

# Ocular and systemic manifestations after oral administration of a high dose of enrofloxacin in cats

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**Objective**—To characterize the effects of oral administration of a high dose of enrofloxacin to cats.

**Animals**—24 (12 male and 12 female) young healthy cats.

**Procedures**—Cats were allocated on the basis of sex into 2 groups (4 males and 4 females/group) from which 3 subgroups for 3 durations (3, 5, or 7 days) of enrofloxacin (50 mg/kg, PO, q 24 h) or control solution (1 mL of water, PO, q 24 h) administration that began on day -1 were created. Funduscopic examinations were performed daily. Electroretinography (ERG) was performed before and every 2 to 3 days after the start of oral administration. Four cats/study group were euthanized on days 3, 5, and 7, and eyes were collected for light and electron microscopic evaluations.

**Results**—Neurologic, funduscopic, and ERG abnormalities were evident only in cats administered enrofloxacin. Funduscopic changes (granular appearance or graying of the area centralis) were noticed on or before day 3 (after only 3 days of enrofloxacin administration), with subsequent similar changes along the visual streak. Vascular attenuation (between days 2 and 4) and generalized tapetal hyperreflectivity (between days 5 and 7) followed. Reduction in b-wave ERG amplitude preceded funduscopic changes. Morphologic changes in the photoreceptor layers correlated with duration of enrofloxacin administration, with generalized degenerative changes evident after 3 doses.

**Conclusions and Clinical Relevance**—The study indicated that a high dose of enrofloxacin (50 mg/kg/d, PO) induced retinal and systemic changes. Enrofloxacin at 10 times the recommended dosage is acutely toxic to the outer retina of clinically normal cats. (*Am J Vet Res* 2007;68:190–202).

Fluoroquinolones are bactericidal antimicrobials widely used in veterinary medicine. Nalidixic acid, the first antibacterial quinolone developed for clinical use and the precursor to other fluoroquinolones, was discovered in 1962 and approved for clinical use in 1967.<sup>1,2</sup> Quinolone carboxylic acid derivatives have a broad spectrum of activity against gram-positive and gram-negative bacteria as well as *Mycoplasma* organ-

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## ABBREVIATIONS

ERG	Electroretinography
STT	Schirmer tear test
IOP	Intraocular pressure
cd	Candela
RPE	Retinal pigmented epithelium
P-OS	Photoreceptor outer segment
P-IS	Photoreceptor inner segment
ONL	Outer nuclear layer
OPL	Outer plexiform layer

isms.<sup>2</sup> Quinolones inhibit bacterial DNA gyrase and deactivate bacterial enzymes necessary for the transcription of DNA. This results in uncoiling of DNA and interference of bacterial DNA synthesis, repair, recombination, and transposition.<sup>3–11</sup> The DNA segments become unreadable, and the bacterial cell dies.

Fluoroquinolones are excreted by renal and hepatic routes. Toxic effects of fluoroquinolones in mammalian cells are low compared with toxic effects in bacterial cells because the enzyme analogue to bacterial DNA gyrase in eukaryotes is 100 to 1,000 times less susceptible to gyrase inhibitors.<sup>12</sup>

Enrofloxacin was approved for use in cats in the United States in 1990 at a dosage of 2.5 mg/kg, PO.<sup>a,b</sup> Enrofloxacin is also approved in the United States for use in dogs and cattle. Prior to FDA approval, enro-

floxacin underwent mandatory toxicologic testing in dogs and cats. Preapproval safety studies conducted in 1989 in dogs and cats revealed the product was safe when administered at a dosage of 25 mg/kg/d for 30 days.<sup>a</sup> In 1992, the general safety of enrofloxacin in young cats was determined at dosages of 5, 15, and 25 mg/kg; there was no evidence of adverse effects.<sup>13</sup> Subsequently, in 1997, a flexible dosing regimen of 5 to 20 mg/kg, PO, once daily or as a divided dose was recommended.<sup>14,a</sup> Once-daily dosing of enrofloxacin was widely accepted after pharmacologic testing revealed that such use achieved higher peak plasma drug concentrations, increased bactericidal efficacy, decreased risk of bacterial resistance, and provided increased ease of administration.<sup>14</sup> After introduction of this flexible dosing regimen, veterinarians reported an increased incidence of vision-related problems that resulted after once-daily oral administration of enrofloxacin. These problems included blindness, partial blindness, and mydriasis.<sup>14-19,a</sup> In 2 reports<sup>15,16</sup> published between 1999 and 2001, investigators suggested a possible link between enrofloxacin administration and vision problems in cats.

In response to the increased incidence of vision-related problems, the manufacturer enlisted input and expertise from members of the American College of Veterinary Ophthalmologists to determine the best means to objectively investigate this matter. A specific safety study was performed to assess potential effects of enrofloxacin on ocular variables in cats. In June 2000, a postapproval study<sup>c</sup> was conducted in which doses of 5, 20, and 50 mg/kg, PO, were administered once daily. In that study, investigators observed dose-related ocular effects attributable to enrofloxacin, with the most severe ocular effects in cats administered 50 mg/kg/d (ie, 2.5 times the high end of the approved dosage range) and with fundic signs that developed within 1 week after onset of administration. No changes were observed in cats administered 5 mg/kg/d (ie, the low end of the approved dosage range). In 2001, the drug label was revised to reflect an FDA-approved dosage for oral administration of 5 mg/kg/d in cats.<sup>19</sup>

A link between enrofloxacin administration and visual deficits in cats has been established, but little is known concerning the mechanisms by which enrofloxacin causes visual compromise. The proven usefulness of fluoroquinolones in the management of cats with a wide array of diseases ensures their continued, common use in veterinary medicine. The objective of the study reported here was to determine the clinical, funduscopic, electroretinographic, and histologic effects of oral administration of a high dose of enrofloxacin on the retinas of clinically normal cats.

## Materials and Methods

**Animals**—Twenty-four healthy, purpose-bred, sexually intact, mixed-breed cats were used in the study. Cats were purchased from a commercial source.<sup>d</sup> Cats ranged in age (females, 10 to 12 months old; males, 24 to 28 months old). The study was performed in accordance with Good Laboratory Practice standards and in compliance with guidelines of the University of Missouri–Columbia Animal Care and Use Committee.

**Study design**—Cats were randomly assigned to 2 treatment groups (control and treatment; 12 cats/group; 6 males and 6 females). Within each treatment group, cats were then further randomly allocated to 3 subgroups (ie, C1, C2, and C3 for the control subgroups and T1, T2, and T3 for the treatment subgroups). Each subgroup comprised 4 cats (2 males and 2 females). Cats were acclimated for a period of 12 days before the start of the study. Baseline data were obtained on day –3. Each cat was administered enrofloxacin<sup>b</sup> (50 mg/kg, PO) or a control substance (1 mL of water, PO) between 5 PM and 6 PM each evening beginning on day –1 and continuing until the end of the study (ie, on days –1, 1, 2, 3, 4, 5, 6, and 7 until cats were euthanized; there was no day 0 designation). All cats were anesthetized for ERG performed on day –3; anesthesia and ERG were repeated on days 1 and 3 (subgroups C1 and T1), 3 and 5 (subgroups C2 and T2); and 1, 5, and 7 (subgroups C3 and T3). Subgroups of cats were euthanized (day 3 for subgroups C1 and T1, day 5 for subgroups C2 and T2, and day 7 for subgroups C3 and T3), and tissues were collected for evaluations.

**Clinical monitoring and collection of data**—During the acclimation and study periods, cats were monitored twice daily for evidence of behavioral, musculoskeletal, respiratory, and skin changes. Evidence of abnormal feces, vomitus, or regurgitated material was also recorded. Food consumption was recorded every morning as a percentage of the food consumed of the total amount of food provided the preceding morning. Cats were weighed on day –3 and the day of euthanasia.

Rectal temperature, heart rate, and respiratory rate were recorded on day –3 and daily between day 1 and the day on which cats were euthanized. Systolic blood pressure<sup>e</sup> was measured each time that a cat was anesthetized for ERG. Blood samples for hematologic and biochemical evaluations were collected from each cat once between days –17 and –14 and again on the day on which each cat was euthanized.

Complete ophthalmic examinations were performed once daily (in the morning) on day –3 and repeated daily between day 1 and the day on which cats were euthanized. Ophthalmic examinations were performed by a board-certified veterinary ophthalmologist (KLN) and included evaluation of pupillary light reflexes,<sup>f</sup> evaluation of a menace response, results of an STT,<sup>g</sup> measurement of IOP,<sup>h</sup> and evaluation after fluorescein staining<sup>i</sup> of the cornea. In addition, complete examinations of the anterior and posterior segments were performed by use of slit-lamp biomicroscopy<sup>j</sup> and indirect funduscopy,<sup>k,l</sup> respectively. Retinal changes were photographed with a digital camera.<sup>m</sup>

**ERG evaluations**—The ERG examinations were conducted after cats had a minimum of 1.5 hours of adaptation in the dark. Cats were then sedated by administration of medetomidine<sup>n</sup> (0.09 mg/kg, IM). Approximately 15 to 20 minutes later, cats were anesthetized by administration of ketamine hydrochloride<sup>o</sup> (5 mg/kg, IM).

Variables recorded included an intensity series (conducted by use of white light in scotopic and photopic conditions) and photopic stimulation (conducted by use

of 30- and 50-Hz flickering lights). A jet corneal contact lens<sup>p</sup> served as the active electrode, and subdermal needle electrodes<sup>q</sup> inserted approximately 10 mm temporal to the lateral canthus of the eye and at the ipsilateral occipital crest served as reference and ground electrodes, respectively. A computerized ERG<sup>r</sup> with Ganzfeld full-field white-light stimulation was used to obtain bilateral dark-adapted scotopic and photopic ERG results. Scotopic ERG responses were recorded by use of stimulation with white light between  $-6$  and  $0.6 \log ([cd \times s]/m^2)$  in increments of  $0.3$  to  $0.5 \log$  units, by removing neutral density filters<sup>s</sup> from the light path. After 10 minutes of light adaptation at  $30 \text{ cd}/m^2$ , photopic stimulation was elicited at  $5.1 \text{ Hz}$ , followed by flicker recordings at  $30$  and  $50 \text{ Hz}$ . All photopic ERG results were recorded at a light stimulus intensity of  $0.0 \log ([cd \times s]/m^2)$ , which is equivalent to  $1 \text{ (cd} \times \text{s)}/m^2$ .

