Effects of enrofloxacin and magnesium deficiency on matrix metabolism in equine articular cartilage

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Objective—To investigate the effects of enrofloxacin and magnesium deficiency on explants of equine articular cartilage.

Sample Population—Articular cartilage explants and cultured chondrocytes obtained from adult and neonatal horses.

Procedure—Full-thickness explants and cultured chondrocytes were incubated in complete or magnesium-deficient media containing enrofloxacin at concentrations of 0, 1, 5, 25, 100, and 500 µg/ml. Incorporation and release of sulfate ^{35}S over 24 hours were used to assess glycosaminoglycan (GAG) synthesis and degradation. An assay that measured binding of dimethylmethylene blue dye was used to compare total GAG content between groups. Northern blots of RNA from cultured chondrocytes were probed with equine cDNA of aggrecan, type-II collagen, biglycan, decorin, link protein, matrix metalloproteinases 1, 3, and 13, and tissue inhibitor of metalloproteinase 1.

Results—A dose-dependent suppression of ^{35}S incorporation was observed. In cartilage of neonates, ^{35}S incorporation was substantially decreased at enrofloxacin concentrations of 25 mg/ml. In cartilage of adult horses, ^{35}S incorporation was decreased only at enrofloxacin concentrations of ≥ 100 µg/ml. Magnesium deficiency caused suppression of ^{35}S incorporation. Enrofloxacin or magnesium deficiency did not affect GAG degradation or endogenous GAG content. Specific effects of enrofloxacin on steady-state mRNA for the various genes were not observed.

Conclusion and Clinical Relevance—Enrofloxacin may have a detrimental effect on cartilage metabolism in horses, especially in neonates. (*Am J Vet Res* 2001;62:160–166)

Fluoroquinolones are antimicrobials used extensively in human and veterinary medicine.[1,2] Enrofloxacin is the only fluoroquinolone approved for systemic use in horses. Quinolones initiate bactericidal activity primarily by inhibiting bacterial DNA gyrase. High potency, low incidence of resistance, high oral bioavailability, extensive tissue penetration, low protein binding, and long elimination half-lives are consistent features of fluoroquinolones.[3,4]

Administration of quinolones in developing adolescents has been associated with acute arthropathy of weight-bearing joints. Cartilage lesions are inducible in juvenile animals of several species including dogs,[a,5-8] rats,[9,10] nonhuman primates,[11] rabbits,[12,13] and guinea pigs.[14] Quinolone-induced arthropathy has been investigated most extensively in immature rats and adolescent dogs. Dogs appear to be the most susceptible and are the only species that develops effusion and associated lameness after treatment with quinolones.[15] In 1 study,[7] enrofloxacin-treated Beagle puppies developed lesions when administered doses at only 5 times the dose cited on the label. In that study, 8 of 10 puppies were lame after 3 days of treatment with high doses of difloxacin, and 9 had articular lesions during postmortem examination.[7]

The exact pathogenesis of quinolone-induced arthropathy remains uncertain. Some investigators favor the idea that chondrocytes are the primary site for the toxic effects of quinolones,[5,9,16] possibly because the drugs interfere with mammalian DNA.[9,10] Alternatively, other published data indicates that quinolones interfere directly with the intracellular matrix of joint cartilage.[14] Additionally, feeding magnesium-deficient diets can induce lesion in joint cartilage of juvenile rats identical to those elicited by administration of fluoroquinolones,[17-21] suggesting a role for magnesium in quinolone-induced arthropathy. However, data that convincingly proves any of these mechanisms are lacking.

Recent research efforts have centered on investigating the effects of quinolones on articular cartilage in vitro. These investigations revealed that synthesis of proteoglycans, DNA, and collagen is susceptible to inhibition by quinolones in canine,[22,23] rat,[24,25] rabbit,[26] murine,[27] bovine,[28] and equine[29] articular cartilage.

To investigate the capacity of in vitro studies to act as an accurate substitute for in vivo testing, Hildebrand et al[22] compared the results of in vitro and in vivo experiments. The effects of various quinolones on integrity of cell membranes, activity of mitochondrial dehydrogenase, and soluble and cellular proteoglycans were evaluated in a model that used canine chondrocytes in a tridimensional suspension culture. Synthesis of soluble proteoglycans in isolated chondrocytes was severely affected by most of the assayed quinolone derivatives. The doses required to inhibit proteoglycan synthesis in vitro were similar to doses that produced macroscopic and microscopic lesions in vivo. Although an in vitro model cannot reproduce metabolic events

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that are evident in vivo, these findings suggest that in vitro testing may be capable of identifying arthropathogenic quinolones without the need to perform in vivo studies.

