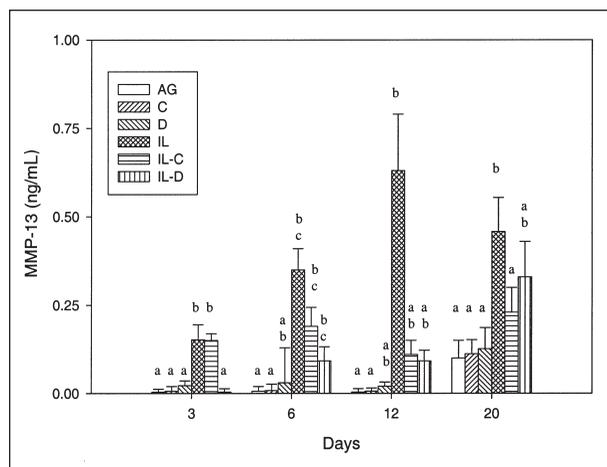


Figure 5—Mean ± SEM matrix metalloproteinase (MMP)-3 concentrations in liquid medium of canine chondrocytes in 3-D culture. See Figure 2 for key.

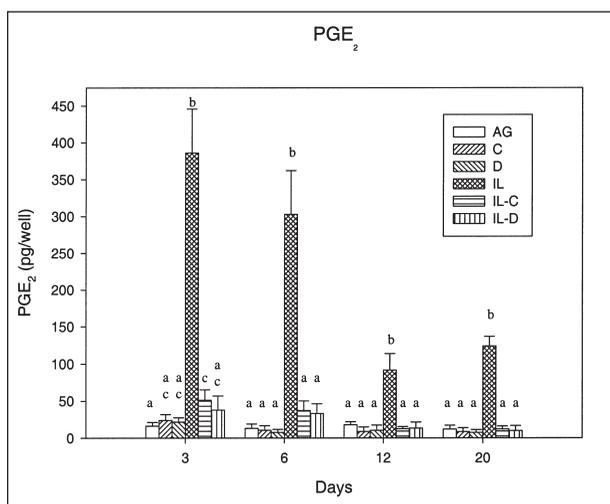


Figure 4—Mean ± SEM prostaglandin E₂ (PGE₂) concentrations in liquid medium of canine chondrocytes in 3-D culture. See Figure 2 for key.

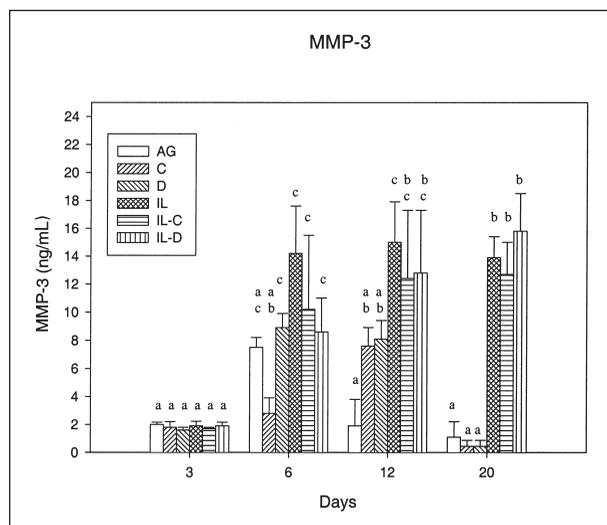


Figure 6—Mean ± SEM MMP-13 concentrations in liquid medium of canine chondrocytes in 3-D culture. See Figure 2 for key.

AG, C, D, and IL-D. There was not a significant difference in MMP-13 concentrations between groups IL and IL-C. On day 6, groups IL, IL-C, and IL-D had significantly higher concentrations of MMP-13, compared with concentrations for groups AG and C. Concentrations of MMP-13 remained significantly higher in the IL group than in groups AG and C on day 12. On day 20, the IL group had significantly ($P = 0.002$) higher MMP-13 concentrations than all groups except IL-D, and the IL-D group had significantly ($P = 0.006$) higher concentrations than groups AG, C, and D. There was not a significant difference in MMP-13 concentrations between groups IL and IL-D on day 20 (Fig 6). We did not detect a significant correlation between MMP-13 concentrations and GAG content, GAG loss, or PGE₂ concentrations when compared among treatment groups over time.