Immediately after the final ERG was recorded, cats were euthanized by IV injection of euthanasia solution.<sup>t</sup> Retinal tissue was harvested immediately after euthanasia from the right eye and fixed in  $2.5\%$  glutaraldehyde in  $0.1M$  cacodylate buffer (pH,  $7.2$ ) for use in light microscopy and transmission electron microscopy. Tissues from the superior and inferior aspects of the peripheral and midperipheral portions of the retina and area centralis were dissected into sections ( $2 \times 3 \text{ mm}$ ) and embedded in plastic.<sup>u</sup> Tissues were labeled with the identification number of the cat and location from which the tissue section was obtained; thus, investigators were not aware of the treatment group at the time light microscopy and transmission electron microscopy examinations were performed.

Structures examined by use of light microscopy were the tapetum; RPE; P-OS; P-IS; ONL; OPL; and inner nuclear, inner plexiform, and ganglion cell layers. Scores were assigned by use of a scale of  $0$  to  $5$  ( $0$ , no abnormalities [normal];  $1$ , minimal abnormalities;  $2$ , mild or slight abnormalities;  $3$ , moderate abnormalities;  $4$ , marked abnormalities; and  $5$ , severe retinal degeneration). In addition, each section evaluated by use of light microscopy was assigned a subjective evaluation that included the quality of the section and the specific abnormalities identified.

**Statistical analysis**—Data for rectal temperature, respiratory rate, and heart rate were analyzed to detect differences between control and enrofloxacin-treated cats. Data were also analyzed to compare differences in values obtained before the start of the study (day  $-3$ ) and on the day of euthanasia between subgroups within treatment groups.

Separate analyses were performed for amplitude, implicit times, and a- and b-waves as well as for the right and left eyes. Baseline data for each light intensity for all 24 cats were used to establish reference ranges (defined as the values between the 5th and 95th percentiles) for each of 15 neutral density filters.<sup>v</sup> For the purposes of the study, the outcome for any cat was defined to be within acceptable limits for a clinically normal cat when the observed value on a specific day was within reference ranges for at least 13 of 15 filters (ie, acceptable number of outliers for each test date was set at 2). Number of outliers between control and treated cats for

a- and b-wave amplitudes and b-wave implicit times were compared among days and between eyes.<sup>20</sup>

The variable used for analysis was the number of days that a cat was observed to be within acceptable limits. For cats evaluated on days 1, 5, and 7, the possible values for the variable (ie, the number of days within acceptable limits) was 0, 1, 2, or 3, whereas for cats evaluated on days 1 and 3 or on days 3 and 5, the possible values for the variable were 0, 1, or 2. The number of days within acceptable limits was examined to detect differences between control and enrofloxacin-treated cats. Because the subgroups of cats differed for the various evaluation days, we used a stratified analysis in which the number of days evaluated was the stratum. A Cochran-Mantel-Haenszel analysis with modified ridit scores was used to test for differences in the treatment and control groups by a method that resulted in a stratified extension of the Wilcoxon rank sum test.<sup>21</sup> One cat (in subgroup T3) did not have a value for day 7. Because this cat conceivably could have had results within acceptable limits, 2 analyses were performed, with the first analysis for this cat assuming that the value for day 7 was within acceptable limits, and the second analysis assuming that the value for day 7 was not within acceptable limits.

For the enrofloxacin-treated cats (subgroups T1, T2, and T3), evidence of decreasing ERG responses with increasing duration of drug administration was examined by use of the Cochran-Mantel-Haenszel option in a commercially available statistical program.<sup>v</sup> Unless otherwise indicated, data were analyzed to detect differences between baseline values (day  $-3$ ) and values obtained during enrofloxacin administration (day 1 to day cats were euthanized) between subgroups within treatment groups by use of paired *t* tests. Comparison between control and enrofloxacin-treated cats were made by use of Student *t* tests.

Results of histologic grading during light microscopic examination were analyzed to detect differences between control and enrofloxacin-treated cats during the study (day of euthanasia) between subgroups within treatment groups by use of paired *t* or Cochran-Mantel-Haenszel tests to determine whether histologic changes were evident with increasing duration of drug administration. A Cochran-Mantel-Haenszel analysis with modified ridit scores was used to determine differences between the treatment and control cats. These differences were tested by a method that resulted in a stratified extension of the Wilcoxon rank sum test.<sup>21</sup> Because a large number of statistical tests were performed for the analysis of histologic changes, a conservative value of  $P \leq 0.01$  was used to define significance for those variables. For all other variables, values of  $P \leq 0.05$  were considered significant.

## Results

**Animals**—Mean age of all cats was 17.25 months (range, 10.9 to 28.9 months). Male cats were significantly ( $P < 0.001$ ) older (mean  $\pm$  SD,  $23.13 \pm 4.24$  months) than female cats ( $11.26 \pm 0.36$  months). There was no significant difference in age within the same sex between control and enrofloxacin-treated cats. Female cats weighed significantly ( $P = 0.004$ ) less than male cats on day  $-3$  and the day of euthanasia for control and

treated cats. Between day -3 and the day of euthanasia for the control cats, female cats lost a significant ( $P = 0.022$ ) amount of weight, whereas no changes were detected for male cats. Change in body weight between day -3 and day of euthanasia did not differ significantly between male and female control cats but was significantly ( $P = 0.035$ ) greater for enrofloxacin-treated females than for enrofloxacin-treated males. In contrast, enrofloxacin-treated cats of both sexes lost a significant ( $P = 0.004$ ) amount of weight during the study. Weight loss of control females was significantly ( $P = 0.013$ ) less than weight loss of treatment females.

**Food consumption and abnormalities of the gastrointestinal tract**—Mean  $\pm$  SD food consumption of treatment cats decreased significantly ( $P = 0.020$ ) between days -3 and -1 ( $80.1 \pm 5.4\%$ ) and over the course of the study period (day 1 to euthanasia;  $31.5 \pm 11.4\%$ ); however, mean food consumption for control cats was significantly ( $P = 0.040$ ) higher between day 1 and day of euthanasia ( $73.8 \pm 3.67\%$ ), compared with mean food consumption during the period of days -3 to -1 ( $63.6 \pm 7.86\%$ ). Mean food consumption of control cats was significantly ( $P = 0.009$ ) higher than that for treatment cats between day 1 and day of euthanasia.

Vomiting was detected for 10 cats. There was variation in the amount of food and hair contained in the vomitus. No association with onset of vomiting and time of enrofloxacin administration was evident. Three control cats vomited hairballs at least once, and 1 control cat vomited partially digested food once during the acclimation period. Clear fluid or partially digested food was vomited once or twice by 6 cats receiving enrofloxacin between day -3 and day of euthanasia. Soft fecal material was evident for 6 control and 4 treatment cats (between 1 and 3 times/cat) during the acclimation period and days -1 to 2.

**Behavioral, musculoskeletal, and neurologic assessment**—Eight of 12 cats receiving enrofloxacin (1

from subgroup T1, 3 from subgroup T2, and 4 from subgroup T3) had behavioral, musculoskeletal, or neurologic abnormalities between day 2 and day of euthanasia. The T1 cat and 3 T2 cats had changes in behavior (lethargy, marked caution, fractiousness, unkempt coat, and marked disorganization of materials in the cage), whereas the 4 cats from subgroup T3 had musculoskeletal and neurologic abnormalities that included incoordination, stiff gait, tremors, convulsions, blindness, ataxia, circling, seizures, ptialism, and nystagmus. Neurologic abnormalities were deemed to be sufficiently serious in 1 female cat from subgroup T3 to warrant euthanasia on day 6 (ie, 1 day before the originally scheduled date of euthanasia). Two control cats had behavioral changes (1 male was fractious for 1 day and 1 female was excessively quiet for 2 days); these short-term behavior changes were not considered to be important to the study. Two control cats and 1 treatment cat came into estrus during the study.

**Rectal temperature, heart rate, and respiration rate**—Mean rectal temperature was significantly ( $P = 0.012$ ) higher on days -3 to -1 (control,  $38.84^\circ\text{C}$ ; treatment,  $39.01^\circ\text{C}$ ) than during the study period (day 1 to day of euthanasia; control,  $38.59^\circ\text{C}$ ; treatment,  $38.67^\circ\text{C}$ ) for all control and treatment cats. For subgroup C3, mean rectal temperature ( $38.89^\circ\text{C}$ ) for days -3 to -1 was significantly ( $P < 0.001$ ) higher than the mean rectal temperature ( $38.48^\circ\text{C}$ ) for day 1 to the day of euthanasia (Table 1). For subgroup T3, mean body temperature ( $39.04^\circ\text{C}$ ) for days -3 to -1 was significantly ( $P = 0.039$ ) higher than the mean rectal temperature ( $38.48^\circ\text{C}$ ) for day 1 to the day of euthanasia. For subgroup T1, mean rectal temperature for days -3 to -1 ( $39.14^\circ\text{C}$ ) was significantly ( $P = 0.026$ ) higher than the mean rectal temperature for day 1 to the day of euthanasia ( $38.68^\circ\text{C}$ ).

Mean heart rate during the period from day 1 to day of euthanasia was significantly ( $P = 0.044$ ) lower for control cats ( $179.32 \pm 21.26$  beats/min), compared

Table 1—Mean  $\pm$  SD rectal temperature, heart rate, and respiratory rate for control and enrofloxacin-treated cats before (days -3 to -1) and during (day 1 to day of euthanasia) the study period.

