**Systemic absorption and adverse ocular and systemic effects after topical ophthalmic administration of 0.1% diclofenac to healthy cats**

Kimberly K. Hsu DVM, MSc  
Chantale L. Pinard DVM, MSc  
Ron J. Johnson DVM, PhD  
Dana G. Allen DVM, MSc  
Butch K. KuKanich DVM, PhD  
Stephanie G. Nykamp DVM  

OBJECTIVE  
To quantify plasma concentrations and determine adverse ocular, renal, or hepatic effects associated with repeated topical ophthalmic application of 0.1% diclofenac to healthy cats.

ANIMALS  
8 healthy sexually intact male cats.

PROCEDURES  
A randomized, placebo-controlled crossover study was conducted. A topical formulation of 0.1% diclofenac was administered 4 times/d for 7 days to 4 cats, and artificial tear (control) solution was administered to the other 4 cats. After a 12-day washout period, cats received the other treatment. Ophthalmic examinations were performed daily. Plasma samples were obtained on days 1 and 7 for pharmacokinetic analysis. A CBC, serum biochemical analysis, urinalysis, determination of urine protein-to-creatinine ratio, and determination of glomerular filtration rate were performed before the start of the study and after each 7-day treatment period.

RESULTS  
Mild conjunctival hyperemia was the only adverse ocular effect detected. Maximal drug concentration and area under the curve were significantly higher on day 7 than on day 1. Diclofenac-treated cats had a significantly lower glomerular filtration rate than did control-treated cats after the second but not after the first treatment period, presumably associated with iatrogenic hypovolemia.

CONCLUSIONS AND CLINICAL RELEVANCE  
Topical ophthalmic administration of 0.1% diclofenac was well tolerated in healthy cats, with only mild signs of ocular irritation. Detectable systemic concentrations of diclofenac were achieved with accumulation over 7 days. Systemic absorption of diclofenac may be associated with reduced glomerular filtration rate, particularly in volume-contracted animals. Topical ophthalmic 0.1% diclofenac should be used with caution in volume-contracted or systemically ill cats. (Am J Vet Res 2015;76:253–265)

**ABBREVIATIONS**

- ALT Alanine aminotransferase
- AUC Area under the curve
- CI Confidence interval
- Cmax Maximum plasma concentration
- CTT Corneal touch threshold
- GFR Glomerular filtration rate
- IOP Intraocular pressure
- STT Schirmer tear test
- TFBUT Tear film break-up time
- Tmax Time to maximum plasma concentration

**Anterior uveitis** is a common ocular condition in domestic cats. Uveitis in cats may be caused by corneal injury or trauma as well as idiopathic, neoplastic, lens-induced, and infectious causes.1–4 The number of cats with anterior uveitis and detectable concurrent systemic disease is substantial but variable, ranging between 38% and 70%.3,5 Sequelae of anterior uveitis include corneal endothelial damage that leads to permanent corneal edema, cataract formation, lens luxation, posterior synechiae, secondary glaucoma, and phthisis bulbi.4,6–8 Given that it is a painful and potentially vision-threatening condition, prompt and aggressive anti-inflammatory treatment is indicated.1,3,4 Although many mediators are involved in ocular inflammation, prostaglandins are considered to be one of the main mediators.9 Thus, anti-inflammatory drugs, including corticosteroids and NSAIDs, are the mainstay of uveitis treatment.1,3,4 Topically administered corticosteroids are commonly used as the primary treatment for anterior uveitis in cats because they are highly effective anti-inflammatory agents and generally are tolerated well by cats.1,3,4 However, topical application of NSAIDs may be used with caution in cases (eg, corneal ulceration or infection) in which topically administered corticosteroids are contraindicated. Topically administered NSAIDs can also be used to treat mild inflammation or in combination with top-
ical application of corticosteroids in cases of severe inflammation.\textsuperscript{13,14,16} In humans, topical administration of NSAIDs has been associated with conjunctival hyperemia,\textsuperscript{10} decreased corneal sensation,\textsuperscript{11–13} and corneal lesions.\textsuperscript{14,15} In cats with experimentally induced uveitis, topical application of NSAIDs has been associated with conjunctivitis and mild increases in IOP.\textsuperscript{16}

It has long been recognized that caution may be needed for use of NSAIDs in cats because of a limited capacity for hepatic glucuronidation, risk of accumulation, and subsequent toxicosis.\textsuperscript{17,18} Thus, compared with the number of NSAIDs licensed for systemic use in dogs, there are few NSAIDs licensed for use in cats, and most licensed products are approved for administration perioperatively or for a period of only a few days.\textsuperscript{19} Currently, all topical ophthalmic applications of NSAIDs are extralabel uses in cats.

