Effects of oral administration of phenylbutazone to horses on in vitro articular cartilage metabolism

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Objective—To evaluate the effects of orally administered phenylbutazone on proteoglycan synthesis and chondrocyte inhibition by IL-1β in articular cartilage explants of horses.

Animals—11 healthy 1- to 2-year-old horses.

Procedure—Horses were randomly assigned to the control (n = 5) or treated group (4.4 mg of phenylbutazone/kg of body weight, PO, q 12 h; n = 6). Articular cartilage specimens were collected before treatment was initiated (day 0), after 14 days of treatment, and 2 weeks after cessation of treatment (day 30). Proteoglycan synthesis and stromelysin concentration in cartilage extracts were assessed after 72 hours of culture in medium alone or with recombinant human interleukin-1β (IL-1β; 0.1 ng/ml).

Results—On day 0, proteoglycan synthesis was significantly less in cartilage explants cultured in IL-1β, compared with medium alone. Mean proteoglycan synthesis in explants collected on days 14 and 30 was significantly less in treated horses, compared with controls. However, incubation of explants from treated horses with IL-1β did not result in a further decrease in proteoglycan synthesis. Significant differences in stromelysin concentration were not detected between or within groups.

Conclusions and Clinical Relevance—Oral administration of phenylbutazone for 14 days significantly decreased proteoglycan synthesis in articular culture explants from healthy horses to a degree similar to that induced by in vitro exposure to IL-1β. Phenylbutazone should be used judiciously in athletic horses with osteoarthritis, because chronic administration may suppress proteoglycan synthesis and potentiate cartilage damage. (*Am J Vet Res* 2001; 62:1916–1921)

Phenylbutazone, a **nonsteroidal anti-inflammatory drug (NSAID)**, is the most common treatment for joint pain associated with osteoarthritis in horses.[1,2] Nonsteroidal anti-inflammatory drugs have been used in horses for decades to relieve other clinical signs of osteoarthritis such as heat and swelling.[3] Phenylbutazone effectively diminishes signs of pain and increases range of motion, thus allowing early return to athletic function.[4] Although phenylbutazone alleviates the clinical signs of osteoarthritis, its effects on articular cartilage have not been thoroughly investigated. However, there is some evidence that phenylbutazone may suppress articular cartilage metabolism.[1,5,6] Suppression of proteoglycan turnover by phenylbutazone could compound the effects of osteoarthritis even though administration of this drug may temporarily alleviate some of the clinical signs.

Depletion of proteoglycan from the articular cartilage matrix is an important feature of osteoarthritis.[7-10] Cartilage matrix metabolism during osteoarthritis is characterized by an increase in proteoglycan degradation and the inability of chondrocytes to synthesize new matrix.[10] The net loss of matrix increases the vulnerability of articular cartilage to mechanical trauma.[11,12] Proteoglycans contribute to cartilage elasticity and compressive stiffness, whereas collagens account for tensile strength.[10]

Results of previous in vitro studies investigating the effects of NSAID on cartilage metabolism in species other than the horse indicate that effects vary depending on drug and concentration. Salicylates, fenoprofen, isoxicam, tolmetin, and ibuprofen induce concentration-dependent inhibition of proteoglycan synthesis in normal canine cartilage.[13] Other compounds such as indomethacin, piroxicam, and diclofenac had no detectable effect on proteoglycan synthesis.[13] Phenylbutazone has been reported to inhibit proteoglycan synthesis in equine articular cartilage explants at concentrations greater than could be achieved in synovial fluid following systemic administration.[6,a]

Systemic administration of therapeutic doses of aspirin inhibits proteoglycan synthesis in cartilage from healthy dogs and dogs with experimentally induced osteoarthritis.[13] In these studies, degeneration was greater in cartilage from affected dogs treated with aspirin, compared with cartilage from healthy dogs treated with aspirin. In vivo administration of NSAID at concentrations less than those known to induce an in vitro effect can introduce other variables that may affect cartilage metabolism. These other variables include exposure of the animal to active drug metabolites, increased uptake of the drug by osteoarthritic cartilage, and motion and weight bearing of the affected limb.

To our knowledge, the effects of systemically administered clinically relevant doses of phenylbutazone on normal equine articular cartilage are not known. We believed that by combining in vivo and in vitro techniques, we could determine the effects of phenylbutazone on equine chondrocytes. In addition,

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this combination of techniques would allow us to investigate the interaction between phenylbutazone and **interleukin- (IL-)**1.