Day of study*	Subgroup†	Rectal temperature (°C)	Heart rate (beats/min)	Respiratory rate (breaths/min)
-3 to -1	C1	38.85 $\pm$ 0.57	167.2 $\pm$ 17.04	32.8 $\pm$ 5.74
	C2	38.81 $\pm$ 0.44	202.7 $\pm$ 12.95	39.3 $\pm$ 5.16
	C3	38.89 $\pm$ 0.13	183.2 $\pm$ 25.86	48.0 $\pm$ 7.12‡
	T1	39.14 $\pm$ 0.45	172.0 $\pm$ 11.80	48.0 $\pm$ 3.27
	T2	38.96 $\pm$ 0.38	184.2 $\pm$ 20.34	39.2 $\pm$ 1.5
	T3	39.04 $\pm$ 0.34	185.5 $\pm$ 19.96	45.0 $\pm$ 5.03
1 to euthanasia	C1	38.67 $\pm$ 0.27	173.0 $\pm$ 15.79	39.4 $\pm$ 5.71
	C2	38.71 $\pm$ 0.62	177.7 $\pm$ 8.02§	44.4 $\pm$ 6.35
	C3	38.48 $\pm$ 0.29§	182.4 $\pm$ 6.51	48.8 $\pm$ 1.45
	T1	38.68 $\pm$ 0.32¶	195.3 $\pm$ 9.15	52.0 $\pm$ 10.16
	T2	38.93 $\pm$ 0.38	200.1 $\pm$ 47.64	43.2 $\pm$ 3.39
	T3	38.48 $\pm$ 0.32¶	189.8 $\pm$ 7.38	43.2 $\pm$ 0.70

\*The first day of the daily administration of enrofloxacin (50 mg/kg, PO; treatment cats) or control solution (1 mL of water, PO; control cats) was designated day -1, and the second day of administration was designated as day 1. †Within each treatment group, cats were randomly allocated to 3 subgroups (ie, C1, C2, and C3 for the control subgroups and T1, T2, and T3 for the treatment subgroups; 4 cats/subgroup [2 males and 2 females]); cats in C1 and T1 were euthanized on day 3, cats in C2 and T2 were euthanized on day 5, and cats in C3 and T3 were euthanized on day 7. ‡Value differs significantly ( $P < 0.05$ ) from values for subgroups C1 and C2 for days -3 to -1. §,¶Within a column, values differ significantly (§ $P < 0.001$ ; ¶ $P < 0.05$ ) from the value for the same subgroup on days -3 to -1. || Value differs significantly ( $P < 0.05$ ) from the value for subgroup C1 for day 1 to day of euthanasia.

Table 2—Mean  $\pm$  SD results of an STT and values for IOP determined on days -3, 1, 2, 3, 4, 5, 6, and 7 of the study period.

Day of study*	STT		IOP	
	Control (mm/min)	Treatment (mm/min)	Control (mm Hg)	Treatment (mm Hg)
-3	14.5 $\pm$ 4.0†	15.3 $\pm$ 5.0†	23.1 $\pm$ 3.6	25.4 $\pm$ 5.0
1	18.0 $\pm$ 7.7	19.8 $\pm$ 9.2	24.8 $\pm$ 7.8	25.6 $\pm$ 4.3
2	18.0 $\pm$ 8.0‡	15.2 $\pm$ 6.7	26.3 $\pm$ 2.5	26.4 $\pm$ 6.6
3	19.5 $\pm$ 7.6	20.7 $\pm$ 7.9	24.4 $\pm$ 3.8	23.2 $\pm$ 2.9
4	18.0 $\pm$ 8.3	16.2 $\pm$ 9.4	23.5 $\pm$ 3.5	21.6 $\pm$ 5.1
5	19.3 $\pm$ 8.4	15.2 $\pm$ 5.8	23.8 $\pm$ 3.4	20.5 $\pm$ 5.3
6	19.8 $\pm$ 8.7	18.8 $\pm$ 6.2	22.6 $\pm$ 3.5	17.4 $\pm$ 1.5
7	22.3 $\pm$ 8.6	16.7 $\pm$ 2.5	23.5 $\pm$ 4.9	20.2 $\pm$ 3.2

\*Values reported represent results for 24 cats on days -3, 1, 2, and 3; 16 cats on days 4 and 5; 8 cats on day 6; and 7 cats on day 7. †Values were significantly lower than mean values obtained on day 1, 2, or 3 for control cats ( $P = 0.005$ ) and day 3 for treatment cats ( $P = 0.014$ ). ‡Value was significantly ( $P < 0.006$ ) higher than the value of the treatment group on the same day.

with the mean heart rate for treatment cats (190.41  $\pm$  27.91 beats/min). For subgroup C2, mean heart rate for the period from days -3 to -1 (202.7 beats/min) was significantly ( $P = 0.049$ ) higher than the mean heart rate from day 1 to the day of euthanasia (177.7 beats/min; Table 1).

Mean respiratory rates for days -3 to -1 for subgroups C1 (32.8 breaths/min) and C2 (39.3 breaths/min) were significantly ( $P = 0.035$ ) lower than the mean respiratory rate for days -3 to -1 for subgroup C3 (48.0 breaths/min; Table 1). Mean respiratory rate for day 1 to day of euthanasia for subgroup C1 (39.4 breaths/min) was significantly ( $P = 0.043$ ) lower than the mean respiratory rate for subgroup C3 (48.8 breaths/min).

**Systolic blood pressure**—On day 3, mean  $\pm$  SD systolic blood pressure in control cats (134.5  $\pm$  20.0 mm Hg) was significantly ( $P = 0.005$ ) lower than that of treatment cats (169.2  $\pm$  7.6 mm Hg). For the treatment cats, mean systolic blood pressure was significantly ( $P = 0.043$ ) lower on day 1 (143.8  $\pm$  24.1 mm Hg) than on day 3 (169.2  $\pm$  21.6 mm Hg) but was significantly ( $P = 0.037$ ) higher on day 3 (169  $\pm$  21.6 mm Hg) than on day 5 (139.2  $\pm$  29.8 mm Hg).

**Hematologic and biochemical analyses**—High blood glucose concentrations were detected in samples obtained from all treatment and control cats on the day of euthanasia. Increased creatinine kinase activity on the day of euthanasia was identified in 6 cats (1 treatment and 5 control cats). Three cats had abnormal hematologic findings (eosinophilia or neutrophilia and lymphopenia, 2 treatment cats; eosinophilia only, 1 control cat).

**External ocular examinations**—Direct and consensual pupillary light reflexes were evident for all cats during the entire study period. Menace responses were questionable on day 5 for 4 cats in subgroup T2 and 3 cats in subgroup T3 and not detectable for cats in subgroup T3 on days 5 ( $n = 1$  cat), 6 (4), and 7 (3; 1 cat was euthanized on day 6). No retention of fluorescein stain was detected in any cat on any day of the study. Similarly, no abnormalities of the anterior segment were observed in any cat on any day of the study. Recorded ocular variables were not significantly different between the left and right eyes for any cat during the course of the study.

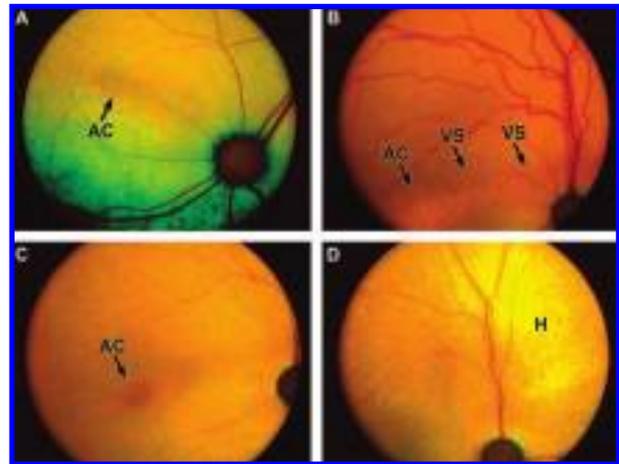


Figure 1—Funduscopy photographs of a representative control cat on day 1 with a normal-appearing fundus (A) and a representative enrofloxacin-treated cat on days 3 (B), 5 (C), and 7 (D). In the enrofloxacin-treated cat, notice the progressive change in granulation and graying of the area centralis (AC) and visual streak (VS), as seen on day 3; vascular attenuation observed on day 5; and marked tapetal hyperreflectivity (H) on day 7. Day of initial daily administration of enrofloxacin (50 mg/kg, PO) or control solution (1 mL of water, PO) was designated as day -1.

On day 2, mean STT value for the control cats (18.0 mm/min) was significantly ( $P = 0.006$ ) higher than that for the treatment cats (15.2 mm/min; Table 2). On day -3, mean STT values (control, 14.5 mm/min; treatment, 15.3 mm/min) were significantly lower than mean values obtained on day 1 (18.0 mm/min), 2 (18.0 mm/min), or 3 (19.5 mm/min) for control cats ( $P = 0.005$ ) and day 3 (20.7 mm/min) for treatment cats ( $P = 0.014$ ). On day -3, mean  $\pm$  SD value for IOP for the control cats (23.1  $\pm$  3.6 mm Hg) was significantly ( $P = 0.001$ ) lower than the mean IOP value on day 2 for those same cats (26.3  $\pm$  2.5 mm Hg). On day -3, mean IOP value (25.4  $\pm$  5.0 mm Hg) was significantly ( $P = 0.026$ ) higher than the mean IOP values obtained on days 4 (21.6  $\pm$  5.1 mm Hg), 5 (20.5  $\pm$  5.3 mm Hg), 6 (17.4  $\pm$  1.5 mm Hg), and 7 (20.2  $\pm$  3.2 mm Hg) for the treatment cats.