Content of collagen type II—Production of collagen type II was not evident by use of immunohistochemical assessment in any section from any treatment group prior to day 20. Most cells of groups AG and C had pericellular and territorial staining for collagen type II on day 20. Pericellular and territorial staining was also evident in sections from groups IL-C and IL-D on day 20, but it was subjectively less than that in groups AG and C. Staining for collagen type II was not evident in any sections for groups IL and D.

Discussion

Chondrocytes cultured in a 3-D construct are phenotypically similar to chondrocytes *in vivo*.²⁴ The 3-D agarose system allows for maintenance of cell viability, differentiation, and production of pericellular and territorial matrix.^{25,28-30} In addition, the liquid medium surrounding the cell constructs is representative of synovial fluid in that it participates in exchange of nutrients, biochemical mediators, and products of cell synthesis and degradation.²⁶ In monolayer cultures, there is a loss of chondrocyte phenotype. Chondrocytes dedifferentiate to a fibroblastic morphology with concomitant loss of type-II collagen and cartilage-specific proteoglycan production.^{23,24} Compared with explant culture, chondrocytes in 3-D culture have a higher mitotic rate, an increase in cellular metabolism, and an increase in synthesis of extracellular matrix, thus mimicking attempted cartilage healing (ie, early OA).^{25,29,30}

The addition of IL-1 to the liquid media creates an environment that addresses a subset of biochemical events found in naturally occurring OA.^{26,31,32} Interleukin-1 produces its effects on OA through access to the chondrocytes from the synovial fluid.^{31,33} Interleukin-1 penetrates the extracellular matrix and initiates chondrocyte production of IL-1 and other cytokines, perpetuating the degradative cycle.³² In another study,²⁶ the addition of IL-1 directly to a 3-D culture construct resulted in severe loss of chondrocyte viability and a lack of *in vitro* matrix production. With regard to chondrocyte response to IL-1, results of the study reported here were consistent with those of other studies,^{26,31,32,34} validating the use of this method for the study of pharmaceutical interventions in OA.

Analysis of results of the study reported here sug-

gests that carprofen and dexamethasone at the concentrations tested were minimally chondroprotective and were not antidegradative. The concentration of carprofen used was selected on the basis of the reported concentration found in the synovial fluid during an *in vivo* study.³⁵ The concentration of dexamethasone used was consistent with the dose used in other *in vitro* studies.^{18,20,36} Concentration-dependent effects of carprofen and dexamethasone were not assessed in the study reported here, and titration studies would be required to further assess dose-dependent effects.

It is believed that GAG synthesis is an important marker of chondrocyte metabolism. Enabling or enhancing GAG production through increased GAG synthesis, decreased matrix degradation, or both is a property of a chondroprotective agent. Carprofen and dexamethasone did appear to prevent a significant decrease in GAG content in the OA model, compared with results for the IL group. However, GAG content in carprofen- and dexamethasone-treated OA models were lower, compared with GAG content for the control group. Higher concentrations of GAG in the liquid media likely indicated increased matrix degradation in the 3-D constructs and subsequent loss of GAG into the liquid media. The IL-C and IL-D groups had significantly higher GAG concentrations in the liquid media, compared with GAG concentrations for the AG, C, and D groups. Carprofen and dexamethasone each decreased the negative effects of IL-1 on GAG production and GAG loss into the liquid media for the OA model.

