Effect of long-term administration of an injectable enrofloxacin solution on physical and musculoskeletal variables in adult horses

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Objective—To evaluate clinical safety of administration of injectable enrofloxacin.

Design—Randomized controlled clinical trial.

Animals—24 adult horses

Procedures—Healthy horses were randomly allocated into 4 equal groups that received placebo injections (control) or IV administration of enrofloxacin (5 mg/kg [2.3 mg/lb], 15 mg/kg [6.8 mg/lb], or 25 mg/kg [11.4 mg/lb] of body weight, q 24 h) for 21 days. Joint angles, cross-sectional area of superficial and deep digital flexor and calcaneal tendons, carpal or tarsal osteophytes or lucency, and midcarpal and tarsocuneal articular cartilage lesions were measured. Physical and lameness examinations were performed daily. Measurements were repeated after day 21, and articular cartilage and bone biopsy specimens were examined.

Results—Enrofloxacin did not induce changes in most variables during administration or for 7 days after administration. One horse (dosage, 15 mg/kg) developed lameness and cellulitis around the tarsal plantar ligament during the last week of administration. One horse (dosage, 15 mg/kg) developed mild superficial digital flexor tendinitis, and 1 horse (dosage, 25 mg/kg) developed tarsal sheath effusion without lameness 3 days after the last administration. High doses of enrofloxacin (15 and 25 mg/kg) administered by bolus injection intermittently induced transient neurologic signs that completely resolved within 10 minutes without long-term effects. Slower injection and dilution of the dose ameliorated the neurologic signs. Adverse reactions were not detected with a 5 mg/kg dose administered IV as a bolus.

Conclusions and Clinical Relevance—Enrofloxacin administered IV once daily at the rate of 5 mg/kg for 3 weeks is safe in adult horses. (J Am Vet Med Assoc 2000;217:1514–1521)

Enrofloxacin (a fluoroquinolone antimicrobial) is rapidly bactericidal, has a broad spectrum of activity and low bacterial resistance, is effective at low tissue concentrations, and can be administered orally or by injection once daily. These features and its effectiveness against many equine pathogens make it an attractive antimicrobial for use in horses. Results of pharmacokinetic studies of enrofloxacin in adult horses suggest that a single dose of 5.5 mg/kg (2.5 mg/lb) of body weight or 3.0 mg/kg (2.3 mg/lb) administered daily would be effective in horses. Parenteral administration at >5 times the manufacturer’s recommended dosage has induced cartilage lesions in juvenile rats, dogs, guinea pigs, rabbits, and nonhuman primates. An anecdotal report has suggested that cartilage lesions may develop in horses after oral administration of enrofloxacin. Concentrations of enrofloxacin (2 to 10 µg/ml) that may be achieved following parenteral administration at 5 mg/kg did not significantly suppress equine chondrocyte explant metabolism in vitro; however, high concentrations of enrofloxacin (>1,000 µg/ml) were toxic. Current limited clinical use has not induced documented joint damage, although the safety of the use of enrofloxacin in horses has not been scientifically evaluated. Injectable enrofloxacin received FDA approval in 1998 for treatment of respiratory tract diseases in cattle. The purpose of the study reported here was to evaluate the clinical safety of administration of injectable enrofloxacin (5, 15, and 25 mg/kg [2.3, 6.8, and 11.4 mg/lb]) given IV once daily for 21 days.

Materials and Methods

Experimental design—A standard general safety study designed to evaluate enrofloxacin administered to healthy mature horses at a dosage of 5, 15, or 25 mg/kg for 21 days was performed. A similar group of horses acted as controls and received saline (0.9% NaCl) solution injections for 21 days. Horses were evaluated to meet inclusion criteria from days −21 to −10 and prior to entering the acclimation phase from days −10 to −1. Twenty-four horses entered the study on day 0. Each treatment group consisted of 6 horses. The study was conducted in 2 replicates of 12 horses (3 from each treatment group). Investigators who performed all daily physical and lameness examinations and the final physical evaluation 24 hours after the last enrofloxacin or saline solution injection were blinded regarding treatment groups, as were investigators who evaluated tendons and articular surfaces.