High concentrations of enrofloxacin can induce toxic effects in equine articular cartilage.[29] The objective of the study reported here was to investigate the effects of physiologic concentrations of enrofloxacin and magnesium deficiency on the synthesis and secretion of proteoglycans in equine articular cartilage in explant culture and the expression of matrix-related genes in cultured chondrocytes.

Materials and Methods

Collection of cartilage—Full-thickness articular cartilage was harvested from the tarsus, carpus, and metacarpophalangeal joints of 21 Thoroughbred horses that were euthanatized for reasons other than musculoskeletal disorders. The population of horses included 12 foals (< 2 months old) and 9 adult horses (> 2 years old). Horses were not included in the study if they had received antimicrobial medication in the 2-week period prior to necropsy. At the time of collection, gross evidence of degenerative joint disease was not detected in any of the joints. All cartilage was collected and handled in an aseptic manner, and the cartilage was constantly bathed in PBS solution. For each experiment, cartilage explants from various joints of 1 horse were pooled before random assignment to multiple treatments. To ensure that only articular cartilage was harvested and not the underlying growth cartilage, only the most superficial 2 mm of cartilage was collected.

Explant culture for cartilage—Cartilage explants were dissected from the pooled harvested cartilage to a dimension of approximately 3 × 3 × 2 mm. Six explants were placed in each well, which corresponded to a wet weight of 50 to 60 mg of cartilage/well. Explants were incubated at 37 C in a humidified atmosphere of 5% CO_2-95% air in 24-well plates covered by 1 ml of maintenance medium (high-glucose **Dulbecco's modified Eagle's medium [DMEM]** that did not contain **fetal calf serum [FCS]** or antibiotics). Explants were allowed to stabilize for 24 hours prior to initiating experimental conditions.

Culture of chondrocytes—Harvested minced cartilage was incubated overnight in 0.5% collagenase.[b] Cells were counted, and 10×10^6 chondrocytes were distributed in 30-mm culture dishes. Prior to addition of chondrocytes, dishes were coated with poly-2 (hydroxyethylmethacrylate) to prevent cell adhesion and maintain phenotype.[30] Chondrocytes were incubated (as described previously for cartilage explants) in DMEM that contained 10% FCS for 48 hours to allow chondrocytes to stabilize prior to initiating experimental conditions.

Procedure—After incubation for 24 hours, pretreatment medium was replaced with 1 ml of treatment medium. Four treatment media were evaluated in the study (media 1, serum-free DMEM; media 2, DMEM and 10% FCS; media 3, **Hank's balanced saline solution (HBSS)** and 4,500 mg of D-glucose/L, 20 m*M* HEPES buffer, 200 mg of $CaCl_2$ (anhydrous)/L, 584 ng of L-glutamine/L, 20 ml of 50× MEM amino acids/L,[c] and 10 ml of 100× MEM vitamin solution/L[d]; media 4, same as media 3 except for the addition of 100 mg of $MgCl_2$ ($6H_2O$)/L (0.82 m*M* Mg/L). Media 3 and 4 (magnesium-testing media) were supplemented with specific additives to achieve media identical to DMEM, except for the magnesium concentration. Seven concentrations of enrofloxacin[e] (0 [control], 1, 5, 25, 50, 100, and 500 µg/ml) were used in each treatment group. For each concentration of enrofloxacin, experiments were conducted in triplicate. For each objective, experiments were repeated with cartilage from at least 2 horses.

To investigate the effect of enrofloxacin on synthesis of **glycosaminoglycans (GAG)**, explants were pulse-labeled with 20 µCi ^{35}S/ml of media and incubated with enrofloxacin for 24 hours in 1 of the treatment media. At termination, all media was collected and frozen at –70 C. Cartilage explants were rinsed 3 times in PBS solution, placed in 1 ml of 0.05% papain,[f] and incubated at 60 C for 24 hours to solubilize the cartilage.