Matrix metalloproteases are primary players in the matrix degradation that is evident in osteoarthritic cartilage, and increased concentrations of MMPs in joint fluid have been found in animals with naturally occurring OA.²² Increased MMP concentrations in the liquid media may represent a degradative process within the tissue.²⁶ Concentrations of MMP-3 and -13 were significantly higher in groups for the OA model than in groups that did not contain IL. These results are consistent with the findings of other studies³⁷⁻⁴¹ in which IL-1 appeared to increase the synthesis of metalloproteases *in vivo* and *in vitro*. Carprofen or dexamethasone at the single doses evaluated did not have a significant effect on MMP concentrations, thereby allowing the degradative process to continue. It is important to mention that the MMP concentrations reported here were evaluated in the liquid media only. Concentrations in the liquid media may not be indicative of MMP concentrations in the extracellular matrix, which are important for inducing the degradative effects of OA.²⁶ The MMPs may become trapped in the matrix; therefore, the MMP concentrations in the liquid media may not correlate with matrix concentrations. In the study reported here, MMP concentrations were not significantly correlated with GAG content, GAG loss, or PGE₂ concentrations. Because the MMP concentrations were evaluated only in the liquid media, it is possible we may have detected a significant correlation if the MMP concentrations had been evaluated in the 3-D construct.

Supraphysiologic concentrations of PGE₂ are considered an indicator of an inflammatory state.

Prostaglandin E₂ is produced by insulted cells in an inflammatory environment. This was true in the study reported here in which PGE₂ concentrations were highest in the IL group. Concentrations of PGE₂ were not significantly different among other treatment groups. As expected, carprofen and dexamethasone each had anti-inflammatory effects in the OA model, which was evidenced by lower concentrations of PGE₂.

In the study reported here, cell viability appeared to be affected by IL-1, as indicated by subjective assessment of histologic features. Cells in the IL group had characteristics indicative of loss of viability, including nuclear pyknosis, karyorrhexis, and lysis, throughout the study. Most cells in the IL-C and IL-D groups maintained characteristics of viability throughout the study. However, there was subjectively more loss of viability in sections from the IL-treated groups than in those from the AG and C groups. This supports our conclusion that carprofen and dexamethasone are chondroprotective in the osteoarthritic environment; however, they do not completely negate the detrimental effects of inflammatory mediators and degradative enzymes.

Matrix production by chondrocytes in 3-D culture was similarly affected by IL-1. Matrix production, as assessed by toluidine blue staining, was lower in the IL group throughout the study, with little or no territorial matrix evident in any sections. For groups AG, C, IL-C, and IL-D, staining of pericellular and territorial matrix increased consistently throughout the study. Proteoglycan staining in group D was similar to staining in those groups on days 3, 6, and 12. However, on day 20, sections of group D had poorly defined, or a total lack of, pericellular matrix staining.

Production of collagen type II was assessed by use of immunohistochemical staining. Control chondrocytes produce pericellular and territorial collagen type II in 3-D culture.^{23,24,26,28,30} We did not detect production of collagen type II in any section from any treatment group prior to day 20, which is consistent with findings of other studies.^{19,20,34} Cells from groups AG, C, IL-C, and IL-D had staining of pericellular and territorial collagen type II on day 20. None of the sections from groups IL and D had staining for collagen type II.

In the study reported here, dexamethasone at the single dose evaluated appeared to have detrimental effects on chondrocyte viability, matrix production, and collagen type-II synthesis. Cells from group D maintained histologic characteristics of cell viability through day 12. However, on day 20, most cells from this group had histologic characteristics indicative of loss of viability and cell death. As determined by use of toluidine blue staining, group D sections on day 20 had poorly defined, or a total lack of, pericellular matrix staining. Finally, we did not detect staining for collagen type II in any sections from group D. These findings are consistent with those of other studies.^{19,20} Glucocorticoids, specifically dexamethasone, can inhibit the mitotic activity of chondrocytes, alter or inhibit synthetic abilities (GAG, collagen type II) of chondrocytes, alter the cartilage matrix, and enhance the formation of degenerate-appearing chondrocytes.^{16,17,19,20,42-44} However, in the study reported here, dexamethasone did have anti-inflammatory effects as

indicated by significantly decreasing the PGE₂ concentrations within the OA cultures.