Preacclimation evaluation—Thirty-eight horses that had completed a quarantine period were initially selected for the study and accepted for preacclimation evaluation. Prior to entering the acclimation period (on or before day −10), each horse underwent a complete physical examination that included a thorough lameness evaluation. The musculoskelet-
tal system was closely examined as part of the physical examination. All limbs were examined visually and palpated for swelling; findings were scored as follows: 0, no swelling; 1, mild swelling; 2, moderate swelling; and 3, severe swelling. A sum score for each limb and horse was recorded. In the initial physical examination, horses were led on a firm level surface in a straight line at the walk and trot; any lameness was graded as follows: 0, no discernible lameness; 1, no lameness at the walk and inconsistent lameness at the trot; 2, no obvious lameness at the walk and slight lameness at the trot; 3, lameness observed at the walk and consistent lameness at the trot; 4, lameness observed at the walk and horse reluctant to trot and severely lame at the trot; and 5, horse not weight-bearing on affected limb at rest, walk, or trot.

A serum biochemical profile and CBC were performed on all horses. Radiographic examinations of the carpi and tarsi were performed and included dorsopalmar, lateromedial, dorsolateral-palmaromedial oblique, and dorsomedial-palmarolateral oblique views to ensure that horses had minimal to no evidence of osteoarthritis; any osteophytes or areas of lucency were measured and recorded. Radiographic evidence of circumscribed osteochondrosis lesions or mild arthritis of the distal intertarsal, tarsometatarsal, or carpometacarpal joints did not exclude horses if other inclusion criteria were met.

Calcaneal tendons (superficial digital flexor and gastrocnemius) and forelimb flexor tendons (superficial digital flexor and deep digital flexor) were evaluated by use of ultrasonography in a transverse and cross-sectional plane. Images of the longitudinal and transverse sections were recorded, and cross-sectional area (cm²) was measured. If an abnormal tendon was detected, the area of abnormality was recorded. Images of forelimb flexor tendons were recorded at equidistant sites between the accessory carpal bone andegot and were labeled. The calcaneal tendon was examined ultrasonographically 10 cm proximal to the point of the hock (tuber calcaneus).

Arthroscopic examination of the midcarpal and tarsocural joints was conducted under general anesthesia. After aseptic preparation, arthroscopic exploration of the middle carpal joint via the lateral portal and the tarsocural joint via the medial portal was performed. Joints were deemed acceptable by the surgeon (ie, minimal cartilage damage estimated as either <10% of the visible superficial area affected by any lesion or by mild [score, 1] lesions scattered over <30% of the visible superficial area); images were recorded on a form and via video camera. Recorded lesions were scored (0, no lesions; 1, cartilage indentations and fibrillation [mild]; 2, cartilage erosions or ulcers) and totaled per joint and horse.

Horses—Inclusion criteria included: age (3 to 11 years); body weight (approx 450 kg [990 lb]); healthy and sound, as determined by physical and lameness examinations; acceptable hemogram and serum biochemical values; acceptable radiographic appearance of carpal and tarsal joints; acceptable results of ultrasonographic examination of flexor and calcanean tendons; and no substantial lesions on the midcarpal and tarsocural joint surfaces, as determined by arthroscopic examination. Of the 38 horses evaluated, 24 (12 geldings, 12 mares) met study criteria and entered the acclimation period; horses were (mean ± SD) 6.8 ± 1.9 years old (range, 3 to 10 years) and weighed 456.8 kg ± 44.8 kg (1,004.9 ± 98.5 lb; range, 393 to 523 kg [864.6 to 1,150.6 lb]).

Horses were treated orally for internal parasites prior to the acclimation period, housed in box stalls (approx 3.7 m × 3.7 m) with ad libitum access to water, and managed in a similar manner during the acclimation and test periods. Horses did not receive other medication in any form during the acclimation or test periods.

Assignment to treatment group—Horses were random-ly assigned to 1 of 4 treatment groups so that at the completion of both replicates there were equal numbers of mares and geldings in each group. Horses were stratified by sex, randomized to replicates by a biometrician (LGH), randomized within replicates, and assigned to treatment groups. This strategy created a randomized complete-block design, with the appropriate number of horses of each sex receiving each treatment in each replicate.