**Funduscopy**—No funduscopy abnormalities were detected in the control cats during the study.

In cats administered enrofloxacin, the fundus appeared abnormal by day 1 (2 females), 2 (3 females and 2 males), or 3 (1 female and 4 males), with a marked granular appearance in the area centralis as the initial

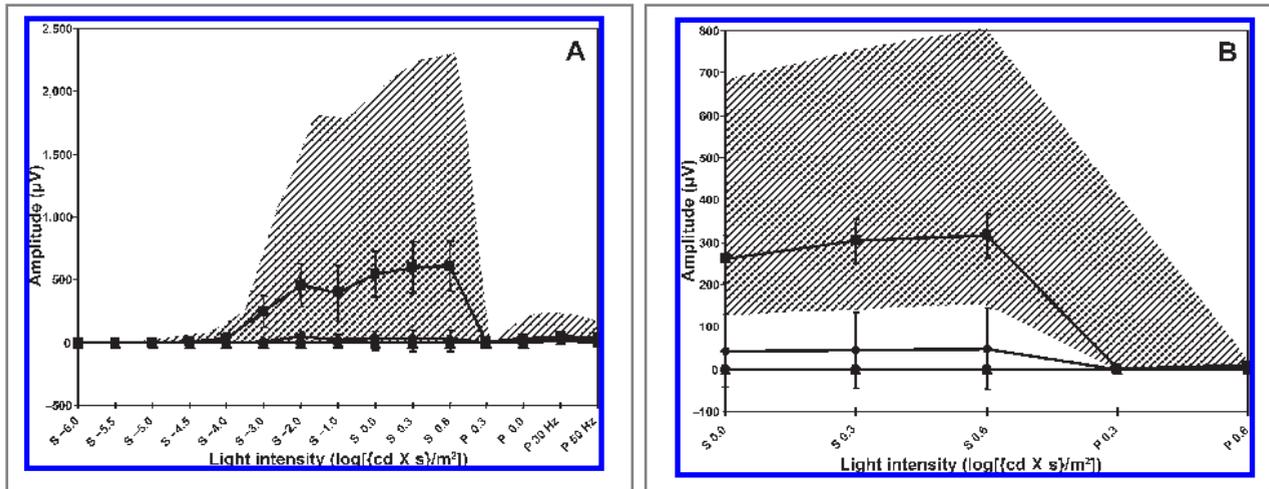


Figure 2—Mean  $\pm$  SD b-wave amplitudes (A) and a-wave amplitudes (B) of enrofloxacin-treated cats on day 1 (squares; 12 cats representing all 3 treatment subgroups), 3 (diamonds; 8 cats representing subgroups T1 and T2), 5 (triangles; 8 cats representing subgroups T2 and T3), and 7 (circles; 4 cats representing subgroup T3). Cats in all 3 treatment subgroups were administered doses of enrofloxacin (50 mg/kg, PO) daily beginning on day -1 and continuing until cats were euthanized (cats in subgroups T1, T2, and T3 [4 cats/subgroup; 2 males and 2 females] were euthanized on days 3, 5, and 7, respectively). The shaded area denotes the 5th to 95th percentile ranges for day -3 for all 24 control and treatment cats. Neutral density filters were used to reduce light intensity from -6 log [(cd X s)/m<sup>2</sup>] to 0.0 log [(cd X s)/m<sup>2</sup>] in steps of 0.5 log units. Then, light intensity was increased in 2 steps in 0.3 log units (ie, 0.3 and 0.6 log [(cd X s)/m<sup>2</sup>]). Intensity 0.0 log [(cd X s)/m<sup>2</sup>] was used for 30 and 50 Hz. S = Scotopic conditions. P = Photopic conditions.

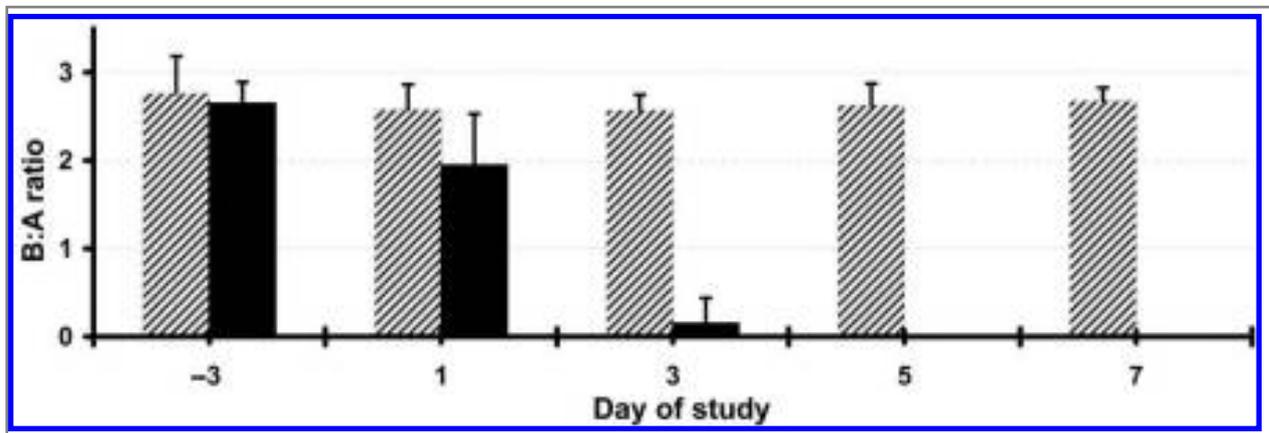


Figure 3—Ratio of the b-wave amplitude to the a-wave amplitude (B:A ratio) in ERG recordings from enrofloxacin-treated (black bars) and control (diagonal-striped bars) cats before (day -3) and after (days 1, 3, 5, and 7) daily administration of enrofloxacin.

change. This was followed by diffuse gray discoloration in the area centralis by day 1 (all 4 cats in subgroup T2), 2 (all 4 cats in subgroup T3), or 3 (all 4 cats in subgroup T1; Figure 1). The visual streak appeared granular and also had gray discoloration by day 2 (2 cats in subgroup T3) or 4 (2 cats in subgroup T2). Focal changes in tapetal reflectivity (hyperreflectivity) were evident between days 3 to 6 (3 females and 1 male from subgroups T2 and T3), and generalized hyperreflectivity was evident between days 5 to 7 (all 4 cats in subgroup T3). Vascular attenuation was mild on days 2 to 4 for most cats in subgroups T1, T2, and T3 (n = 8), moderate for some cats on day 3 in subgroups T1 and T3 (2), and severe on days 4 to 7 for all 8 cats in subgroups T2 and T3. Increased pigmentation of the fundus, which was not consistent with a dark coat color, was evident in 1 cat of subgroup T1. Increased pallor of the fundus was detected late in the study period in 2 cats (1 each from subgroups T1 and T2).

**ERG**—Amplitudes and implicit times were calculated (Figure 2). There was a significant ( $P = 0.006$ ) correlation between treatment group and decreases in ERG responses, whereby an increase in the duration of enrofloxacin administration was correlated with a decrease in ERG responses.

The ERG results were compared between eyes with regard to a- and b-wave amplitudes and amplitude changes over time, and significant differences were detected (values of  $P = 0.003$  to  $P < 0.001$ ). Treatment cats had significantly fewer days when they were considered within acceptable limits, compared with the number of days control cats were considered within acceptable limits. Statistical analysis revealed that the number of days treatment cats were within acceptable limits was significantly fewer for a- and b-wave amplitude and implicit time variables for both the right and left eyes. Ratio for the b-wave amplitude to the a-wave amplitude decreased by day 1 (compared with the ratio on day -3)

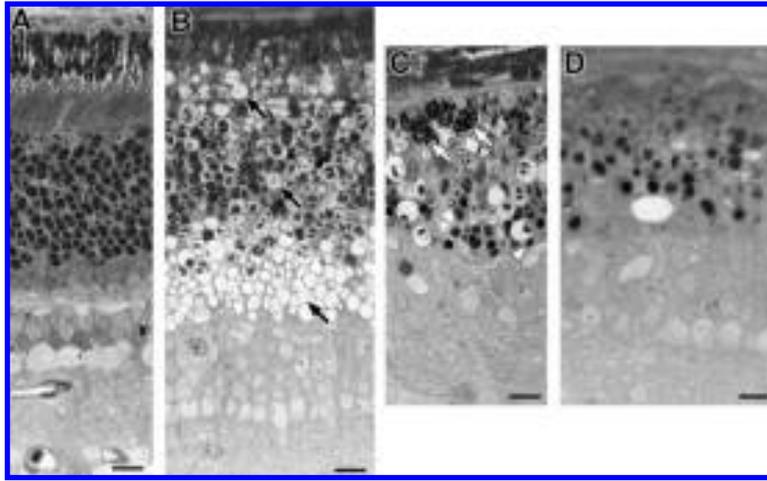


Figure 4—Photomicrographs of sections of retinal tissues obtained from enrofloxacin-treated cats on days –1 (A), 3 (B), 5 (C) and 7 (D). Notice the progressive change from severe vacuolization (black arrows) in the P-IS, ONL, and OPL on day 3 to degeneration of photoreceptors with pyknosis of photoreceptor nuclei on day 5; swollen RPE cells are evident (white arrows). By day 7, only a few abnormal photoreceptor nuclei (white arrowhead) remain and the P-IS and P-OS are not visible. Toluidine blue stain; bar = 10 µm.

in enrofloxacin-treated cats and was significantly lower in treatment cats than the ratio calculated for control cats by day 3 ( $P < 0.001$ ; Figure 3). Ratio for the b-wave amplitude to the a-wave amplitude for control cats did not change significantly between days –3 and the end of the study.