Carprofen did not appear to have any detrimental effects on chondrocyte viability, matrix production, or collagen type-II synthesis when used at a concentration of 4 µg/mL. In 1 in vitro study,¹³ it was reported that carprofen at concentrations of 1 and 10 µg/mL stimulated a significant increase in GAG synthesis, but carprofen at concentrations of ≥ 20 µg/mL inhibited GAG and total protein synthesis. We did not observe increased GAG synthesis by chondrocytes exposed to carprofen at a concentration of 4 µg/mL. Carprofen did have anti-inflammatory effects as indicated by a decrease in PGE₂ concentrations within the OA cultures.

The 3-D agarose culture model provides an appropriate environment to study chondrocytes in vitro. The in vitro events recorded in the study reported here correspond to the biochemical events that occur in naturally occurring OA. In this study, carprofen and dexamethasone each had significant anti-inflammatory effects; however, neither therapeutic agent caused a significant decrease in MMP concentrations nor a decrease in detrimental cell and matrix effects that occur with this in vitro model of OA. The authors believe it is important to again acknowledge that only single doses of dexamethasone and carprofen were evaluated in this study and that these doses may not be physiologic with respect to in vivo concentrations achieved in diarthrodial joints. The model used here only considers a subset of the events that happen during naturally occurring OA. In clinical patients, other factors must also be addressed, including body weight, body condition, and amount of activity.

^aHuman recombinant IL-1β, Upstate Biotech, Lake Placid, NY.

^bZenecarp, C-Vet Veterinary Products, Leyland, UK.

^cDexamethasone sodium phosphate, Steris Laboratories Inc, Phoenix, Ariz.

^dAvidin-biotin-peroxidase kit, Vector Laboratories Inc, Burlingame, Calif.

^eRabbit anti-bovine collagen type II, Chemicon International Inc, Temecula, Calif.

^fPGE₂ ELISA, Amersham International PLC, Buckinghamshire, UK.

^gMMP-3 ELISA, Amersham International PLC, Buckinghamshire, UK.

^hMMP-13 ELISA, Amersham International PLC, Buckinghamshire, UK.

ⁱSigma Stat, Jandel Scientific, San Rafael, Calif.

References

1. Johnston SA. Osteoarthritis. Joint anatomy, physiology, and pathobiology. *Vet Clin North Am Small Anim Pract* 1997;27:699-723.
2. Herman JH, Sowder WG, Hess EV. NSAID induction of interleukin 1/catabolin inhibitor by osteoarthritic synovial tissue. *J Rheumatol* 1991;18(suppl 27):124-126.
3. Dingle JT. Cartilage maintenance in osteoarthritis: interaction on cytokines, NSAID and prostaglandins in articular cartilage damage and repair. *J Rheumatol* 1991;28(suppl):30-37.
4. Hugenberg ST, Brandt KD, Cole CA. Effect of sodium salicylate, aspirin, and ibuprofen on enzymes required by the chondrocyte for synthesis of chondroitin sulfate. *J Rheumatol* 1993;20:2128-2133.
5. Bassler CT, Henrotin YE, Reginster JL, et al. Effects of tiaprofenic acid and acetylsalicylic acid on human articular chondrocytes in 3-dimensional culture. *J Rheumatol* 1992;19:1433-1438.
6. Lane-Smith R, Kajiyama G, Lane NE. Nonsteroidal anti-inflammatory drugs: effects on normal and interleukin 1 treated

human articular chondrocyte metabolism in vitro. *J Rheumatol* 1995; 22:1130–1137.

7. Lane NE, Williams RJ, Schurman DJ, et al. Inhibition of interleukin 1 induced chondrocyte protease activity by a corticosteroid and a nonsteroidal antiinflammatory drug. *J Rheumatol* 1992;19:135–139.