Acclimation period—Horses were allowed to acclimate for 9 days prior to receiving enrofloxacin or placebo. During the acclimation period, each horse underwent a daily physical examination. From day 3 to 5, horses were jogged daily to evaluate soundness. On day 3, serum biochemical analyses and CBC were performed, and body weight was recorded. Within 24 hours prior to administration of injections, each horse underwent a complete physical and lameness examination that included hoof-tester evaluation, jogging for soundness, and limb flexion tests. Goniometric measurements of metacarlo- and metatarsophalangeal ( fetlock) joints were recorded with the horse standing squarely at rest. In brief, an arm of the goniometer was placed parallel to the metacarpus, and the other arm was placed parallel to the pastern. The angle measurement was read 3 times, and the mean was calculated.

Enrofloxacin administration—On day 0 of the study, enrofloxacin injectable solution (100 mg/ml) was administered IV via bolus injection through an indwelling catheter at either 5, 15, or 25 mg/kg. After day 0, horses that received 5 mg of enrofloxacin/kg received an IV bolus injection through an indwelling catheter once daily for 20 days. Because of adverse reactions in the 15- and 25-mg/kg groups during bolus injection, enrofloxacin was administered IV via slow injection (30 minutes) through an indwelling catheter once daily for 20 days in the 15-mg/kg group and via slow infusion (30 to 60 minutes) through an indwelling catheter once daily for 20 days after dilution in 300 mL of sterile saline solution in the 25-mg/kg group. Control horses received an IV bolus injection of saline solution once daily for 21 days at a volume equal to a dose of 5 mg/kg of the enrofloxacin formulation.

Adverse reactions to enrofloxacin—Adverse reactions that could be related to administration of enrofloxacin were recorded as any unusual observation or change in the horse's actions during or for 30 minutes after administration, including benign behavioral changes such as Flehman lip curl, increased respiratory rate, and yawning.

Evaluation during the study—Horses were observed twice daily for signs of disease (eg, signs of depression or anorexia) or effects of enrofloxacin administration (eg, bizarre behavior, signs of apprehiension, vocalization, or altered locomotion) during 1 of the observation periods, prior to administration of the next injection, each horse underwent a physical examination and was jogged in hand for evaluation of soundness. The catheter site was evaluated, and a score was given for signs of pain (0, absent; 1, mild; 2, moderate; 3, severe), heat (0, absent; 1, present), and swelling (0, absent; 1, 0.5 to 1 cm wide; 2, 1 to 2 cm wide; 3, 2 to 5 cm wide; 4, >5 cm wide). If an adverse effect or lameness was detected, a more thorough lameness examination was conducted to characterize the finding. On day 10, body weight of each horse was obtained, and dosage was adjusted accordingly.

On day 21 (24 hours after the last enrofloxacin injection), serum biochemical analyses and CBC were performed, and body weight of each horse was recorded. Each horse underwent a complete physical examination that included a thorough lameness evaluation, as described. Goniometric measurements of fetlock joints were recorded. If
lameness was detected, additional diagnostic tests were performed to localize and diagnose the lameness, including regional perineural diagnostic nerve blocks, if necessary. From days 22 through 28, radiographic, ultrasonographic, and arthroscopic examinations were performed, as described. Additionally, during arthroscopic surgery of the tarsocrural joints, a full-thickness biopsy specimen (approx 0.5 cm) that included articular cartilage and subchondral bone was obtained with an osteotome from the abaxial surface of the lateral trochlear ridge of each talus. Biopsy specimens were immediately fixed in neutral-buffered 10% formalin, decalcified briefly, sectioned at 6 μm, and stained with toluidine blue. Sections were evaluated and scored by an investigator (ALB) and a pathologist (SEW), without knowledge of treatment group, for: structure (0, normal; 1, surface irregularities; 2, pannus and surface irregularities; 3, clefts to intermediate zone; 4, clefts to deep zone; 5, clefts to calcified zone; 6, complete disorganization); cells (0, normal; 1, diffuse hypercellularity; 2, cloning; 3, hypocellularity); intensity of matrix staining (0, normal; 1, slight reduction; 2, moderate reduction; 3, severe reduction; 4, no dye noted); and tibial mark integrity (0, intact; 1, crossed by blood vessels).

Subsequent to completion of these evaluations, any horse that was lame at the termination of the study was euthanized and necropsied. Necropsy included gross and histologic evaluation of all body systems (except spinal cord) and all appendicular joints and was performed within 48 hours after the last injection.

Statistical analyses—A check was made to ensure that the initial treatment groups were equal before enrofloxacin or saline solution administration began; this was confirmed for all outcomes and initial weight. Data recorded before acclimation were compared among groups by use of contingency table analysis. Each of the model factors was assessed separately if the factor had acceptable influence of this factor on the treatment effect was resolved before administration of enrofloxacin. All horses were deemed to be clinically healthy prior to administration of enrofloxacin or saline solution and had acceptable CBC results on day 3.