Although one goal of topical treatment is to limit systemic absorption and adverse systemic effects, a considerable proportion of topically applied medications may be systemically absorbed. Medications instilled into the conjunctival sac may rapidly enter the systemic circulation through conjunctival absorption, drainage via the nasolacrimal system, and absorption through the nasal mucosa.\textsuperscript{20–22} Topically applied medications may also be drained via the nasolacrimal system into the oropharynx and be swallowed, with absorption via the gastrointestinal system.\textsuperscript{20–22} To the authors' knowledge, there are no published reports of studies that have characterized the amount of systemic absorption in cats that results from topical administration of NSAIDs, and little is known about adverse systemic effects associated with repeated topical application of NSAIDs.

To the authors' knowledge, topical administration of NSAIDs has not been associated with reports of adverse systemic effects in cats. Despite the perceived risk of toxicosis with systemic use of NSAID in cats, few prospective, placebo-controlled studies have been conducted to examine the risks associated with systemic administration of NSAIDs. Because of confounding factors and insufficient diagnostic testing, it is difficult to draw conclusions from retrospective studies of systemic use of NSAIDs, with many studies\textsuperscript{23–25} confounded by advancing age of cats, dehydration, and concurrent systemic disease. Studies\textsuperscript{26–27} that involved the use of more sensitive measures of renal health, such as GFR and renal scintigraphy, have failed to reveal adverse effects in healthy young cats that received a number of commercially available systemic NSAIDs for up to 6 weeks.

The objective of the study reported here was to determine whether 0.1% diclofenac can be safely administered topically to cats in accordance with a relevant clinical regimen. We wanted to investigate adverse ocular effects, systemic absorption, and adverse systemic effects by use of a dosing regimen applied 4 times/d for 1 week. In addition to determining whether diclofenac reaches plasma concentrations high enough to cause adverse systemic effects, another objective was to determine whether there is accumulation with repeated doses. It was hypothesized that topical administration of diclofenac would be associated with signs of mild ocular irritation, decreased corneal sensitivity, and a mild increase in IOP but would not have substantial effects on any other variables examined. Although adverse gastrointestinal tract effects are of concern, the emphasis was on renal and hepatic function. It was hypothesized that topical ophthalmic administration of 0.1% diclofenac would not be associated with substantial accumulation or adverse renal or hepatic effects.

**Materials and Methods**

**ANIMALS**

Eight sexually intact male purpose-bred barrier-raised domestic shorthair cats\textsuperscript{b} were included in the study. Data for 1 additional sexually intact male cat, used in a preliminary experiment to verify the study design for sample collection, was included in the systemic absorption and adverse effects portions of the study because none of the systemic variables required subjective evaluation. Data for that cat were not included in the adverse ocular effects portion of the study because investigators were aware of the treatments administered during the preliminary study and several of the ocular variables required subjective assessment. Sample size was determined with a power analysis based on IOP differences detected in a previous study.\textsuperscript{16} All 9 cats were approximately 1 year old (range, 8 to 14 months). All 9 cats had a starting body weight between 5.02 and 6.63 kg (mean ± SE, 5.60 ± 0.20 kg). Cats were exposed to an automated cycle of 12 hours of light to 12 hours of darkness (light phase from 7 AM to 7 PM and dark phase from 7 PM to 7 AM). Cats were acclimatized to handling and ocular procedures for 1 month prior to the start of the study. The study was approved by the University of Guelph Animal Care and Use Committee, and all procedures were performed in accordance with Canadian Council on Animal Care guidelines.

A general physical examination was performed on each cat prior to inclusion in the study. A CBC, serum biochemical analysis, urinalysis, and determination of urine protein-to-creatinine concentration ratio\textsuperscript{b} were performed on each cat approximately 2 weeks before the start of the study to ensure that all cats were healthy and to collect baseline values. Baseline urine samples were collected via cystocentesis. Ocular examination including neuro-ophthalmic examination (pupillary light reflex, dazzle reflex, menace response, and pupillary light reflexes), slit-lamp biomicroscopy,\textsuperscript{c} STT,\textsuperscript{d} fluorescein staining,\textsuperscript{c} rebound tonometry,\textsuperscript{c} and indirect ophthalmoscopy\textsuperscript{c} after topical administration of 1% tropicamide\textsuperscript{d} were performed before the study.