To investigate the effect of enrofloxacin on degradation of newly synthesized proteoglycan, explants were incubated in media 2 without enrofloxacin and pulse-labeled with 20 µCi ^{35}S/well. After 24 hours of incubation, cartilage explants were rinsed 3 times in PBS solution and incubated in media 2 supplemented with enrofloxacin for 24 hours. After that incubation, the cartilage and media were harvested as described for synthesis determination.

Determination of GAG synthesis and degradation—Radiolabeled sulfate uptake by the cartilage explants was determined, using liquid scintillation counting. Five microliters of papain-digested cartilage was added to 4.5 ml of scintillation cocktail,[g] and scintillation counting was performed.

To account for the ^{35}S-labeled proteoglycan fragments in spent media, the spent media were separated, using gel exclusion chromatography.[h] Radioactivity in the filtrate represented the ^{35}S-labeled proteoglycan fragments. An aliquot (250 µl) of filtered media was added to 4.5 ml of scintillation cocktail, and scintillation counting was performed.

All samples were assayed in duplicate. Results were expressed as number of **counts per minute (CPM)** per µg of DNA and number of CPM per mg of cartilage (wet-weight basis).

Quantification of GAG content—Total GAG content of the explants and spent media was determined by reaction with 1,9-dimethylmethylene blue.[31,i] Shark chondroitin sulfate[j] (0 to 25 µg/ml) was used as a standard. Cartilage explants and media were assayed in duplicate, and GAG content was adjusted on the basis of µg of GAG per µg of DNA (to minimize site variation in cartilage structure) and µg of GAG per mg of cartilage (wet-weight basis).

Determination of DNA Content—Total DNA content was determined via fluorometric assay, using a commercially available product[k] and reported methods. Samples were assayed in duplicate and quantified, using a standard curve for calf thymus DNA, which was assayed concurrently. For each measurement, 30 ml of papain-digested cartilage was placed in a cuvette and mixed manually with 2 ml of dye solution by gentle shaking. Enhancement of dye fluorescence was specific for DNA, as indicated by sensitivity to DNase and resistance to RNase digestions. All solutions and samples were used within 2 hours of preparation.

Northern blot analysis—Chondrocytes were incubated in 5 concentrations of enrofloxacin (0 [control], 5, 50, 100, and 500 µg/ml) for 24 hours in each of the treatment media. After 24 hours of incubation, RNA was extracted, using a monophasic phenol,[l] and electrophoresed in denaturing 1% agarose gels (10 µg of RNA/lane). Gels were capillary blotted to charged nylon membranes[m] and hybridized with random primed[n] ^{32}P-labeled cDNA probes that had been purified in gel-filtration centrifugation columns.[o] Probes specific for equine fibronectin, aggrecan, biglycan, decorin, type-II collagen, link protein, matrix metalloproteinases 1, 3, and 13, and tissue inhibitor of metalloproteinase 1 were used. All cDNA inserts were cloned[p] and ranged in size from 756 (tissue inhibitor of

metalloproteinase 1) to 3,777 (type-II collagen) base pairs. All cDNA clones were isolated from an equine chondrocyte library constructed in our laboratory. Prehybridization and hybridization incubations were performed at 65 C for 30 to 60 minutes and 2 to 12 hours respectively, using a commercial hybridization buffer.[q] Blots were washed 3 times (20 min/wash) at 55 C with 0.5X, 0.25X, and 0.1X saline sodium citrate buffer and 0.1% sodium dodecyl sulfate and exposed to radiographic film[r] for 6 to 24 hours at –70 C. Resulting images were quantified by use of computer-integrated densitometry.[s] Corrections for variations in gel loading were accomplished by means of densitometry of ribosomal bands in photographic negatives of the ethidium-stained gels.

Statistical analysis—Results were analyzed, using linear regression.[32] When appropriate, robust techniques were used to suppress the influence of extreme values.[33,34] Values of $P < 0.05$ were considered significant.

The **critical enrofloxacin dose (CED)** was defined as the dose of enrofloxacin that was associated with a substantial decrease in GAG synthesis (substantial was defined as values that were decreased by > 2 SD).

Results

Results were similar when expressed on the basis of number of units per µg of DNA or number of units per mg of cartilage (wet-weight basis). All results reported here were expressed on the basis of number of units per mg of cartilage (wet-weight basis).