8. Trotter GW, McIlwraith CW, Yovich JV, et al. Effects of intra-articular administration of methylprednisolone acetate on normal equine articular cartilage. *Am J Vet Res* 1991;52:83–87.

9. Vasseur PB, Johnson AL, Budsberg SC, et al. Randomized, controlled trial of the efficacy of carprofen, a nonsteroidal anti-inflammatory drug, in the treatment of osteoarthritis in dogs. *J Am Vet Med Assoc* 1995;206:807–811.

10. O'Brien WM, Bagby GF. Carprofen: a new nonsteroidal anti-inflammatory drug; pharmacology, clinical efficacy and adverse effects. *Pharmacotherapy* 1987;7:16–24.

11. Tursi A, Loria MR, Specchia G, et al. In vitro studies of anti-inflammatory activity of carprofen. *Eur J Rheumatol Inflamm* 1982;5: 488–491.

12. Fox SM, Johnston SA. Use of carprofen for the treatment of pain and inflammation in dogs. *J Am Vet Med Assoc* 1997;210:1493–1498.

13. Benton HP, Vasseur PB, Broderick-Villa GA, et al. Effect of carprofen on sulfated glycosaminoglycan metabolism, protein synthesis, and prostaglandin release by cultured osteoarthritic canine chondrocytes. *Am J Vet Res* 1999;60:286–292.

14. Armstrong S, Lees P. Effects of R and S enantiomers and a racemic mixture of carprofen on the production and release of proteoglycan and prostaglandin E₂ from equine chondrocytes and cartilage explants. *Am J Vet Res* 1999;60:98–104.

15. Johnston SA, Budsberg SC. Nonsteroidal anti-inflammatory drugs and corticosteroids for the management of canine osteoarthritis. *Vet Clin North Am Small Anim Pract* 1997;27:841–862.

16. Simmons DJ, Kunin AS. Autoradiographic and biochemical investigations of the effect of cortisone on the bones of the rat. *Clin Orthop* 1967;55:201–215.

17. Kunin AS, Meyer WL. The effect of cortisone on the intermediary metabolism of epiphyseal cartilage from rats. *Arch Biochem Biophys* 1969;129:421–430.

18. Hainque B, Dominice J, Jaffray P, et al. Effects of dexamethasone on the growth of cultured rabbit articular chondrocytes: relation with the nuclear glucocorticoid-receptor complex. *Ann Rheum Dis* 1987;46:146–152.

19. Oikarinen AI, Vuorio EI, Zaragoza EJ, et al. Modulation of collagen metabolism by glucocorticoids: receptor-mediated effects of dexamethasone on collagen biosynthesis in chick embryo fibroblasts and chondrocytes. *Biochem Pharmacol* 1988;37:1451–1462.

20. Srinivas GR, Chichester CO, Barrach HJ, et al. Effects of certain antiarthritic agents on the synthesis of type II collagen and glycosaminoglycans in rat chondrosarcoma cultures. *Agents Actions* 1994;41:193–199.

21. Pelletier JP, Martel-Pelletier J. Protective effects of corticosteroids on cartilage lesions and osteophyte formation in the Pond-Nuki dog model of osteoarthritis. *Arthritis Rheum* 1989;32:181–193.

22. Pelletier JP, DiBattista JA, Raynauld JP, et al. The in vivo effects of intraarticular corticosteroid injections on cartilage lesions, stromelysin, interleukin-1, and oncogene protein synthesis in experimental osteoarthritis. *Lab Invest* 1995;72:578–586.

23. Thompson AY, Peiz KA, Seyedin SM. Chondrogenesis in agarose culture: a model for chondrogenic induction, proliferation, and differentiation. *Exp Cell Res* 1985;157:483–494.

24. Buschmann MD, Gluzband YA, Grodzinsky AJ, et al. Chondrocytes in agarose culture synthesize a mechanically functional extracellular matrix. *J Orthop Res* 1992;10:745–758.