Cytokines are key determinants of cartilage damage following joint trauma.[2,7,8,10,14,15] Synovial fluid concentrations of IL-1 are high in horses with osteoarthritis. This cytokine functions to inhibit proteoglycan synthesis and promote matrix degradation by induction of neutral metalloproteinases such as stromelysin (**matrix metalloproteinase 3 [MMP-3]**).[16] Interleukins are produced by all cellular constituents of diarthrodial joints and by certain inflammatory cells (eg, monocytes and macrophages). The precursor form of IL-1 is enzymatically cleaved to form IL-1α and IL-1β.[17] Experimentally, IL-1β is more potent than IL-1α,[17] and it has been linked to the development of osteoarthritis in horses. In addition, IL-1β has been isolated from cartilage explants and synovial fluid of horses.[17] Results of both in vivo and in vitro studies indicate that IL-1β inhibits proteoglycan synthesis and facilitates degeneration of proteoglycans via induction of neutral metalloproteinases.[6,7,15,18]

Nonsteroidal anti-inflammatory drugs modify the inflammatory response by blocking prostaglandin synthesis via the cyclooxygenase pathway of arachidonic acid metabolism.[19-22] Phenylbutazone does not directly inhibit IL-1 but may indirectly influence articular cartilage via interference with proteoglycan synthesis.[23] Our hypotheses for the study reported here were that phenylbutazone administered orally for 2 weeks would suppress proteoglycan synthesis and exaggerate IL-1-induced inhibition of chondrocyte metabolism. The specific objectives of this study were to evaluate the effects of oral administration of phenylbutazone on proteoglycan synthesis and induction of matrix-degrading enzymes (eg, stromelysin) in articular cartilage explants of horses.

Materials and Methods

Horses and groups—Eleven 18- to 30-month-old horses were used in this study. Inclusion criteria included no abnormal findings on physical examination, no clinical signs of lameness, and no radiographic abnormalities of the metacarpophalangeal and metatarsophalangeal (fetlock) joints. All horses received a tetanus toxoid vaccination when entered into the study and a physical examination (determination of rectal temperature and heart and respiratory rates) daily throughout the study. Hay and water were available ad libitum except for the 12 hours preceding a surgical procedure. Horses were randomly assigned to the control or treated group (control, n = 5; treated, 6); those in the treated group received phenylbutazone[b] (4.4 mg/kg of body weight, PO, q 12 h) for 14 days. The protocol was approved by The Ohio State University Institutional Laboratory Animal Use and Care Committee.

Cartilage explant cultures—Specimens of articular cartilage were collected aseptically from anesthetized horses at the 3 following times: 3 hours before drug administration (day 0), on day 14 of drug administration (day 14), and immediately after euthanasia 2 weeks after cessation of drug administration (day 30). The first specimen was collected from a randomly selected metacarpophalangeal joint, the second specimen from the opposite metacarpophalangeal joint, and the third from a randomly selected metatarsophalangeal joint.

Food was withheld from horses for 12 hours prior to collection of the cartilage specimens. Horses were then premedicated with xylazine hydrochloride[c] (0.2 mg/kg, IV), and general anesthesia was induced with diazepam[d] (0.2 mg/kg, IV) and ketamine hydrochloride[e] (2.2 mg/kg, IV). Anesthesia was maintained with halothane[f] vaporized into 100% oxygen in a semiclosed circle system. To collect specimens, horses were positioned in lateral recumbency with the selected joint uppermost. A 4-cm linear incision was made through the skin, subcutaneous tissues, and joint capsule 1 cm lateral to the intermediate ridge of the distal portion of the third metacarpal or metatarsal bone. A quarter-inch chisel was used to harvest 300 to 400 mg of full-thickness articular cartilage from the dorsal surface of the condyle of the third metacarpal or metatarsal bone. After collection of the first 2 specimens, the joint capsule and subcutaneous tissues were closed with absorbable suture[g] in a simple continuous pattern. The skin was apposed with nonabsorbable suture[h] in a simple interrupted pattern. A sterile bandage was placed over the joint, and bandages were maintained until the sutures were removed. Horses were evaluated daily for signs of lameness, and the surgery site was monitored for signs of infection. Before collection of the third specimen, horses were euthanatized with pentobarbital sodium.[i] All horses received procaine penicillin G (22,000 U/kg, IM) before the first and second biopsy. Horses in the treatment group received the first dose of phenylbutazone 3 hours after the initial biopsy.