Synthesis of GAG—Enrofloxacin caused a dose-dependent decrease in rate of GAG synthesis in cartilage obtained from neonatal and adult horses. This dose-dependent decrease was evident in all media conditions, as determined by ^{35}S uptake **(Fig 1-3)**. The amount of ^{35}S released into the media was directly proportional to the amount of ^{35}S uptake into the explants. All results reported here were expressed as ^{35}S uptake into explants.

Age—Uptake of ^{35}S was significantly greater in explants harvested from neonates than in explants harvested from adults. Magnitude of the response to a dose of enrofloxacin was approximately 10 times greater in cartilage from neonates than in cartilage from adults (**Table 1**, Fig 1). In cartilage from neonates, ^{35}S uptake was substantially decreased at an enrofloxacin concentration of 25 µg/ml. Uptake of ^{35}S in cartilage obtained from adults was not substantially

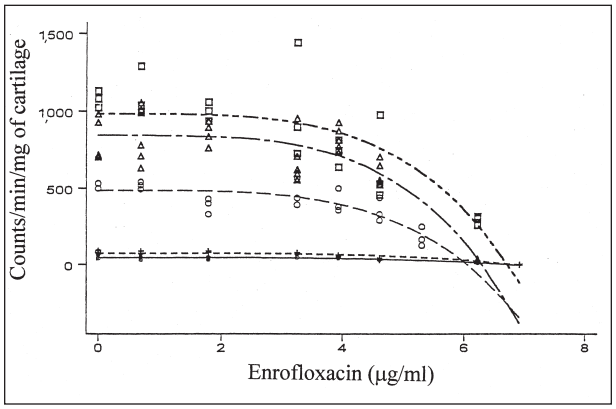

Figure 1—Effects of enrofloxacin and age on incorporation of sulfate ^{35}S in cartilage harvested from 2 adults and 3 neonates. + = Data from adult 2, media 2 (line of short dashes represents best-fit curve). ○ = Data from neonate 1, media 2 (line of long dashes represents best-fit curve). □ = Data from neonate 3, media 2 (line of 1 long and 4 short dashes represents best-fit curve). △ = Data from neonate 2, media 2 (line of a long dash and a short dash represents best-fit curve). ● = Data from adult 1, media 2 (solid line represents best-fit curve).

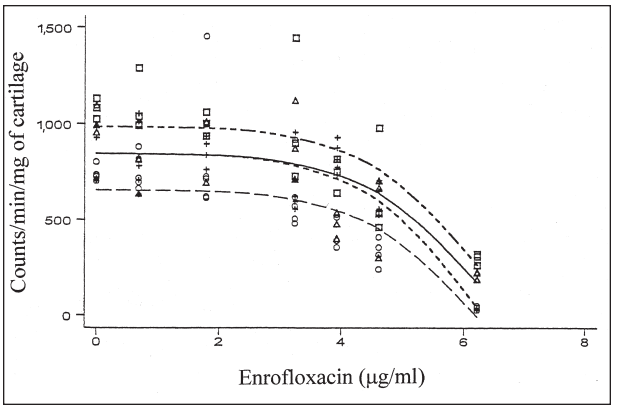

Figure 2—Effects of enrofloxacin and media supplemented with and without fetal calf serum (FCS) on ^{35}S incorporation in cartilage harvested from 2 neonates. + = Data from neonate 5, media 1 (line of short dashes represents best-fit curve). ○ = Data from neonate 4, media 1 (line of long dashes represents best-fit curve). □ = Data from neonate 5, media 2 (line of 1 long and 4 short dashes represents best-fit curve). △ = Data from neonate 4, media 2 (solid line represents best-fit curve).