Results

Acclimation period—During this 9-day period, 2 horses had increased rectal temperature (> 38.1 °C [100.5°F]) for 2 to 3 days, and 2 horses had mild lameness (grade ≤ 1) for 1 to 2 days. Lameness and fever resolved before administration of enrofloxacin. All horses were deemed to be clinically healthy prior to administration of enrofloxacin or saline solution and had acceptable CBC results on day 3.

Adverse reactions to enrofloxacin—Reactions were detected in all 3 horses in the first replicate in the 15-mg/kg group and all 3 horses in the first replicate in the 25-mg/kg group (most severe reaction) on the first day (day 0) of enrofloxacin administration when enrofloxacin was administered IV by bolus injection. Reactions were detected during the injection or immediately after the injection. In the 25-mg/kg group, 1 horse became recumbent with seizure-like activity, and 1 horse almost became recumbent with seizure-like activity. No horses in the 5-mg/kg group or the control group had abnormal behavior or adverse reactions. Few adverse reactions (total, 6 [all in 1 horse]) developed after the rate of injections was decreased and enrofloxacin was diluted, as described; reactions were mild (head jerking) and resolved within 20 minutes.

Physical examination—All 24 horses had normal results of physical examinations and were sound after acclimation and before administration of enrofloxacin or saline solution. One horse (15-mg/kg group) developed grade-1 lameness of the left hind limb associated with a swelling that developed on the back of the tarsus on day 14. Most horses gained weight during the study, and 5 horses gained > 13.6 kg (30 lb; 3 in the control group, 1 in the 3-mg/kg group, and 1 in the 25-mg/kg group). One horse in the 5-mg/kg group lost > 13.6 kg. A significant effect of enrofloxacin on weight gain or loss was not detected.

Respiratory rate was increased in 6 horses on either 1 (6 horses) or 2 (2 horses) days of the 21-day period of injections. The number of days of increased respiratory rate was not significantly different among groups or replicates during the acclimation period, compared with the injection period. Respiratory rate appeared to be affected by activity and noise in the barns (eg, feeding, stall cleaning), anticipation of physical and lameness examinations, and general handling rather than drug effect.

Nine horses (some from each group) had mild swelling in 1 or more joints after acclimation and before administration of enrofloxacin or saline solution. In some horses, the swelling was in the operated joints, but in 1 horse, swelling was detected in the metacarpophalangeal and metatarsophalangeal joints. Nine horses had mild swelling in 1 or more joints after enrofloxacin or saline solution administration; 6 horses that did not have swelling before administration. On day 21, significant differences were not detected among groups for joint swelling, and joint swelling was not significantly different from that detected before administration of enrofloxacin or saline solution.

One horse in the 25-mg/kg group and 1 horse in the 15-mg/kg group developed musculoskeletal abnormalities that were not associated with lameness 48 to 72 hours after enrofloxacin administration had been discontinued (days 23 to 24). One horse (25-mg/kg group) developed effusion of the tarsal sheath of the left hind limb. One horse (15-mg/kg group) developed a local swelling (tendinitis) of the distal portion of the superficial digital flexor tendon of the left forelimb.

Hematologic and serum biochemical analyses—Hematologic and serum biochemical values were considered acceptable (values within or slightly lower than reference ranges) for all horses at initial evaluation and 3 days before administration of enrofloxacin, except for 1 horse in the 25-mg/kg group that had mildly
increased activity of \( \gamma \)-glutamyl transaminase (GGT) at initial evaluation (72 U/L; reference limit, < 40 U/L); for confirmation, the test was repeated, and similar results were obtained (GGT, 66 U/L). Ultrasonography of the lower and determination of serum concentrations of bile acids and direct and indirect bilirubin were performed during the initial evaluation period (days -21 to -10); results were within reference ranges. The GGT values for this horse were lower after the acclimation period (47 U/L) and within reference range after enrofloxacin administration (33 U/L). Hematologic and serum biochemical values were acceptable after 21 days of enrofloxacin administration, except for 1 horse in the 15-mg/kg group that had increased serum activity of aspartate aminotransferase (776 U/L) and GGT (116 U/L). During the evaluation period after enrofloxacin administration (days 21 to 28), a liver biopsy specimen was obtained from this horse, and vacuolization of hepatocytes was detected histologically. No liver inflammation or necrosis was evident, and the biliary system had normal features.