25. Cook JL, Kreegar JM, Payne JT, et al. Three-dimensional

culture of canine articular chondrocytes on multiple transplantable substrates. *Am J Vet Res* 1997;58:419–424.

26. Cook JL, Anderson CC, Kreegar JM, et al. Effects of human recombinant IL-1 β on articular chondrocytes in three-dimensional culture. *Am J Vet Res* 2000;61:766–770.

27. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* 1986;883:173–177.

28. Benya PD, Shaffer JD. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell* 1982;30:215–224.

29. Brittberg M, Lindahl A, Nilsson A, et al. Cellular aspects on treatment of cartilage injuries. *Agents Actions Suppl* 1993;39:237–240.

30. Cook JL, Kreegar JM, Payne JT, et al. Effects of EGF, FGF, and PDGF on canine chondrocytes in three-dimensional culture. *Vet Comp Orthop Traumatol* 1997;10:210–213.

31. Pujol JP, Loyau G. Interleukin-1 and osteoarthritis. *Life Sci* 1987;41:1187–1198.

32. Tiku K, Thakker-Varia S, Ramachandru A, et al. Articular chondrocytes secrete IL-1, express membrane IL-1, and have IL-1 inhibitory activity. *Cell Immunol* 1992;140:1–20.

33. Goldring MB. Degradation of articular cartilage in culture: regulatory factors. In: Woessner JF, Howell DS, eds. *Joint cartilage degradation: basic and clinical aspects*. New York: Marcel Dekker Inc, 1993;281–345.

34. Goldring MB, Birhead J, Sandell LJ, et al. Interleukin 1 suppresses expression of cartilage-specific types II and IX collagens and increases types I and III collagens in human chondrocytes. *J Clin Invest* 1988;82:2026–2037.

35. Schneider TA, Budsberg SC. Plasma and synovial concentrations of carprofen in dogs with chronic osteoarthritis. *Vet Comp Orthop Traumatol* 2001;14:19–24.

36. Pelletier JP, McCollum R, DiBattista J, et al. Regulation of human normal and osteoarthritic chondrocyte interleukin-1 receptor by antirheumatic drugs. *Arthritis Rheum* 1993;36:1517–1527.

37. Caron JP, Tardif G, Martel-Pelletier J, et al. Modulation of matrix metalloproteinase 13 (collagenase 3) gene expression in equine chondrocytes by interleukin 1 and corticosteroids. *Am J Vet Res* 1996; 57:1631–1634.

38. Martel-Pelletier J, McCollum R, Fujimoto N, et al. Excess of metalloproteinases over tissue inhibitor of metalloproteinase may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis. *Lab Invest* 1994;70:807–815.

39. Okada Y, Shinmei M, Tanaka O, et al. Localization of matrix metalloproteinase 3 (stromelysin) in osteoarthritic cartilage and synovium. *Lab Invest* 1992;66:680–690.

40. Shinmei M, Kikuchi T, Masuda K, et al. Effects of interleukin-1 and anti-inflammatory drugs on the degradation of human articular cartilage. *Drugs* 1988;35(suppl 1):33–41.

41. Quintavalla JC, Berg RA, Beavis AJ, et al. Differential induction of stromelysin mRNA by bovine articular chondrocytes treated with interferon- γ and interleukin-1 α . *J Cell Physiol* 1993;154:113–121.

42. Kolibas LM, Goldberg RL. Effect of cytokines and antiarthritic drugs on glycosaminoglycan synthesis by bovine articular chondrocytes. *Agents Actions* 1989;27:245–249.

43. Dearden LC, Mosier HD, Espinosa T. Cortisone induced alterations of costal cartilage in single and in parabiosed rats. *Cell Tissue Res* 1978;189:67–89.

44. Bonucci E, Dearden LC, Mosier HD. Effects of glucocorticoid treatment on the ultrastructure of cartilage and bone. In: Avioli LV, Gennari C, Imbimbo B, eds. *Glucocorticoid effects and their biological consequences*. New York: Plenum Press Inc, 1984;269–278.