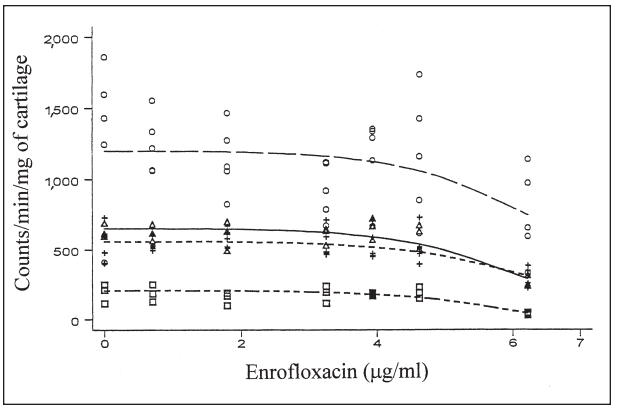

Figure 3—Effects of enrofloxacin and magnesium deficiency on ^{35}S incorporation in cartilage harvested from 1 adult and 1 neonate. + = Data from neonate 6, media 4 (line of short dashes represents best-fit curve). ○ = Data from neonate 6, media 3 (line of long dashes represents best-fit curve). □ = Data from adult 3, media 4 (line of 1 long and 4 short dashes represents best-fit curve). △ = Data from adult 3, media 3 (solid line represents best-fit curve).

Table 1—Effects of enrofloxacin and age of horse on incorporation of sulfate [^{35}S] in cartilage harvested from 2 adult and 3 neonatal horses

Source of cartilage	Constant (CPM/mg)	Coefficient	CED/µg/ml
Adult			
	48.18 ± 2.56	−0.0222 ± 0.0024	100
	74.67 ± 4.35	−0.0331 ± 0.0058	100
Neonate			
	488.74 ± 22.45	−0.3656 ± 0.0626	25
	846.05 ± 27.05	−0.5392 ± 0.0452	25
	976.91 ± 50.23	−0.5580 ± 0.0568	25

Values reported are mean ± SD of 2 replicates for each specimen. CPM = Counts per minute. CED = Critical enrofloxacin dose.

Table 2—Effects of enrofloxacin and media on [^{35}S] incorporation in cartilage harvested from 2 neonatal horses

	Media*			
	1		2	
Source of cartilage	Constant (CPM/mg)	Coefficient	Constant (CPM/mg)	Coefficient
Neonate	655.96 ± 25.79	−0.4456 ± 0.0431	846.05 ± 27.05	−0.5322 ± 0.0452
Neonate	822.04 ± 55.33	−0.5042 ± 0.0626	976.0 ± 50.23	−0.5586 ± 0.0568

*Media 1 and 2 were identical except that medium 1 was deficient in 10% FCS.

Table 3—Effects of enrofloxacin and magnesium deficiency on [^{35}S] incorporation in cartilage harvested from 1 adult and 1 neonatal horse

Source of cartilage	Medium*	Constant (CPM/mg)	Coefficient
Neonate			
	3	569.71 ± 18.40	−0.168 ± 0.023
	4	1193.90 ± 68.20	−0.290 ± 0.105
Adult			
	3	191.79 ± 13.48	−0.093 ± 0.013
	4	957.47 ± 24.13	−0.238 ± 0.023

*Media 3 and 4 were identical except that medium 3 was deficient in magnesium.

Table 4—Effects of enrofloxacin and magnesium deficiency on total proteoglycan content in cartilage harvested from 1 adult and 1 neonatal horse, as determined by use of the 1,9-dimethylmethylene blue assay

Source of cartilage	Media*	Constant (CPM/mg)
Neonate		
	3	228 ± 0.008
	4	305 ± 0.005
Adult		
	3	216 ± 0.012
	4	279 ± 0.009

Coefficient = Not determined, because the value did not exceed 2 SD.
See Table 3 for remainder of key.

decreased until an enrofloxacin concentration of 100 µg/ml was reached.

Media condition—Incorporation of ^{35}S was significantly greater in explants incubated in media 2 (FCS-supplemented medium) than in media 1 (serum-free medium). There was not a significant difference between the magnitude of the dose-response (coefficient of the curve) for explants incubated in media 1 or 2 (**Table 2**, Fig 2).

Magnesium deficiency (media 3) caused significant suppression of ^{35}S incorporation in cartilage from neonates and adults. Magnesium deficiency did not significantly alter the response to enrofloxacin, compared with results for the medium supplemented with magnesium (media 4; **Table 3**, Fig 3).

Degradation of GAG—Enrofloxacin did not have an effect on GAG degradation in cartilage explants obtained from neonatal or adult horses. Incorporation of ^{35}S in the explants and release into the media were significantly greater in explants harvested from neonates (123.18 ± 12.98 CPM/mg) than in explants harvested from adults (67.29 ± 6.39 CPM/mg).