Cartilage specimens were transported to the laboratory in chilled (4 C) DMEM.[j] On arrival to the laboratory, specimens for each horse were pooled, minced, and aliquoted (50 to 100 mg wet weight/well) into 6-well tissue culture plates and cultured in standard medium (DMEM supplemented with 10% fetal bovine serum,[k] 100 U of penicillin/ml,[j] and 100 µg of gentamicin/ml[j]) for 24 hours at 37 C in 5% carbon dioxide and 95% air. At 24 hours, medium was changed, and explants were incubated for an additional 48 hours in standard medium alone or with 400 µl of recombinant human IL-1β[l] (0.1 ng/ml); this medium was also changed after 24 hours. The concentration of recombinant human IL-1β used in the present study was chosen on the basis of results of a pilot study performed to determine the amount of recombinant human IL-1β required to effectively reduce proteoglycan synthesis by approximately half. In that study, cartilage explants were harvested from 3 horses and cultured as described for the present study except that medium was supplemented with 400 µl of recombinant human IL-1β at 1 of the following concentrations: 0, 0.01, 0.1, 1.0, 10.0, and 100.0 ng/ml. Data from the pilot study suggested that addition of recombinant human IL-1β at a concentration of 0.01 ng/ml would be sufficient to reduce proteoglycan synthesis in the cartilage explants.

Preparation of cartilage extracts—After culture in medium alone or with recombinant human IL-1β for 48 hours, duplicate explants from each horse were single-pulse radiolabeled with 40 µCi of sulfur 35 (^{35}S)[l] and incubated for an additional 24 hours. Radiolabeled explants were washed 4 times with 4 ml of PBS solution (pH 7.2) and extracted with 4*M* guanidine hydrochloride for 48 hours on a stir plate at 4 C. Cartilage extract (guanidine solution) from each well was collected, placed in presoaked dialysis tubing (molecular weight cutoff, 6,000 to 8,000), and dialyzed against deionized water for 48 hours at 4 C to remove free ^{35}S and allow for reaggregation of proteoglycan with endogenous hyaluronic acid. The dialysis water was changed every 24 hours. Following dialysis, the volume of guanidine solution in each bag was measured, and a 100-µl aliquot was reserved for scintillation counting to determine amount of incorporated ^{35}S. The remaining extract was stored at –20 C for determination of stromelysin (MMP-3) concentration.

Determination of proteoglycan synthesis—Newly synthesized proteoglycan was quantitated on the basis of the amount of radioactivity (ie, ^{35}S) in the cartilage extract (dialyzed guanidine solution). Total protein concentration in cartilage extracts served as a reference standard for proteoglycan concentration and was determined by use of a commercial assay.[m] Briefly, 100 µl of cartilage extract or standard (0 to 10.0 µg of protein/ml) was added to duplicate wells of a microtiter plate. Plates were covered and incubated at 37 C for 30 minutes, and absorbance at 562 nm was determined by use of a spectrophotometer.[n] Comparisons between or within groups did not change based on whether proteoglycan synthesis was expressed as µCi of ^{35}S per g of total protein or per g of wet weight cartilage. Proteoglycan synthesis was expressed as mean ± SEM in units of µCi of ^{35}S per g of wet weight cartilage.

Determination of stromelysin concentration—A commercially available human MMP-3 ELISA[o] was used to measure stromelysin concentration in cartilage extracts. Standards were prepared from stock solutions of MMP and ranged from 0 to 400 ng of MMP/ml. Aliquots (100 µl) of cartilage extract and standards were added to duplicate wells of a 96-well microtiter plate precoated with anti-MMP-3 antibody. Microtiter plates were incubated for 1 hour at 24 C. After washing wells 4 times with wash buffer (0.01*M* phosphate buffer, pH 7.5, containing 0.05% Tween 20), 100 µl of peroxidase conjugate (horseradish peroxidase-labeled Fab' fragment specific for MMP-3 in 0.03*M* phosphate buffer [pH 7.0] containing 0.1*M* sodium chloride, 1% [wt/vol] bovine serum albumin, and 0.01*M* EDTA) was added to each empty well. Plates were incubated for an additional 2 hours at 24 C, wells were washed 4 times with wash buffer, emptied, and 100 µl of 3,3',5,5'-tetramethylbenzidine-hydrogen peroxide in 20% (vol/vol) dimethylformamide was added to each well. Plates were incubated at 24 C for 30 minutes on a plate shaker. The reaction was stopped by the addition of 100 µl of 1*M* sulfuric acid to each well, and absorbance at 450 nm was read on a spectrophotometer[n] within 30 minutes of terminating the reaction.