Lameness examinations—All horses were sound after the acclimation period. Significant differences among groups were not detected for number of lameness days. One horse (15-mg/kg group) was lame from days 14 to 21 of the study. Four additional horses (1 from the 5-mg/kg group; 2 from the 15-mg/kg group; 1 from the control group) had intermittent days of mild (grade-1) lameness. These horses were sound at the end of the study. Horses with intermittent lameness were only mildly lame, and the lameness resolved while receiving enrofloxacin. There was no indication that these horses showed higher dosages of enrofloxacin had more lameness, because no horses in the 25-mg/kg group were lame. Fourteen of the 18 horses that received enrofloxacin were sound for the entire 21-day period of administration. In 80% of the horses with any lameness, lameness was mild, of short duration (few days), and resolved during the administration period.

Each horse had a lameness examination performed each day lameness was detected. Intermittently lame horses had negative results to flexion tests and hoof testers. Ancillary diagnostic tests were not performed (such as nerve blocks), because horses could not receive any other drugs during the study, and horses were not lame at the end of the study when ancillary diagnostic tests could be performed. The horses that developed tarsal sheath effusion and superficial digital tendinitis after the administration of enrofloxacin was discontinued were not lame at the walk or trot.

Ancillary diagnostic tests and postmortem examination—Ancillary diagnostic tests were performed on the only horse that was lame the day after the last day of enrofloxacin administration. This horse had grade-1 lameness and had positive responses to flexion tests of the lower and upper portions of the limbs and negative results with hoof testers. Swelling of the plantar aspect of the calcaneus and lower portion of the tarsal region was visible; this region felt warm, and palpation (pressure) elicited signs of pain. A proximal metatarsal nerve block to anesthetize structures distal to the tarsus did not change the lameness. A diagnosis of cellulitis and local inflammation was made, and desmitis of the long plantar tarsal ligament (cub) was suspected. Ultrasonographic examination of the tendons and long plantar tarsal ligament was performed and revealed fluid surrounding the tendon and ligament. The fiber patterns of the tendons and ligament were considered normal. At necropsy examination of this horse, all organs and joints appeared grossly normal, including the long plantar tarsal ligament of the left hind limb. Some edema of the subcutaneous tissues of the calcaneal area was noticed. On histologic evaluation, the fibers of the long plantar tarsal ligament had normal appearance, and all joints had normal findings for each of the 4 evaluated categories. Sites where specimens were obtained for histologic examination represented weight-bearing areas of the following joints: shoulder, elbow, carpal, metacarpal, and metatarsophalangeal, proximal interphalangeal, distal interphalangeal, tarsometatarsal, and distal intertarsal joints. In the tarsocural joints, histologic specimens were obtained from non-weight-bearing surfaces. The pathologic diagnosis was cellulitis and edema of the tendinous and ligamentous structures rather than desmitis of the long plantar tarsal ligament. Traumatic injury to this area was suspected. Other adverse effects of drug administration were not identified by necropsy or histologic examination.

Goniometric evaluation—No significant differences in fetlock joint angles were detected after administration of enrofloxacin or saline solution, compared with values before administration, in any group for any limb. Therefore, enrofloxacin administration had no effect on fetlock joint angle, an estimate of flexor tendon laxity.

Radiographic findings—Lesions were detected before and after administration of enrofloxacin or saline solution in 14 of the 24 horses and were almost always in the distal tarsal joints. Importantly, no horses had any clinical signs associated with the lesions. Two horses had osteochondral fragments associated with osteochondrosis at the distal portion of the intermediate ridge of the tibia of both tarsi; these findings were considered abnormal but acceptable and did not change during the study. Administration of enrofloxacin did not significantly affect the number or size of osteophytes or lucent areas among groups.