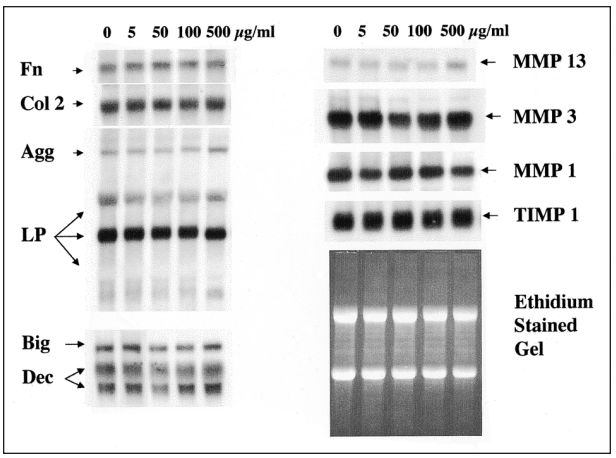

Figure 4—Northern blots of total RNA from cultured equine chondrocytes incubated in media 2 (Dulbecco's modified Eagle's medium [DMEM] that contained 10% FCS). Each lane contained the concentration of enrofloxacin indicated. Enrofloxacin did not affect gene expression in the cultured equine chondrocytes. Fn = Fibronectin. Agg = Aggrecan. Big = Biglycan. Dec = Decorin. Col 2 = Type-II collagen. LP = Link protein. MMP 1 = Matrix metalloproteinase 1. MMP 3 = Matrix metalloproteinase 3. MMP 13 = Matrix metalloproteinase 13. TIMP 1 = Tissue inhibitor of metalloproteinase 1.

Endogenous proteoglycan content of explants and media—Enrofloxacin did not affect the proteoglycan content of explants or media, as determined by use of the 1,9-dimethylmethylene blue assay, in any of the 4 media conditions used for incubation of cartilage obtained from neonatal or adult horses. Magnesium deficiency significantly decreased the total GAG content in the explants, as determined by use of the 1,9-dimethylmethylene blue assay, in cartilage harvested from neonates and adults (**Table 4**).

Northern blot analysis—Enrofloxacin did not have an effect in cartilage obtained from neonates or adults on the steady-state amounts of mRNA for aggrecan, biglycan, decorin, type-II collagen, link protein, matrix metalloproteinases 1, 3, and 13 and tissue inhibitor of metalloproteinase 1. This result was similar for all 4 media conditions (**Fig 4**).

Discussion

In the study reported here, enrofloxacin inhibited ^{35}S incorporation in equine articular cartilage in a dose-dependent manner, and inhibition was most pronounced in cartilage harvested from neonates. The lowest dosage of enrofloxacin that substantially inhibited GAG synthesis was 25 µg/ml, whereas in cartilage harvested from adults, the lowest dosage of

enrofloxacin required to substantially inhibit ^{35}S incorporation was 100 µg/ml.

Cartilage of neonatal horses may be more susceptible to enrofloxacin than cartilage of adult horses because of an increased rate of metabolism. Chondrocytes of growing animals produce a greater quantity of matrix macromolecules.[35] Because the study reported here documented that enrofloxacin inhibited GAG synthesis, increased turnover of GAG in immature cartilage may increase the susceptibility of cartilage of neonatal animals to the toxic effects of fluoroquinolones. However, the exact pathogenesis of quinolone-induced arthropathy remains unclear. To our knowledge, convincing data documenting the mechanism by which the cartilage of neonates appears to be more sensitive to fluoroquinolones has not been published.

Data currently are not available regarding the minimum in vivo arthropathic concentration of enroflo xacin in horses. This information cannot be extrapolated from other species, because studies have documented differing susceptibility to fluoroquinolones among species. The minimal arthropathic doses of norfloxacin reportedly were 25, 50, and 100 mg/kg for juvenile rabbits, dogs, and rats, respectively.[36]

Researchers investigating the pharmacokinetics of enrofloxacin in horses have documented that the systemic concentration of enrofloxacin 10 minutes after IV administration (5 mg/kg) was 9.44 µg/ml.[37] In the study reported here, the CED for cartilage harvested from neonates was 2.5 times that systemic concentration, and the CED for cartilage harvested from adults was 10 times that systemic concentration. Also, in that previous study,[37] the synovial concentration of enrofloxacin 4 days after twice-daily oral administration (5 mg/ml) was 4.71 µg/ml. Thus, the CED reported here for cartilage harvested from neonates was 5 times that synovial concentration, and the CED for cartilage harvested from adults was 20 times that synovial concentration.