**Light microscopy**—Pathologic changes in the ONL, P-IS, and P-OS were significantly ( $P = 0.006$ ) correlated with duration of enrofloxacin administration. Degenerative changes were evident in these retinal layers in all regions of the retina examined by day 3 of the study (after only 3 doses of enrofloxacin administered at 10 times the recommended dosage). There was also severe vacuolization in the P-IS, ONL, and OPL. Furthermore, some disorganization of the P-OS lamellae was observed. By day 5, degenerative changes had progressed, severe photoreceptor disruption was evident, and there was an obvious loss of rod photoreceptors. Although degenerative changes were also observed in cone photoreceptors, these photoreceptors appeared to be relatively spared, compared with the degenerative changes in rod photoreceptors. However, by day 7, generalized loss of both rods and cones was identified.

Various structures in the light microscopic sections of the retinas were evaluated to determine histologic scores (Figure 4). Histologic scores of light microscopic sections were summarized (Figure 5). Some minor changes were observed in the RPE when sections were examined by use of light microscopy; these changes included swelling (hypertrophy) of extended areas of the RPE cells. This change was not significantly (values ranged from  $P = 0.06$  to  $P = 0.71$ ) correlated with any specific region of the retina. No changes were observed in the tapetum, inner nuclear layer, inner plexiform layer, or ganglion cell layer during enrofloxacin administration in any of the retinal areas evaluated. Furthermore, none of the control cats had any histopathologic changes during the study.

**Transmission electron microscopy**—Results of transmission electron microscopy corroborated the light microscopic findings of cats that received enrofloxacin. The least severe changes were observed in cats of subgroup T1 and ranged from vacuolization of solitary P-IS and disorganization of solitary P-OS lamellar disks in 1 cat to vacuolization and disruption in most of the P-IS and P-OS in 3 cats (Figure 6). Changes in the ONL included pyknosis of individual nuclei in 1 cat to pyknosis and degenerative changes in most of the nuclei in 3 cats. Mild to severe vacuolization was observed in the ONL and OPL, whereas the remainder of the inner retina and RPE were normal in appearance (4 cats).

More severe changes were found in retinas from cats of subgroup T2 (Figure 6). Less vacuolization was observed in the P-OS and P-IS, compared with that observed for cats of subgroup T1. The P-OS and P-IS of both rods and cones were all markedly shortened and severely disorganized. Severe disruption was observed in rod outer segments. Phagocytes containing engulfed P-OS lamellar disks were observed in the subretinal space, and a large number of phagosomes were observed in the RPE in 1 cat, which caused the cells to appear distended. In conjunction with a large number of pyknotic nuclei in the ONL, several normal-appearing nuclei were also apparent. However, a large number of these were enclosed in large vacuoles. No changes were observed in the OPL of cats in subgroup T2, and the inner retina was considered to be normal in appearance.

Substantial alterations were observed in cats of subgroup T3. In the subretinal space, primarily only remnants of P-IS and P-OS were observed, although some severely shortened P-IS of both rods and cones remained. In addition, a few solitary, normal-appearing but shortened cone P-IS and P-OS were observed. Pyknosis was severe in the ONL, and severe vacuolization was observed around some otherwise normal-appearing nuclei. The RPE cell layer appeared thin and

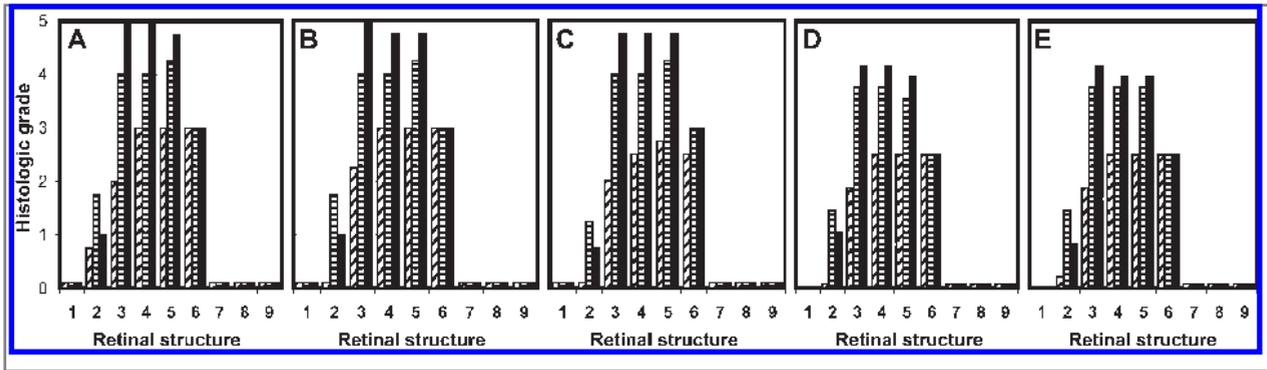


Figure 5—Histologic score of retinal layers for tissues obtained from 5 regions (superior peripheral [A], superior midperipheral [B], area centralis [C], inferior midperipheral [D], and inferior peripheral [E]) of the eyes of enrofloxacin-treated cats euthanized on days 3 (diagonal-striped bars), 5 (horizontal-striped bars), or 7 (black bars). Structures examined were the tapetum (1), RPE (2), P-OS (3), P-IS (4), ONL (5), OPL (6), inner nuclear layer (7), inner plexiform layer (8), and ganglion cell layer (9). Scores were assigned by use of a scale of 0 to 5 (0, no abnormalities [normal]; 1, minimal abnormalities; 2, mild or slight abnormalities; 3, moderate abnormalities; 4, marked abnormalities; and 5, severe retinal degeneration).

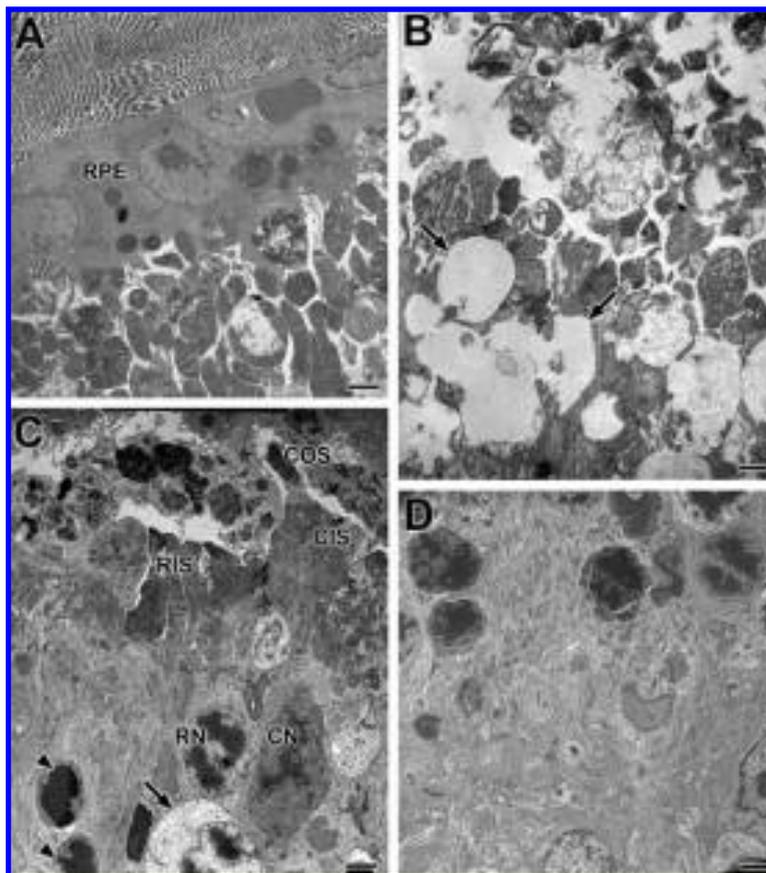


Figure 6—Electron photomicrographs of sections of retinal tissues obtained from enrofloxacin-treated cats on days 3 (A and B), 5 (C), and 7 (D). Disorganization of rod outer segments is evident on day 3 with severe vacuolization and disruption of inner segments (arrows). On day 5, there is primarily a normal-appearing cone, whereas most rod outer and inner segments are disorganized or disrupted. Also on day 5, notice the vacuolization around photoreceptor cell nuclei (arrow) and pyknosis of photoreceptor cell nuclei (arrowheads). On day 7, only a thin layer of abnormal photoreceptor cell nuclei remain, and cell structures in the OPL appear condensed. COS = Cone outer segment. CIS = Cone inner segment. RIS = Rod inner segment. RN = Rod nucleus. CN = Cone nucleus. Uranyl acetate stain; bar = 2  $\mu$ m.

condensed with fewer cytoplasmic inclusion bodies observed than in clinically normal cats. The RPE was otherwise normal in appearance with respect to the configuration of apical microvilli, and basal infold-

ings, tapetal cells, and adjacent choriocapillaries were normal. The OPL appeared condensed, whereas other structures of the inner retina were considered to be normal in appearance.

## Discussion

Enrofloxacin is a bactericidal antimicrobial that is widely used in canine and feline populations. At 10 times the recommended dosage, enrofloxacin disrupts the transmission of nerve impulses within the retina and has adverse effects on neurologic and retinal function of cats. Retinal effects after oral administration of enrofloxacin to cats, as determined on the basis of electrophysiologic and histologic variables, are related to drug dosage and duration of administration. Enrofloxacin administration in the study reported here altered food consumption and body weight and resulted in musculoskeletal, behavioral, and neurologic abnormalities that included blindness. Analysis of the results of this study does not support sex or age of an animal as a contributor to these adverse effects.

In the study reported here, female cats were significantly younger and weighed significantly less than male cats. Female cats did not have an increased frequency of retinotoxic effects. The numbers of male and female cats in each treatment group and subgroup that had adverse clinical, funduscopic, histologic, and ERG effects after enrofloxacin administration were equally distributed.