Ultrasonographic findings—Administration of enrofloxacin did not significantly increase cross-sectional size of the forelimb flexor tendons or the hind limb calcaneal tendons in 16 measurements in each horse (proximal, middle, and distal measurements of the superficial digital flexor tendons and deep digital flexor tendons of both forelimbs plus measurements of the superficial digital flexor and gastrocnemius tendons of both hind limbs). However, there was an association between deep digital flexor tendon cross-sectional area, treatment, time, and initial body weight. Deep digital flexor cross-sectional area was greater after treatment in heavier horses that were administered larger dosages of enrofloxacin (P = 0.045).
All horses had identifiable articular cartilage lesions was not evident, and a change in tendon fiber density within the tarsal sheath of the horse that developed subclinical or no effects, because swelling or lameness was not evident, and a change in tendon fiber density or linear pattern was not detected. Administration of drugs did not induce any substantial clinical evidence of tendinitis or tendonopathy.

Ultrasonography of the deep digital flexor tendon within the tarsal sheath of the horse that developed tarsal sheath effusion did not reveal any abnormalities of the deep flexor tendon. Excessive fluid was evident in the sheath but did not contain debris. Ultrasonography of the flexor tendons of the horse that developed focal swelling of the distal portion of the superficial digital flexor tendon of the left forelimb revealed a focal central hypoechoic area in that tendon, which confirmed the tentative diagnosis of superficial digital flexor tendinitis.

Catheter scores—Significant differences were not detected among groups for the number of days during the study in which catheter scores for signs of pain, heat, or swelling were 0. All horses had some lateral swelling (score, 1; not painful and <1 cm wide) at the catheter site for at least 2 of the 21 days of the study, except for 1 horse that did not develop swelling at any time. Mean number of days with any catheter score >0 was 8 (38%). Therefore, some catheter-site reaction was associated with long-term maintenance of catheters within veins and was not related to treatment. Enrofloxacin was not irritating to veins or destructive to catheters. Administration of enrofloxacin, an antimicrobial, did not decrease the likelihood of catheter swelling, as may be expected if local infection was a cause of swelling.

Arthroscopic findings—Arthroscopy scores were not different among groups for each joint or in total. All horses had identifiable articular cartilage lesions before acclimation despite being sound and without joint effusion or radiographic changes. In almost all instances, these lesions were small, focal, considered minor, and judged to be clinically unimportant for horses not engaged in athletic work.

Histologic findings—Histologic scores were not significantly different among groups for any of the 4 histologic categories (cells, structure, staining intensity, and tidemark integrity) Of 192 histologic evaluations (24 horses × 2 tarsocrural joints/horse × 4 histologic categories), only 4 abnormalities were detected; 1 control horse and 2 horses that received 25 mg of enrofloxacin/kg had mild reduction in proteoglycan matrix staining in the intermediate zone (score, 1), and 1 horse that received 15 mg of enrofloxacin/kg had hypocellularity (score, 3; thin cartilage rather than cell death) with healthy appearing cells and without chondrocyte clustering. In all instances, the biopsy specimens from the opposite tarsocrural joint of these horses was histologically normal.

Discussion

Injectable enrofloxacin administered to adult horses at 5 mg/kg IV once daily for 3 weeks did not induce detectable adverse reactions, physical abnormalities, or joint or tendon disease. At higher dosages, adverse reactions that consisted of muzzle twitching, excitability, and, ultimately, seizure-like behavior have been noticed, and, therefore, slow injection via an indwelling catheter is recommended. Judged on the basis of published pharmacokinetics of enrofloxacin in horses, higher dosages should not be necessary for effective antimicrobial action. In other animal species and humans administered 5 mg/kg twice daily, reported adverse reactions to fluoroquinolones are rare and consist mainly of gastrointestinal tract disturbances. High concentrations (3 to 100 times those expected from parenterally administered dosages) of fluoroquinolones (ciprofloxacin, difloxacin, ofloxacin, norfloxacin) cause gastrointestinal, neurologic, and musculoskeletal adverse effects. Specifically, adverse neurologic reactions in humans consist of headache, malaise, agitation, sleep disorders, and, in rare instances, convulsions. Convulsions have been correlated with quinolone binding at the \( \text{gamma amino butyric acid (GABA}_A \) receptors in the brain, which blocks \( \text{GABA}_A \) (an inhibitory neurotransmitter) binding, thereby resulting in CNS stimulation. In a different study of seizure-prone rats, administration of enrofloxacin decreased seizure activity and, therefore, would not seem likely to enhance seizures. Peracute reactions were allergic or anaphylactic (5 horses had mild to moderate cutaneous reactions that consisted of muzzle twitching, excitability, and, ultimately, seizure-like activity seen in the horses of the study reported here. The most likely explanation for the adverse reaction seen in our study is rapid transfer of enrofloxacin across the blood-brain barrier, inciting neurologic stimulation. The mechanism or direct cause of the neurologic stimulation is speculative but could include direct drug effects on \( \text{GABA}_A \) receptors in the CNS or secondary release of mediators stimulated by drug or carrier.