Determination of drug concentrations in synovial fluid—Synovial fluid samples were obtained by arthrocentesis of the selected metacarpophalangeal or metatarsophalangeal joint at the same times cartilage specimens were collected. Synovial fluid was stored at –70 C in tubes containing EDTA until concentrations of phenylbutazone and its major metabolite, oxyphenbutazone, were determined by use of high-performance liquid chromatography.

Statistical analyses—Data were expressed as mean values ± SEM. Quantitative data were analyzed by use of a repeated-measure ANOVA to determine effect of phenylbutazone, followed by a Dunnett post test. Effect of IL-1β on cartilage synthesis was assessed by use of 1-way ANOVA. For all analyses, $P < 0.05$ was considered significant.

Results

Proteoglycan synthesis—In explants collected on day 0 from both groups, incorporation of ^{35}S was considered excellent (1.53 ± 0.19 µCi/g). Incubation of day-0 explants with IL-1β (0.1 ng/ml) resulted in a significant ($P = 0.04$) decrease in ^{35}S incorporation in all explants (1.05 ± 0.09 µCi/g), indicating that IL-1β inhibited proteoglycan synthesis. Oral phenylbutazone administration resulted in a significant decrease in mean ^{35}S incorporation determined for all explants collected on days 14 and 30 from treated horses (0.88 ± 0.11 µCi/g), compared with control horses (1.40 ± 0.13 µCi/g). However, incubation of explants with IL-1β did not result in a further decrease in mean incorporation determined for all explants collected on days 14 and 30 from treated horses (0.96 ± 0.01 µCi/g).

Incubation of day-0 explants from treated horses with IL-1β significantly ($P = 0.04$) suppressed proteoglycan synthesis (**Table 1**). Oral phenylbutazone administration for 14 days also resulted in a significant ($P = 0.002$) suppression of proteoglycan synthesis, compared with that determined prior to drug administration. Although proteoglycan synthesis increased from day 14 to day 30 in cartilage explants from treated horses, this increase was not significant ($P = 0.056$), and incorporation of ^{35}S in the treated group remained significantly greater on day 30, compared with the day-0 value (1.80 ± 0.23 vs1.07 ± 0.15 µCi/g). Incubation of cartilage explants collected from treated horses on either day 14 or day 30 with IL-1β did not result in further decreases in ^{35}S incorporation. Significant differences were not detected in ^{35}S incorporation on day 30 between groups, regardless of whether explants were incubated with IL-1β.

Table 1—Proteoglycan synthesis by articular cartilage explants from control horses or horses treated with phenylbutazone (4.4 mg/kg of body weight, PO, q 12 h) for 14 days

	Proteoglycan synthesis (mCi of ^{35}S/g of cartilage)		
Group (n)	**Day 0***	**Day 14**	**Day 30**
Control (5)			
Medium alone	1.21 ± 0.26[a,b,c]	1.05 ± 0.04[a]	1.38 ± 0.22
Medium + rhIL-1β	0.84 ± 0.14[c]	0.76 ± 0.03[b]	1.03 ± 0.13
Phenylbutazone (6)			
Medium alone	1.80 ± 0.23[a,†]	0.72 ± 0.07[b]	1.07 ± 0.15
Medium + rhIL-1β	1.24 ± 0.08[b]	0.74 ± 0.12[b,†]	1.27 ± 0.19

Data are reported as mean ± SEM.
*Articular cartilage specimens collected 3 hours before first dose of phenylbutazone administered to treated horses.
rhIL-1β = Recombinant human interleukin-1β.
[a,b,c]Within a column, values with different superscripts are significantly ($P < 0.05$) different. [†]Significantly ($P < 0.05$) different from other values within a row.