Studies with cultured ovine chondrocytes revealed that inhibition of ^{35}S incorporation was observed at concentrations of 5 µg of levofloxacin/ml, a concentration lower than the minimum in vivo arthropathic concentration of levofloxacin in rabbits.[26] Analysis of those results suggested that an in vitro model may be of use to identify arthropathogenic quinolones without the need to perform studies that involve the use of animals. Analysis of results of the study reported here indicated that enrofloxacin may not be chondrotoxic in horses, particularly adult horses. Tolerance of orally administered enrofloxacin in adult horses was investigated.[38] Enrofloxacin (15 mg/kg) was administered to adult horses for 21 days without the development of quinolone-induced arthropathy. In addition, GAG content and proteoglycan synthesis of articular cartilage were not different between enrofloxacin-treated horses and control horses.

In vitro inhibition of ^{35}S incorporation by fluoroquinolones has been reported. Inhibition of ^{35}S incorporation by ciprofloxacin at concentrations of 10 µg/ml was documented in suspension culture of canine chondrocytes.[22] Rate of synthesis of GAG was reduced by concentrations of difloxacin that were ≥ 80 µg/ml in explant cultures of canine articular cartilage.[23] In a model that used explants of bovine articular cartilage in short-term culture, 200 µg of nalidixic acid/ml inhibited ^{35}S incorporation.[28] Investigators examined the effects of enrofloxacin on equine articular cartilage in explant culture in another study.[30] Dosages used in that study included 0, 5, 10, 1,000, 10,000, and 50,000 µg/ml. Inhibition of ^{35}S incorporation was observed at 1,000 mg/ml, an extremely high dosage, which is likely to be more than 100-fold higher than concentrations reached in vivo. Results of that study and the data from the study reported here support the conclusion that high doses of quinolone derivatives can exert a specific inhibitory effect on proteoglycan biosynthesis.

In our study, magnesium deficiency caused substantial suppression of ^{35}S incorporation in cartilage from neonatal and adult horses. It is unclear whether the suppression of ^{35}S incorporation caused by magnesium deficiency is a result of a generalized cytotoxic response or whether it is associated with the chondrotoxic effects of magnesium deficiency that have been documented in vivo.[17] It has been reported that magnesium deficiency enhances cytotoxic effects by increasing membrane permeability, particularly for calcium.[39]

Magnesium deficiency did not significantly alter the response to various doses of enrofloxacin. These results contradict results of in vivo studies in rats in which magnesium deficiency exacerbated cartilage lesions induced by ofloxacin.[17] Quinolones are potent chelating agents for magnesium. For example, bioavailability of ciprofloxacin is reduced by > 90% when the drug is taken simultaneously with antacids containing magnesium and aluminum.[t] Because quinolones accumulate in articular cartilage,[40] it is possible that quinolones form stable chelated complexes with magnesium, resulting in a local deficiency of magnesium in articular cartilage and subsequent arthropathy. Magnesium-dependent integrin receptors of the β1-subcell family may have a primary role in quinolone-induced arthropathy.[17] In a recent study,[19] investigators documented a decrease in the β1-integrin receptors in cartilage in juvenile rats following treatment with ofloxacin.[19] The disparity of results in those studies and results of the study reported here may represent limitations of in vitro studies. For example, it is not possible to precisely mimic in vivo weight-bearing forces in explant culture. The importance of magnesium deficiency in horses is unclear, and its possible role in quinolone-induced arthropathy requires further investigation.

Incorporation of ^{35}S was greater in explants incubated in media supplemented with FCS than in serum-free media. Fetal calf serum is mitogenic and contains various growth factors, so its addition may more closely mimic the in vivo environment of chondrocytes. There was not a significant difference between the magnitude of the dose-response (coefficient of the curve) for explants incubated in media supplemented with FCS and media without FCS. This suggests that the enrofloxacin dose-response curve is independent of mitogenic activity and the effect of growth factors.