Liver enzyme abnormalities have been detected in 2 to 3% of patients receiving fluoroquinolone treatment. Of these, a small number developed hepatic necrosis, hepatitis, or liver failure. One horse in our study developed increased serum activity of liver enzymes while receiving enrofloxacin (15 mg/kg) Fluoroquinolones, including enrofloxacin, inhibit hepatic cytochrome P-450 1A1 and P-450 I A2 activity. In rats, enrofloxacin induces a slight stimulation of the P-450 IIB subfamily of hepatic cytochromes, but, overall, P-450 activity is reduced. In the horse in our study, vacuolization of hepatocytes may have represented endoplasmic reticulum and, possibly, induction.
of liver enzymes. The relationship of this change to enrofloxacin is unknown, particularly because enrofloxacin is reported to reduce, not induce, specific liver enzymes.

Numerous ultrasonographic tendon measurements and fetlock joint angle measurements were made in the study reported here to assess functional alterations in tendon strength or the development of tendinitis. Tendinitis and tendon rupture are associated with fluoroquinolone administration in humans. In a retrospective cohort study that investigated 97 cases of tendinitis or tendon rupture in 1,841 human users of fluoroquinolones, compared with 9,406 users of other antimicrobials, the relative risk of gastrointestinal tendinitis was 3.7; risk for tendinitis involving other tendons was 1.3. For ofloxacin, the relative risk of tendinitis was highest at 10.1. Typical tendon lesions in humans have prominent intratendinous changes, although peritendinous changes are also seen. Histologically, the lesions represent degenerative changes, including cyst formation and local necrosis. Results of in vitro studies of cultured rabbit gastrocnemius tendon indicate cytotoxicity that affects mitochondria. The suspected mechanism of toxic effects on articular cartilage is also cytotoxicity. In our study, 3 horses spontaneously developed abnormalities of tendons, ligaments, or tissues adjacent to ligaments while confined to a box stall. Although a direct association with enrofloxacin was not proven with these data, development of these lesions caused that suspicion. It would be prudent to recommend a slow return to exercise in horses that have been treated with long-term administration of enrofloxacin, particularly if known risk factors are present such as corticosteroid administration, renal failure, and advanced age.

Lameness was not associated with IV administration of as much as 25 mg of enrofloxacin/kg (> 3 times the recommended dosage for cattle of 2.5 to 3.0 mg/kg for multiple days) once daily. A sum index of lameness days for the duration of the study was selected as a stringent method to assess the lameness data rather than a mean lameness score, comparison among groups, or comparison among lameness scores determined before and during drug administration. Our findings that horses with lameness were only mildly lame (median grade, 1), that most lameness resolved while horses received enrofloxacin, that horses that received a greater dose of enrofloxacin did not have more lameness, and that no horses that received 25 mg of enrofloxacin/kg had any lame days all support our conclusion that enrofloxacin did not cause lameness.

Articular cartilage damage was not identified in association with administration of enrofloxacin at any dosage used in the study reported here. Parenteral administration of fluoroquinolone at > 25 mg/kg induces cartilage lesions in juvenile rats, dogs, guinea pigs, rabbits, and nonhuman primates. Results of in vitro studies of nalidixic acid, ciprofloxacin, and norfloxacin indicate suppression of chondrocyte metabolism at concentrations > 10 times those expected in synovial fluid. In our study, we observed articular cartilage lesions before administration of enrofloxacin in most horses despite soundness, full range of joint motion, normal radiographic findings, and lack of joint effusion. Failure to quantify articular cartilage condition prior to drug administration may have contributed to inappropriate conclusions that articular cartilage lesions were associated with enrofloxacin administration or inability of studies to prove or not prove association with enrofloxacin administration. Although expensive and time consuming, arthroscopic inspection of the articular cartilage before administration of enrofloxacin allowed close evaluation of the gross condition of the articular cartilage. Judged on the basis of results of in vitro studies of equine articular cartilage explants, enrofloxacin at a dosage of approximately 5 mg/kg would not be expected to induce suppression of chondrocyte metabolism, toxic effects on chondrocytes, or depletion of proteoglycan. In vitro, higher concentrations of enrofloxacin suppress equine chondrocyte proteoglycan synthesis, and histologic evaluation reveals chondrocyte necrosis characterized by pyknotic nuclei and empty lacunae. These classical histologic changes were not identified in our histologic evaluation of articular cartilage. Proteoglycan degradation is revealed by increased release of newly synthesized proteoglycan without detection of endogenous proteoglycan loss, a finding similar to results of studies of ciprofloxacin and norfloxacin in other species. Endogenous proteoglycan degradation has only been demonstrated in articular cartilage explants harvested from juvenile dogs treated parenterally with high doses of norfloxacin (100 mg/kg [45 mg/lb] for 7 days). We did not expect to find articular cartilage damage in our adult horses, because much higher dosages than used in our study induced lesions in toxicity studies, and in vitro equine articular cartilage studies did not reveal abnormalities at lower dosages.