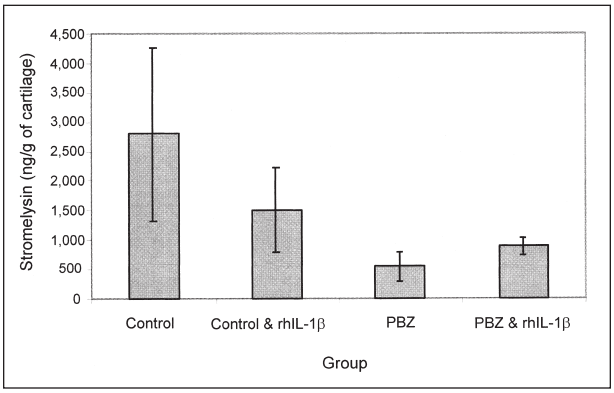

Figure 1—Stromelysin concentration in articular cartilage explants from control horses (n = 5) and horses treated with phenylbutazone (PBZ; 4.4 mg/kg of body weight, PO, q 12 h; n = 6) for 14 days. Articular cartilage specimens were collected 3 hours prior to initiation of phenylbutazone treatment, after 14 days of treatment, and 2 weeks following cessation of treatment and cultured in medium alone or with recombinant human interleukin-1β (rhIL-1β; 0.1 ng/ml) for 48 hours. Stromelysin concentration was determined by use of a human matrix metalloproteinase-3 ELISA. Columns represent the mean of values for explants collected at day 14; error bars represent the SEM.

Stromelysin concentration—Concentrations of stromelysin were low in all media samples from cartilage explants collected on day 14 from both control and treated horses. Mean values were then compared between and within groups. Stromelysin concentrations in explants from either group incubated with IL-1β were not significantly different from concentrations in explants incubated in medium alone (**Fig 1**). In addition, although concentration of stromelysin in explants from treated horses incubated in medium alone was less than that in explants from control horses, this difference was not significant ($P = 0.16$).

Phenylbutazone and oxyphenbutazone concentrations in synovial fluid—Neither phenylbutazone nor its metabolite, oxyphenbutazone, were detected in synovial fluid samples collected on days 0 or 30. On day 14, phenylbutazone was detected in synovial fluid samples from all treated horses (mean ± SEM, 0.93 ± 0.18 μg/ml; range, 0.49 to 1.62 μg/ml). Oxyphenbutazone was also detected in 4 of these 6 horses (0.36 ± 0.13 μg/ml; 0.15 to 0.73 μg/ml).

Discussion

Phenylbutazone administered at 4.4 mg/kg orally twice daily for 14 days resulted in a significant decrease in proteoglycan synthesis by articular cartilage explants. As expected, in vitro incubation of cartilage explants with IL-1β depressed chondrocyte metabolism. However, IL-1β did not induce further decreases in proteoglycan synthesis by explants from phenylbutazone treated horses. To our knowledge, this is the first study to indicate that proteoglycan synthesis is suppressed following oral administration of phenylbutazone to healthy horses.

The concentration of IL-1β selected for this study (0.1 ng/ml) is relatively low, compared with that used in another in vitro study.[18] We selected a concentration that would effectively reduce proteoglycan synthesis by approximately half on the basis of results of a dose response curve, because this would potentially allow us to evaluate increases or decreases in proteoglycan synthesis secondary to treatment. Although IL-1β significantly suppressed proteoglycan synthesis in explants from control horses when values determined at all time points were pooled, IL-1β did not significantly suppress proteoglycan synthesis by control explants on days 0 or 30. This was probably attributable to the low concentration of IL-1β used, the large variability in data, and the small number of horses. A higher concentration of IL-1β may have resulted in suppression of proteoglycan synthesis in day-0 and day-30 control explants. However, it is possible that higher concentrations would have resulted in maximum suppression, and any effect of phenylbutazone would have been masked. Incubation with IL-1β did not further reduce proteoglycan synthesis in explants from phenylbutazone-treated horses.

Synovial fluid concentrations of phenylbutazone ranged from 0.49 to 1.62 μg/ml after 14 days of oral administration of phenylbutazone. Synovial fluid concentrations following a single dose of 4.4 mg of phenylbutazone/kg range from 0.7 to 4.4 μg/ml and following administration of 10 mg of phenylbutazone/kg, range from 7 to 11 μg/ml.[22,24-26] Phenylbutazone concentrations in synovial fluid following chronic administration have not been reported. However, concentrations detected in our study were within ranges reported by others after administration of a single dose of 4.4 mg of phenylbutazone/kg.[22,24-26] Oxyphenbutazone was detected in synovial fluid samples from 4 of 6 treated horses in the present study; concentrations ranged from 0.15 to 0.73 μg/ml. Synovial fluid concentrations of oxyphenbutazone following a single dose of 4.4 mg of phenylbutazone/kg ranged from 0.2 to 1.9 μg/ml.[22,24]