Weight loss was associated with administration of enrofloxacin and decreased food consumption in male and female cats that received enrofloxacin. The actual amount of weight lost per cat was greater for female enrofloxacin-treated cats than male enrofloxacin-treated cats. Food consumption was significantly increased for all male cats (treatment and control) and female control cats during the study. Male control cats did not lose weight during the study, whereas all female control cats lost a significant amount of weight during the study, despite an increase in food consumption. However, the amount of weight lost per kilogram of body weight for male enrofloxacin-treated and female control cats was less than that for the enrofloxacin-treated female cats in which food consumption was significantly decreased during the study period.

Body weight was not significantly correlated with retinotoxic effects of enrofloxacin because the same dosage per kilogram of body weight was administered to each cat. The proportional decrease in body weight of female cats, compared with that for male cats, probably reflected initial body reserves as an indicator of ability to withstand potential systemic toxic effects of enrofloxacin, such as progressive musculoskeletal changes, nonclinical upset of the gastrointestinal tract, or neurologic changes. In contrast, the increase in food consumption in control cats may have been associated with increased familiarity with surroundings and handling, lack of systemic abnormalities, and decreased amounts of stress.

Numerous possible causes exist for deviations in hematologic variables. Changes may have been attributable to laboratory or sample variation, parasites (eosinophilia), or stress (neutrophilia and lymphopenia).<sup>22</sup> Hyperglycemia has been associated with stress,<sup>23,24</sup> and stress may have been associated with venipuncture. Baseline glucose concentrations were within the reference range; however, glucose concentrations were increased above the reference range in control and

enrofloxacin-treated cats at the time of euthanasia. Increased blood glucose concentrations may be associated with recent food consumption or abnormal production or use of insulin.<sup>25</sup> These possibilities were deemed unlikely to have developed in the large number of cats. Because baseline glucose concentrations were not increased above the reference range, stress associated with recent handling was not considered a valid cause of high blood glucose concentrations at the time of euthanasia. This hypothesis was further refuted because cats were anesthetized at the time of blood collection on the day of euthanasia. Similarly, because food was withheld from all cats prior to blood collection on the day of euthanasia, recent food consumption was an unlikely contributor to increases in glucose concentrations. However, increases in blood glucose concentrations above the reference range in anesthetized control and enrofloxacin-treated cats at the time of euthanasia may have been attributable to chronic stress or anesthetic effects.<sup>26-28</sup>

Behavioral changes were evident in enrofloxacin-treated cats and may have represented clinical manifestations of changes associated with enrofloxacin administration. In the study, neurologic abnormalities worsened with increasing duration of enrofloxacin administration. These neurologic changes are not unique to cats. In human patients receiving quinolone, mild symptoms such as dizziness, headache, and insomnia have been reported.<sup>29,30</sup> It has been suggested<sup>30</sup> that these drugs competitively inhibit receptor binding of  $\gamma$ -aminobutyric acid, an inhibitory transmitter of the retina and CNS. Structural similarity of substituents of some quinolones at the C7 position with the binding region of the  $\gamma$ -aminobutyric acid molecule may be the reason for this phenomenon.<sup>29</sup>

Variations between mean rectal temperatures before and during the study were inconsistent between treatment groups and not related to food consumption, weight change, heart rate, respiratory rate, or administration of enrofloxacin. Mean rectal temperature was significantly higher before the study than during the study for all control and treatment cats. However, mean rectal temperatures were not outside of reference ranges for any cats at any time during the study, and as such, the higher mean rectal temperature recorded for days -3 to -1 was likely a stress response to first-time handling and restraint.<sup>23,24</sup>

During the study period, heart rate of control cats was significantly lower than the heart rate of enrofloxacin-treated cats. The reason for the difference in heart rate is unclear because no correlation with changes in blood pressure was identified. Presumably, enrofloxacin-treated cats that had behavioral, musculoskeletal, and neurologic abnormalities (including blindness) were under greater stress and, consequently, had a higher heart rate.

Respiratory rate was variable in the control cats, with the respiratory rate of C3 cats significantly higher than the respiratory rate of C1 and C2 cats during days -3 to -1 and during the study. The respiratory rate of C3 cats did not change significantly between days -3 to -1 and the study period, nor were significant differences identified between control and enrofloxacin-treated

cats. The importance of the high respiratory rate identified in C3 cats remains unclear.

The effect of fluoroquinolones on blood pressure is also unclear. In the study reported here, high doses of enrofloxacin resulted in blood pressure changes that were variable between control and treatment cats, and the changes were not correlated with treatment group or duration of enrofloxacin administration. An increase in blood pressure was detected on day 3, but the value did not differ significantly from blood pressure measurements obtained during the period of days -3 to -1. The importance of this finding, if any, remains unclear.

Direct and consensual pupillary light responses were detected for all cats during the entire study period and suggest that enrofloxacin primarily affects the outer retina and not the melanopsin-containing ganglion cells of the inner retina. This conclusion is supported by reports<sup>31,32</sup> in which investigators identified that melanopsin is responsible for the pupillary light response to stimulation with bright light. Menace responses were questionable for nearly all cats in subgroups T2 and T3 by day 5 and were not detected for all cats in subgroup T3 on days 6 and 7. Abnormal menace responses indicate reduced visual capacity, and complete lack of menace responses suggests blindness.<sup>33,34</sup>

The visual problems observed were probably the result of effects of enrofloxacin administration on the retinal or cortical tissues (or both). No abnormalities were detected in the anterior segment in any cat on any day of the study. Results for STTs varied between treatment groups and day of study; however, the STT results were not outside of the reference range at any point during the period from days -3 to -1 or during the study period. The IOPs were significantly lower for enrofloxacin-treated cats on days 4 to 7, compared with IOPs for days -3 to -1; however, values were not outside the reference range at any time point. The increase in IOP identified on day -3 may have been secondary to initial stress attributable to handling at the start of the study.<sup>35-37</sup>

Fundus abnormalities were detected early in enrofloxacin-treated cats and were manifested as a focal increase in granularity in the area centralis, followed by gray discoloration in the area centralis region and along the visual streak. Subsequently, there were focal changes in tapetal reflectivity and a slight vascular attenuation. Vascular attenuation became marked and was followed by generalized tapetal hyperreflectivity. These fundoscopic changes were consistent with generalized progressive retinal degeneration.<sup>38,39</sup> The ophthalmoscopically observed changes in color were probably secondary to disorganization and degeneration of the visual cell outer and inner segments,<sup>15,40</sup> which progressed to a more generalized hyperreflectivity evident as a complete loss of visual cells with subsequent thinning of the neuroretina. The fundoscopic alterations were most readily observed in the central portion of the retina (area centralis and visual streak). Concentrations of rod and cone photoreceptors are higher in the central portion than in other parts of the retina.<sup>41,42</sup>

Increased granularity may reflect hypertrophy observed in RPE cells in the outer retina, but it could also have been caused by altered transmission of light

through the neurosensory retina as a result of disorganization and degeneration of photoreceptors.<sup>40,43</sup> Retinal oxygen tension increases in concert with a reduction in photoreceptor numbers resulting from generalized retinal disease.<sup>44-46</sup> As a result of physiologic autoregulation of retinal vessels, there is a reduction of the vascular diameter when the oxygen tension of the retina increases.<sup>47,48</sup> Vascular attenuation observed early in the course of diffuse retinopathies is a sign of generalized loss of the oxygen-consuming photoreceptor cells.<sup>47</sup>

Female cats had fundoscopic changes after fewer doses of enrofloxacin than did male cats. The reasons for this sex distribution are unclear but may reflect an increased susceptibility of lighter-weight female cats to the effects of enrofloxacin. In contrast, retinal function, as defined by results of ERG, was not influenced by sex, with similar decreases in retinal function observed in cats of both sexes. Fundoscopic changes in control cats were considered normal variations because these findings were observed before the start of the study, and no progression of changes was detected.

Scotopic or dark-adapted ERG responses at low amounts of light are generated by rod photoreceptors because these photoreceptors are sensitive to single photons of light.<sup>49</sup> In contrast, cone photoreceptors, the visual cells responsible for color and day vision, function at high amounts of light under scotopic conditions and, even more specifically, after strong light adaptation when the rod system is desensitized.<sup>50</sup> Dark-adapted ERG responses were already decreased by 24 hours after the first dose of enrofloxacin, which was 2 days before a noticeable reduction in light-adapted responses. The ERG responses were then successively reduced and became rapidly nonrecordable as a consequence of photoreceptor degeneration. The rapid reduction of scotopic responses followed by reduction of photopic responses is compatible with the successive loss of rod photoreceptor cells and subsequent loss of cone photoreceptor cells, which is not an unusual finding in generalized inherited retinal disease processes or drug-induced retinopathies.<sup>15,51-56</sup> The early decrease in the ratio of the b-wave amplitude to the a-wave amplitude primarily reflects the significant decrease in b-wave amplitude, which is also a consequence of the loss of photoreceptor function.<sup>57,58</sup>

Clinical findings and results of ERG support the results of light- and electron-microscopic evaluations of the retina, which revealed profound early loss of rod photoreceptors with relative sparing of cones followed by a generalized loss also of cone photoreceptors. Severe vacuolization was identified histologically as an early change in the P-IS, ONL, and OPL in each of the 5 examined retinal regions (area centralis, superior midperipheral, superior peripheral, inferior midperipheral, and inferior peripheral) of enrofloxacin-treated cats. Severe disorganization in the rod P-OS structures were also observed early in the course of the disease. At later time points, degenerative changes were marked, then followed by a complete loss of normal rod and cone cell structures. Mild-to-moderate hypertrophy was identified by day 5 in the RPE of all cats that received enrofloxacin, with the severity of these changes reduced by day 7. Thus, the earliest

morphologic changes were observed in photoreceptors and appeared to indicate a direct toxic effect on these cellular elements.