The CED of cartilage harvested from neonates and

incubated in HBSS, with or without supplemental magnesium, was 100 µg/ml, compared with a CED of 25 µg/ml in cartilage harvested from neonates and incubated in DMEM. This suggests that the enrofloxacin dose-response curve can be affected by variations in media composition. Thus, results of studies in which investigators used various culture media should be compared with caution.

In the study reported here, enrofloxacin did not have an effect on GAG degradation in cartilage explants obtained from neonatal or adult horses. Duration of the pulse-chase experiment was only 24 hours; therefore, it is possible that results may have differed had the chase segment of the experiment been prolonged. Analysis of our results suggests that if GAG metabolism plays a primary role in quinolone-induced arthropathy, it is more likely to be related to alterations in GAG synthesis than GAG degradation. Data presented here supports the report[23] that difloxacin at concentrations of 80 and 160 µg/ml did not alter the net rate of catabolism of sulfated GAG in canine articular cartilage in explant culture. However, GAG synthesis in that study was inhibited at a concentration of 80 µg of difloxacin/ml.

The 1,9-dimethylmethylene blue assay is an assay that measures total GAG content. In the study reported here, alterations in synthesis of GAG by explants were detected by use of liquid scintigraphy; however, enrofloxacin did not significantly affect the proteoglycan content of the explants or media, as determined by results of the 1,9-dimethylmethylene blue assay. This may have been attributable to a lack of sensitivity of the assay or other factors such as a decrease in GAG degradation in response to enrofloxacin, which could have resulted in the total GAG content remaining unchanged. Analysis of data for our 1,9-dimethylmethylene blue assay did not confirm other results that documented inhibition of the synthesis of soluble proteoglycans by canine chondrocytes in suspension culture containing 3 µg of ciprofloxacin/ml.[22]

Enrofloxacin did not have an effect on the steady-state amounts of mRNA in chondrocytes obtained from neonatal or adult horses investigated in the study reported here. Dose-dependent suppression of synthesis of GAG by enrofloxacin was not paralleled by transcriptional effects in these major matrix molecules. We are not aware of any other studies that investigated the effects of fluoroquinolones on gene transcription.

Equine articular cartilage in explant culture was susceptible to a toxic effect of enrofloxacin. Additional studies appear warranted to evaluate other potential effects of enrofloxacin on equine chondrocytes. Until the specific mechanism of quinolone-induced arthro pathy is elucidated, we recommend that quinolone administration should be limited in juvenile horses.

[a]Howard LC, VanSickle DC, Deshmukh K, et al. Cinoxacin induced arthropathy in juvenile beagle dogs (abstr). *Toxicol Appl Pharmacol* 1979;48:A145.

[b]Collagenase D, Boehringer Mannheim, Indianapolis, Ind.

[c]MEM Amino Acids Solution (50X), Gibco BRL Life Technologies, Gaithersburg, Md.

[d]MEM Vitamin Solution (100X), Gibco BRL Life Technologies, Gaithersburg, Md.

[e]Enrofloxacin, Bayer Co, Shawnee Mission, Kan.

[f]Papain, Sigma Chemical Co, St Louis, Mo.

[g]ScintiVerse, Fisher Scientific, Springfield, NJ.

[h]Sephadex G-50, Sigma Chemical Co, St Louis, Mo.

[i]1,9-Dimethyl-methylene blue, Aldrich Chemical Co, Milwaukee, Wis.

[j]Chondroitin sulfate sodium salt, Sigma Chemical Co, St Louis, Mo.

[k]Hoechst 33258, American Hoechst Corp, Somerville, NJ.

[l]TRIzol, Gibco BRL Life Technologies, Gaithersburg, Md.

[m]Hybond N$^+$, Amersham, Arlington Heights, Ill.

[n]Rediprime, Amersham, Arlington Heights, Ill.

[o]Microspin G-50 columns, Amersham, Arlington Heights, Ill.

[p]pBluescript, Stratagene, La Jolla, Calif.

[q]Rapid-hyb, Amersham, Arlington Heights, Ill.

[r]Reflection, NEN Life Sciences, Boston, Mass.

[s]NIH IMAGE. Available at: http://rsb.info.nih.gov/nih-image. Accessed Oct 1, 1998.

[t]Hoffken GK, Borner K, Glatzel PD, et al. Reduced enteral absorption of ciprofloxacin in the presence of antacids (abstr). *Eur J Clin Microbiol* 1985;4:345.

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