In the present study, oral administration of 4.4 mg of phenylbutazone/kg suppressed proteoglycan synthesis by articular cartilage explants. When phenylbutazone is added in vitro to cartilage explants, suppression is typically not detected until concentrations are ≥ 100 μg/ml.[6,a] Clinically relevant concentrations of phenylbutazone (2 to 20 μg/ml) added in vitro did not inhibit proteoglycan synthesis. The difference between results of these previous studies and our study may reflect the influence of chronic in vivo drug exposure (2 weeks), exposure to active metabolites such as oxyphenbutazone, and motion and weight bearing.

Our results were similar to those of another study[13] that examined the in vitro effects of a number of NSAID, including salicylates, fenoprofen, isoxicam, tolmetin, ibuprofen, and carprofen, on articular cartilage. Results of that study revealed that these NSAID induced a concentration-dependent inhibition of proteoglycan synthesis in normal canine articular cartilage. Further studies[13,27] have been performed to investigate the effects of salicylates and carprofen on normal and osteoarthritic canine cartilage. In vitro salicylate treatment of cartilage from dogs with experimentally induced osteoarthritis resulted in an increase in inhibition of proteoglycan synthesis, compared with cartilage from control dogs.[13] In that same study, in vivo administration of salicylate to dogs with osteoarthritis enhanced articular cartilage degeneration. Carprofen decreased proteoglycan synthesis to a greater extent in cultured articular explants from acetabular joints of dogs with naturally occurring osteoarthritis, compared with explants from healthy dogs.[27] Enhanced inhibition of proteoglycan synthesis in osteoarthritic cartilage may be related to increased uptake of NSAID by osteoarthritic cartilage.

We did not evaluate the effects of oral phenylbutazone administration on articular cartilage from horses with naturally occurring osteoarthritis. Instead, we chose first to establish the effects of oral administration of phenylbutazone at a clinically relevant dose on articular cartilage from clinically normal horses. To simulate conditions similar to naturally occurring osteoarthritis, articular cartilage explants were exposed in vitro to IL-1β. We found that in vivo administration of phenylbutazone did not further inhibit proteoglycan synthesis beyond that induced by IL-1β alone.

Interleukin-1 has been isolated from synovial fluid of horses with naturally occurring osteoarthritis and is considered a primary mediator of degradative processes characteristic of osteoarthritis.[18,28] In vitro exposure

of equine chondrocytes to IL-1 induces production or activation of metalloproteinases such as collagenase and stromelysin, serine proteases, and tissue plasminogen activator.[18] In addition, IL-1 inhibits collagen and proteoglycan synthesis by chondrocytes.[15,29] Recombinant human IL-1β has been used in vitro to induce changes in articular cartilage from horses consistent with damage characteristic of osteoarthritis. Our results indicating that IL-1β inhibited proteoglycan synthesis were similar to results of in vitro studies that revealed a dose-dependent suppression of proteoglycan synthesis in equine articular cartilage explants treated with recombinant human IL-1β.[15,18,30] We hypothesized that the suppressive effects of IL-1β would be additive to those of phenylbutazone. However, we found that after oral phenylbutazone administration, proteoglycan synthesis was not further inhibited by IL-1β. This result suggests a possible competitive interaction between these 2 agents and indicates that phenylbutazone and IL-1β may have a similar or shared mechanism of action.

Phenylbutazone does not directly inhibit IL-1β[23] but rather suppresses some mediators of inflammation such as prostaglandin E_2, prostaglandin I_2, and thromboxane A_2. Phenylbutazone has little effect on other eicosanoids not derived from arachidonic acid.[19-22] Interleukin-1 stimulates production of prostaglandin E_2 by chondrocytes in vitro.[14] Our data suggest that prostaglandin E_2 production may be a predominant mechanism of action for suppression of proteoglycan synthesis in vitro by IL-1β and that phenylbutazone is capable of interfering with this process.