The RPE cells host many functions within the retinas, including furnishing metabolites to photoreceptors and, on a regular basis, removing the outermost parts of the outer segments of the photoreceptors as part of the process of continual renewal of the outer segments.<sup>59,60</sup> Hypertrophy of RPE cells was most evident in enrofloxacin-treated cats on day 5, but the RPE cells in cats of subgroup T3 were subsequently reduced in size such that they were even smaller than cells of clinically normal cats. This may reflect a reduction in the amount of outer segment debris in the subretinal space because most of the disrupted outer segment structures had been phagocytized by that time point. Thus, changes in the RPE are probably secondary to the degenerative process that affects photoreceptor cells. No substantial abnormalities were observed in inner retinal cell layers, including the ganglion cell layer; thus, it appeared that these cell layers were spared from the direct toxic effects of enrofloxacin.

The specific mechanism or mechanisms of action by which enrofloxacin exerts the effects detected in the study reported here is unknown. In other reports,<sup>14,51</sup> investigators have speculated on effects of blood pressure, chemical induction or interaction, or phototoxic reactions. As previously stated, systolic blood pressure did not change significantly after enrofloxacin administration; therefore, it has been discounted as playing a major role in the retinal changes identified.

Enrofloxacin is structurally similar to other known ocular toxins, including cinchona alkaloid, chloroquine, halogenated hydroquinolones,<sup>51</sup> and nalidixic acid.<sup>14</sup> Nalidixic acid, the first fluoroquinolone developed for human use and the precursor to modern-day fluoroquinolones, causes prolonged ERG implicit times and decreased amplitudes<sup>54,61</sup> with atrophy of the OPL and degenerative changes in the ONL in cats.<sup>15</sup> Nalidixic acid is not approved for use in domestic animals, and when administered to cats at a rate of 10 mg/kg/d in 1 study,<sup>54</sup> it resulted in prolonged implicit times and decreased amplitudes. An increase in vision-related problems has been identified in rats and humans when fluoroquinolones are concurrently administered with drugs that increase plasma concentrations of fluoroquinolones through decreased renal excretion.<sup>62-64</sup> Such drugs include probenecide, furosemide, and cimetidine.

Although other fluoroquinolones are structurally less related to nalidixic acid than to enrofloxacin, the effects of such other fluoroquinolones on the retina have been investigated and, in some cases, found to be retinotoxic in cats<sup>14</sup> and phototoxic *in vitro*.<sup>65</sup> Mild retinotoxic effects have been detected in rabbits administered ofloxacin,<sup>62,66-68</sup> norfloxacin,<sup>69,70</sup> and ciprofloxacin.<sup>64,71-74</sup> Orbifloxacin is recommended for once-daily oral administration at a dosage of 2.5 to 7.5 mg/kg. No adverse ocular effects have been detected in animals administered 15 mg of orbifloxacin/kg, PO, once daily; however, retinal degeneration has

been reported<sup>14</sup> at dosages of 45 and 75 mg/kg, PO, once daily. Marbofloxacin is labeled for use at 2.75 to 5.5 mg/kg as a once-daily oral administration; no retinal effects have been observed in cats administered marbofloxacin at dosages up to 55 mg/kg, PO, once daily.<sup>14</sup>

Ultraviolet A light (wavelengths of 315 to 400 nm) in combination with administration of quinolones results in retinal degeneration in albino mice as a result of photodegradation by-products.<sup>65,75</sup> These by-products cause degeneration of P-OS cells; result in vacuoles in ONL cells and pyknotic nuclei; and, finally, lead to loss of the P-OS.<sup>75</sup> To our knowledge, comparable studies have not been performed in cats.

In the study reported here, we conducted a rigorous examination of the fundoscopic, ERG, and histologic effects after oral administration of enrofloxacin (50 mg/kg/d) on the retinas of adult cats. Enrofloxacin at 10 times the recommended dosage in cats resulted in decreased retinal function by day 1 (ie, after only 1 dose of enrofloxacin administration), visible fundic changes by day 3 (after 3 doses of enrofloxacin administration), and clinically obvious visual deficits by day 4 (after 4 doses of enrofloxacin administration). Rod degeneration preceded cone degeneration. Changes in the ONL and OPL were simultaneous with degeneration of the P-IS and P-OS. In addition, adverse neurologic effects were observed. Results of this study provide additional insights into the adverse effects of oral administration of a high dose of enrofloxacin to healthy cats. Investigation of the effects of administration of the recommended dosage of enrofloxacin on the retinas and CNS of cats is warranted.

- a. Evans E, Bayer Health Care LLC, Animal Health Division, Shawnee Mission, Kan. Personal communication, 2000, 2001.
- b. Baytril, Shawnee Mission, Kan.
- c. Bayer Health Care LLC, Animal Health Division, Shawnee Mission, Kan: Corporate data.
- d. Liberty Research Inc, Waverly, NY.
- e. Parks Medical Doppler ultrasound, model 811.B, Aloha, Ore.
- f. Finnoff transilluminator, Welch Allyn Distributors, Skaneateles Falls, NY.
- g. Schirmer tear test standardized strips, Schering-Plough Animal Health, Omaha, Neb.
- h. Tono-Pen XL, Mentor O&O Inc, Norwell, Mass.
- i. Fluor-I-Strips AT, Ayerst Laboratories, New York, NY.
- j. Slitlamp biomicroscope, SL14, KOWA Co Ltd, Tokyo, Japan.
- k. All-pupil indirect ophthalmoscope, Keeler Instruments Inc, Broomhall, Pa.
- l. Volk double aspheric lens, 20 diopter, Veatch Ophthalmic Instruments, Tempe, Ariz.
- m. Nidek NM-100 hand-held digital fundus camera, Nidek Co Ltd, Freemont, Calif.
- n. Domitor (Medetomidine), Novartis, Pfizer Animal Health, Exton, Pa.
- o. Ketaset III, Fort Dodge Animal Health, Fort Dodge, Iowa.
- p. ERG jet electrodes, LKC Technologies Inc, Gaithersburg, Md.
- q. Grass telefactor subdermal needle electrodes, model E2, Astro-Medica, West Warwick, RI.
- r. ERG System TOR, Global Eye Program, Rejmyre, Sweden.
- s. Kodak Wratten neutral density filters No. 96, Eastman Kodak Co, Rochester, NY.
- t. Beuthanasia-D-Special, Schering Plough Animal Health, Omaha, Neb.
- u. Epon-araldite resin, Electron Microscopy Sciences, Hatfield, Pa.
- v. PROC Freq, version 9.1, SAS Institute Inc, Cary, NC.