In vivo studies have also revealed adverse effects of fluoroquinolones (nalidixic acid, ciprofloxacin, difloxacin, norfloxacin, pefloxacin, and levofloxacin) administered for > 2 weeks on articular cartilage, particularly at high concentrations in foals, juvenile rats, and dogs. Lesions develop primarily on weight-bearing surfaces and are sometimes accompanied by lameness. Typical gross lesions associated with quinolone arthropathy in vivo are degeneration and necrosis of chondrocytes, decreased extracellular matrix, as evidenced by decreased staining intensity for proteoglycan, and clumping of unmasked collagen fibrils. For the only horse that was euthanized in our study, choice of sites for histologic examination was made on the basis of predilection sites for fluoroquinolone-induced arthropathy. Our scoring system for the articular cartilage biopsy specimens obtained from all horses specifically evaluated chondrocyte death (hypocellularity) and fissure formation in the intermediate zone (structure, specifically clefts, in any zone). None of these effects were identified at any drug dosage evaluated in our study.

Enrofloxacin chondrotoxicity is dose-dependent for equine articular cartilage explants incubated with ≥ 1,000 μg of enrofloxacin/ml, and the chondrotoxicity does not correlate with age of the horse. Foals, however, may be more susceptible to developing clinical
lesions after fluoroquinolone administration. Not only has articular cartilage damage been identified in juveniles of other species, but any abnormality in chondrocyte function is more likely to cause detectable articular cartilage lesions in rapidly metabolic and synthesizing cells. Articular cartilage explants from neonatal horses had > 100-fold the rate of proteoglycan synthesis than explants from adult horses that were approximately 8 years old. The results of our study of adult horses should not be extrapolated to assume clinical safety in foals. The use of enrofloxacin in horses younger than those of our study (3 years) is not recommended.

The soft-tissue abnormalities that developed in 3 horses (edema around the long plantar ligament, tarsal sheath effusion, and superficial digital flexor tenosynovitis) were not statistically correlated with enrofloxacin administration but are unusual in horses maintained in stalls. Also, the cross-sectional area of the deep digital flexor tendon increased with enrofloxacin dosage in heavier horses, although this did not correlate with any clinical or ultrasonographic abnormalities. Histologic evaluation of the collagen in the articular cartilage in all horses in the study and all appendicular joints and the long plantar ligament in the only horse that was necropsied did not reveal any evidence of collagen abnormality such as fiber disruption, necrosis, or loss of cells. The importance of these findings is not known.

In clinical practice, 3 weeks is a considerable period for continuous antimicrobial administration. In the study reported here, we evaluated horses for 4 weeks after initiation of enrofloxacin administration, but, nevertheless, it cannot be completely ruled out that delayed musculoskeletal abnormalities may develop at a later time. Exercise-induced damage of weight-bearing articular cartilage that has been injured by enrofloxacin administration may theoretically take time to develop or cause clinical signs. Arthroscopy allows close inspection of articular cartilage with magnification but has limitations regarding magnification and ability to see all articulating surfaces. Our biopsy specimens did not have substantial histologic abnormalities, including chondrocyte death, but were obtained from non-weight-bearing areas. It is possible that weight-bearing areas could develop lesions early in the course of enrofloxacin administration. In the 1 horse that was necropsied, 28 joint specimens from weight-bearing surfaces were examined; histologic lesions were not identified. Long-term complications in these horses would not be expected, because lesions in previous studies developed during the period when fluoroquinolones were being administered.

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