In vitro stimulation of chondrocytes with IL-1 also results in activation and production of metalloproteinases such as stromelysin, collagenase, and gelatinase. Stromelysin (MMP-3) degrades the noncollagen components of cartilage (ie, proteoglycans and fibronectin) and functions to activate latent collagenase.[15,31] Stromelysin is one of the primary enzymes responsible for proteoglycan degradation leading to cartilage softening and fibrillation.[31] Results of a previous study[32] that compared metalloproteinase activity in synovial fluid from normal equine joints with that in osteoarthritic joints indicate that osteoarthritis induces an overall increase in metalloproteinase activity. Evaluation of specific metalloproteinases revealed that stromelysin activity increased more than overall matrix metalloproteinase activity in osteoarthritic joint fluid.[33]

We attempted to measure stromelysin concentrations in equine articular cartilage explants, using a human MMP-3 ELISA. Homology between equine and human stromelysin DNA is reported to be 89%.[31] Stromelysin (MMP-3) was detected in cartilage extracts by use of this human ELISA. In contrast to results of other in vitro studies in which treatment with IL-1 resulted in up-regulation of stromelysin transcriptional activity and production by equine chondrocytes,[18,31] we did not detect a significant increase in stromelysin concentration in equine cartilage explants following exposure to recombinant human IL-1β. Possible explanations for our inability to detect changes in stromelysin concentration include our culture conditions and use of recombinant human IL-1β. An in vitro study investigating the response of equine chondrocytes and synovial cells to human recombinant IL-1α and β, compared with equine mononuclear cell supernatant, revealed that mononuclear cell supernatant induced a greater increase in stromelysin production.[14] The authors of that study also noted that stromelysin concentrations were inversely proportional to the amount of fetal bovine serum in the culture media.[14] Culture conditions, particularly the addition of fetal bovine serum, can affect the detection of metalloproteinases, because fetal bovine serum contains metalloproteinase inhibitors such as α_2-macroglobulin.[14,34]

We did detect a slight but not significant decrease in stromelysin concentration in explants from treated horses, compared with the control group. Results of an in vitro study[35] indicate that phenylbutazone can inhibit production of stromelysin from equine synovial lining cells. However, concentrations of phenylbutazone required to induce this inhibitory effect (150 μg/ml) were greater than could be achieved following systemic administration of the drug. Results of another study[6] indicate that lower concentrations of phenylbutazone (2 to 20 μg/ml) suppressed proteoglycan degradation in explants of equine articular cartilage. These results together with results of the present study suggest that phenylbutazone may function overall to suppress chondrocyte metabolism.

Results of the present study confirmed our hypothesis that oral administration of phenylbutazone to horses suppressed proteoglycan synthesis by articular cartilage. However, phenylbutazone did not aggravate IL–1β-induced inhibition of proteoglycan synthesis. Despite these potentially detrimental effects, phenylbutazone will continue to be used because of its low cost and efficacy. Our results, however, suggest that phenylbutazone should be used judiciously in athletic horses with osteoarthritis. Chronic administration of phenylbutazone may suppress proteoglycan synthesis, which could potentiate cartilage damage, particularly when combined with mechanical trauma to affected joints resulting from early return to athletic function. Our data also suggest that recovery of chondrocyte metabolism after cessation of treatment with phenylbutazone may require more than 2 weeks. Therefore, phenylbutazone may prolong cartilage healing in horses. Further studies are warranted to determine whether other NSAID result in fewer adverse effects.

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[b]Phenylbutazone, Vedco Inc, St Louis, Mo.

[c]Tranquived injection, Vedco Inc, St Louis, Mo.

[d]Diazepam injection USP, Schein Pharmaceutical Inc, Florham Park, NJ.

[e]Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa.

[f]Halocarbon Laboratories, River Edge, NJ.

[g]Vicryl, Ethicon Inc, Somerville, NJ.

[h]Surgilene, Davis & Geck Monfil Inc, Manati, Puerto Rico.

[i]Beuthanasia-D Special, Schering-Plough Animal Health Corp, Kenilworth, NJ.

[j]Sigma Chemical Co, St Louis, Mo.

[k]Gibco BRL, Gaithersburg, Md.
[l]$NaSO_4$ [^{35}S], DuPont NEN, Boston, Mass.
[m]Micro BCA protein assay reagent kit, Pierce Chemical Co, Rockford, Ill.
[n]Tri-carb Packard Beckman, model 3255, Packard, Palo Alto, Calif.
[o]Matrix metalloproteinase-3 (MMP-3), human ELISA system, Amersham Corp, Arlington Heights, Ill.

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