## References

1. Leshner GY, Froelich EJ, Gruett MD, et al. 1-8 naphthyridine derivatives. A new class of chemotherapeutic agents. *J Med Pharm Chem* 1962;91:1063-1065.
2. Andersson M, MacGowan A. Development of the quinolones. *J Antimicrob Chemother* 2003;51(suppl S1):1-11.
3. Hooper D, Wolfson J. Fluoroquinolone antimicrobial agents. *N Engl J Med* 1991;324:384-394.
4. Wolfson J, Hooper D. Fluoroquinolone antimicrobial agents. *Clin Microbiol Rev* 1989;2:378-424.
5. Lamp K, Bailey E, Rybak M. Ofloxacin clinical pharmacokinetics. *Clin Pharmacokinet* 1992;22:32-46.
6. Crumplin G, Smith J. Nalidixic acid and bacterial chromosome replication. *Nature* 1976;260:643-645.
7. Wang J. DNA topoisomerases. *Annu Rev Biochem* 1985;54:665-667.
8. Gootz T, Brightly K. Fluoroquinolone antibacterials: SAR mechanism of action, resistance, and clinical aspects. *Med Res Rev* 1996;16:433-486.
9. Gellert M, Mizuuchi K, O'Dea MH, et al. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. *Proc Natl Acad Sci U S A* 1977;74:4772-4776.
10. Gellert M, Mizuuchi K, O'Dea MH, et al. DNA gyrase: an enzyme that introduces superhelical turns into DNA. *Proc Natl Acad Sci U S A* 1976;73:3872-3876.
11. Walker R. The fluoroquinolones. *Mayo Clin Proc* 1999;74:1030-1037.
12. Boothe DM. Enrofloxacin revisited. *Vet Med* 1994;8:744-753.
13. Altreuther P. Safety and tolerance of enrofloxacin in dogs and cats, in *Proceedings*. 1st Int Symp Baytril 1992;15-19.
14. Wiebe V, Hamilton P. Fluoroquinolone-induced retinal degeneration in cats. *J Am Vet Med Assoc* 2002;221:1568-1571.
15. Gelatt K, van der Woerd A, Ketring KL, et al. Enrofloxacin-associated retinal degeneration in cats. *Vet Ophthalmol* 2001;4:99-106.
16. Giuliano E, van der Woerd A. Feline retinal degeneration: clinical experience and new findings (1994-1997). *J Am Anim Hosp Assoc* 1999;35:511-514.
17. Grahn B, Storey E, Cullen C. Diagnostic ophthalmology. *Can Vet J* 2002;43:137-138.
18. Crispin S, Gould DJ, Carter WJ, et al. Idiosyncratic reaction to enrofloxacin in cats. *Vet Rec* 2002;150:555-556.
19. Hampshire VA, Doddy FM, Post LO, et al. Adverse drug event reports at the United States Food and Drug Administration Center for Veterinary Medicine. *J Am Vet Med Assoc* 2004;225:533-536.
20. Narfström K, Ekesten B, Rosolen SG, et al. Committee for the Harmonized ERG Protocol, European College of Veterinary Ophthalmology. Guidelines for clinical electroretinography in the dog. *Doc Ophthalmol* 2002;105:83-92.
21. Stokes ME, Davis CS, Koch GG. In: Hoaglin DC, Mosteller F, Tukey JW, eds. *Categorical data analysis using the SAS system*. 2nd ed. Cary, NC: SAS Institute Inc, 2000.
22. Hall RL. Leukocyte responses and abnormalities. In: Fenner WR, ed. *Quick reference to veterinary medicine*. Philadelphia: JB Lippincott Co, 1982;369-371.
23. Diagnostic procedures for the office laboratory. In: Fraser CM, Mays A, eds. *The Merck veterinary manual*. Rahway, NJ: Merck & Co Inc, 1986;877-893.
24. Bruyette DS. Feline endocrinology update. *Vet Clin North Am Small Anim Pract* 2001;31:1063-1081.
25. The pancreas (endocrine diseases). In: Fraser CM, Mays A, eds. *The Merck veterinary manual*. Rahway, NJ: Merck & Co Inc, 1986;266-277.
26. Ambrisko TD, Hikasa Y. Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. *Can J Vet Res* 2002;66:42-49.
27. Ambrisko TD, Hikasa Y, Sato K. Influence of medetomidine on stress-related neurohormonal and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. *Am J Vet Res* 2005;66:406-412.
28. Hikasa Y, Hisahiro K, Katsuaki T, et al. Comparisons of sevoflurane, isoflurane, and halothane anesthesia in spontaneously breathing cats. *Vet Surg* 1996;25:234-243.
29. Brown S. Fluoroquinolones in animal health. *J Vet Pharmacol Ther* 1996;19:1-14.
30. Hooper D, Wolfson J. Adverse effects. In: Hooper D, Wolfson J, eds. *Quinolone antimicrobial agents*. Washington, DC: American Society for Microbiology, 1993;489-512.
31. Semo M, Peirson S, Lupi D, et al. Melanopsin retinal ganglion cells and the maintenance of circadian and pupillary responses to light in aged rodless/coneless (rd/rd cl) mice. *Eur J Neurosci* 2003;17:1793-1801.
32. Gooley J, Lu J, Chou TC, et al. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat Neurosci* 2001;4:1165.
33. Scagliotti R. Neuroophthalmology. In: Gelatt K, ed. *Veterinary ophthalmology*. Baltimore: Lippincott Williams & Wilkins, 1986;1316.
34. Martin L, Kaswan R, Chapman W. Four cases of optic nerve blindness in the horse. *Equine Vet J* 1986;18:133-137.
35. Bhattacharjee P, Paterson CA, Spellman JM, et al. Pharmacological validation of a feline model of steroid-induced ocular hypertension. *Arch Ophthalmol* 1999;117:361-364.
36. Miyazaki Y, Matsuo T, Kurabayashi Y. Immobilization stress induces elevation of intraocular pressure in rabbits. *Ophthalmic Res* 2000;32:270-277.
37. Li Voon Chong JS, Sen J, Johnson Z, et al. Hydrocortisone replacement dosage influences intraocular pressure in patients with primary and secondary hypocortisolism. *Clin Endocrinol (Oxford)* 2001;54:267-271.
38. Narfström K, Ekesten B. Diseases of the canine ocular fundus. In: Gelatt K, ed. *Veterinary ophthalmology*. Baltimore: Lippincott Williams & Wilkins, 1999;869-933.
39. Glaze M, Gelatt K. Feline ophthalmology. In: Gelatt K, ed. *Veterinary ophthalmology*. Baltimore: Lippincott Williams & Wilkins, 1999;997-1052.
40. Narfström K. Progressive retinal atrophy in the Abyssinian cat: clinical characteristics. *Invest Ophthalmol Vis Sci* 1985;26:193-200.
41. Steinberg RH, Reid M, Lacy PL. The distribution of rods and cones in the retina of the cat (*Felis domesticus*). *J Comp Neurol* 1973;148:229-248.
42. Tucker GS, Hamasaki DI, Labbie A, et al. Anatomic and physiologic development of the photoreceptor of the kitten. *Exp Brain Res* 1979;37:459-474.
43. Peiffer R, Wilcock B, Dubielzig R, et al. Fundamentals of veterinary ophthalmic pathology. In: Gelatt K, ed. *Veterinary ophthalmology*. Baltimore: Lippincott Williams & Wilkins, 1999;359-360, 411.
44. Yu D, Cringle S, Valter K, et al. Photoreceptor death, trophic factor expression, retinal oxygen status, and photoreceptor function in the P23H rat. *Invest Ophthalmol Vis Sci* 2004;45:2013-2019.
45. Linsenmeier R. Electrophysiological consequences of retinal hypoxia. *Graefes Arch Clin Exp Ophthalmol* 1990;228:143-150.
46. Braun R, Linsenmeier R, Goldstick T. Oxygen consumption in the inner and outer retina of the cat. *Invest Ophthalmol Vis Sci* 1995;36:542-554.
47. Padnick-Silver L, Kang-Derwent JJ, Giuliano E, et al. Retinal oxygenation and oxygen metabolism in Abyssinian cats with a hereditary retinal degeneration. *Invest Ophthalmol Vis Sci* 2006;47:3683-3689.
48. Alm A, Bill A. The oxygen supply in the retina. II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. *Acta Physiol Scand* 1972;84:306-319.
49. Baylor DA, Lamb TD, Yau KW. Responses of retinal rods to single photons. *J Physiol (Lond)* 1979;288:613-634.
50. Fain GL, Matthews HR, Cornwall C, et al. Adaptation in vertebrate photoreceptors. *Physiol Rev* 2001;81:117-151.
51. Vrabec T, Sergott RC, Jaeger EA, et al. Reversible visual loss in a patient receiving high-dose ciprofloxacin hydrochloride (Cipro). *Ophthalmology* 1990;97:707-710.
52. Pittman F, Westphal M. Optic atrophy following treatment with diiodohydroxyquin. *Pediatrics* 1974;54:81-83.
53. Tsuchiya M, Hayasaka S, Mizuno K. Affinity of ocular acid-insoluble melanin for drugs in vitro. *Invest Ophthalmol Vis Sci* 1987;28:822-825.

54. Imai R, Sugimoto S, Ando T, et al. A procedure for recording electroretinogram and visual evoked potential in freely moving cats. *J Toxicol Sci* 1990;15:263–274.
55. Narfström K, Nilsson SE. Progressive retinal atrophy in the Abyssinian cat. Electron microscopy. *Invest Ophthalmol Vis Sci* 1986;27:1569–1576.
56. Narfström K, Ehinger B, Bruun A. Immunohistochemical studies of cone photoreceptors and cells of the inner retina in feline rod-cone degeneration. *Vet Ophthalmol* 2001;4:141–145.
57. Asi H, Perlman I. Relationships between the electroretinogram a-wave, b-wave and oscillatory potentials and their application to clinical diagnosis. *Doc Ophthalmol* 1992;79:125–139.
58. Ruether K, Kellner U. Inner retinal function in hereditary retinal dystrophies. *Acta Anat (Basel)* 1998;162:169–177.
59. Young RW, Bok D. Participation of the retinal pigmented epithelium in the rod outer segment renewal process. *J Cell Biol* 1969;42:392–403.
60. Young RW. Visual cells and the concept of renewal. *Invest Ophthalmol Vis Sci* 1976;15:700–725.
61. Nomura M, Yamada M, Yamamura H, et al. Ophthalmotoxicity and ototoxicity of the new quinolone antibacterial agent levofloxacin in Long Evans rats. *Arzneimittelforschung* 1992;43:398–403.
62. Foote E, Halstenson C. Effects of probenecid and cimetidine on renal disposition of ofloxacin in rats. *Antimicrob Agents Chemother* 1998;42:456–458.
63. Sudoh T, Fujimura A, Shiga T, et al. Renal clearance of lomefloxacin is decreased by furosemide. *Eur J Clin Pharmacol* 1994;46:267–269.
64. Jaehde U, Sorgel F, Reiter A, et al. Effect of probenecid on the distribution and elimination of ciprofloxacin in humans. *Clin Pharmacol Ther* 1995;58:532–541.
65. Przybilla B, Georgii A, Bergner T, et al. Demonstration of quinolone phototoxicity in vitro. *Dermatologica* 1990;181:98–103.
66. Sakai H, Mochizuki K, Torisaki M, et al. Nontoxic intravitreal dose of ofloxacin for rabbit retina. *Ophthalmic Res* 1994;26:344–351.
67. Cohen RG, Raizman M, Callina C, et al. Retinal safety of oral and topical ofloxacin in rabbits. *J Ocul Pharmacol Ther* 1997;13:369–379.
68. Wiechens B, Neuman D, Grammer JB, et al. Retinal toxicity of liposomal incorporated and free ofloxacin after intravitreal injection in rabbit eyes. *Int Ophthalmol* 1998;22:133–143.
69. Mochizuki K, Higashide T, Torisaki M, et al. Effects of norfloxacin on the retina in rabbits. *Graefes Arch Clin Exp Ophthalmol* 1995;233:173–180.
70. Tanahashi T, Mochizuki K, Torisaki M, et al. Effects of intravitreal injection of norfloxacin on the retina in pigmented rabbits. *Lens Eye Toxic Res* 1992;9:493–503.
71. Wiechens B, Grammer JB, Johannsen U, et al. Experimental intravitreal application of ciprofloxacin in rabbits. *Ophthalmologica* 1999;213:120–128.
72. Marchese AL, Slana VS, Holmes EW, et al. Toxicity and pharmacokinetics of ciprofloxacin. *J Ocul Pharmacol* 1993;9:69–76.
73. Rootman DS, Savage P, Hasany SM, et al. Toxicity and pharmacokinetics of intravitreally injected ciprofloxacin in rabbit eyes. *Can J Ophthalmol* 1992;27:277–282.
74. Stevens SX, Fouraker BD, Jensen HG. Intraocular safety of ciprofloxacin. *Arch Ophthalmol* 1991;109:1737–1743.
75. Shimoda K, Yoshida M, Wagai N, et al. Phototoxic lesions induced by quinolone antibacterial agents in auricular skin and retina of albino mice. *Toxicol Pathol* 1993;21:554–561.