**PROCEDURES**

A blinded placebo-controlled crossover design was used. The study consisted of two 7-day treatment pe-
periods (phases 1 and 2), with a 12-day washout period between treatment periods. Given that there is a lack of information on the half-life for diclofenac in cats, the washout period was chosen on the basis of the design of a recent study in cats for which a 6-day washout period was used; this was doubled to decrease the likelihood of residual effects of diclofenac. Before beginning phase 1 of the study, cats were assigned by use of a randomization procedure (by drawing slips of paper out of a hat) to control and diclofenac treatment groups (4 cats/group). During phase 1, cats in the diclofenac treatment group received 1 drop (50 µL) of 0.1% diclofenac ophthalmic solution in each eye and cats in the control treatment group received 1 drop (50 µL) of an artificial tear solution in each eye. Drops were administered 4 times/d (8 AM, 12 PM, 4 PM, and 8 PM) for 7 days (first day of each phase was designated as day 1). After the washout period, cats received the other solution during phase 2. To ensure that the principal investigator (KKH) remained unaware of the treatment administered to each cat during both phases, drops were administered with identical sterile syringes by another member of the research team.

OCULAR ADVERSE EFFECTS

Baseline ocular data were collected on 3 days within a 2-week period before the beginning of the study. Ocular examinations and tests were performed at 8 AM on each day of each 7-day study phase and for the first 3 days of the washout period. On the final day of the washout period, all ocular tests were repeated to ensure that there were no abnormalities or lingering treatment effects. During each phase, an STT test was performed at 8 AM prior to drop administration. Fluorescein staining and TFBUT were measured at 12 PM prior to drop administration. The TFBUT was evaluated by use of methods described elsewhere. A stopwatch was used to measure STT and TFBUT. After measurement of TFBUT, the cornea was rinsed with sterile eye wash solution and examined for uptake of fluorescein stain by use of slit-lamp microscopy.

Intraocular pressure (measured with a rebound tonometer) and pupillary diameter were evaluated 4 times/d during each phase of the study prior to drop administration at 8 AM, 12 PM, 4 PM, and 8 PM. Horizontal pupillary diameter was measured with Jameson calipers held adjacent to the cornea. Measurements were always performed in the same room, where lighting was maintained between 172 and 196 luxes (16 to 18 candles).

Slit-lamp biomicroscopy was performed before and after drop administration. Ocular irritation was scored in accordance with a modified Hackett-MacDonald scoring system to assess blepharospasm, ocular discharge, conjunctival hyperemia, conjunctival chemosis, and prolapse of the nictitating membrane (ie, third eyelid). Scores were assigned on a scale of 0 to 4 (0 represented an absence of signs, and 4 represented maximum severity of signs). After topical application of the drop to each eye, the amount of time that the eyes remained closed was recorded and used as an indicator of ocular irritation.

The CTT test was performed 3 days before the start of each treatment phase and at 8:30 PM on day 7 of each treatment phase. The CTT was measured with a Cochet-Bonnet aesthesiometer with a 0.12-mm-diameter nylon monofilament, as previously described. Measurements of filament length were converted to units of pressure by use of a chart provided by the manufacturer.

SYSTEMIC ABSORPTION AND ADVERSE EFFECTS

Physical examinations, including measurement of vital parameters, were performed at 8 AM each day during the study. Each cat’s cage and litter tray were also monitored throughout the study for signs of vomiting or diarrhea.

Two days before each phase, an indwelling catheter was inserted in each jugular vein of each cat to facilitate collection of serial blood samples. Cats were sedated with ketamine hydrochloride (5 mg/kg, IM), buprenorphine (0.02 mg/kg, IM), and acepromazine maleate (0.05 mg/kg, IM); cats were anesthetized by IV administration of propofol given to effect. Jugular catheters were monitored and flushed twice daily with heparinized saline solution (2 mL) and replaced as needed. To prevent self-trauma, neck bandages and Elizabethan collars were applied to each cat for the duration of the study. The same number of blood samples were collected from cats when receiving the control and diclofenac solutions.

One day before the beginning of phase 1, GFR was measured in all cats by use of plasma clearance of technetium Tc 99m pentatate (metastable 99Tc-labeled diethyleneetriamine pentaacetic acid) used in accordance with an established protocol. The protocol was modified to minimize the volume of blood collected. Two milliliters of blood was collected from a jugular catheter at 15, 30, 120, and 240 minutes for counting in a gammacounter; 2 mL of heparinized saline solution then was used to flush the jugular catheter and replace the blood volume withdrawn during each sample collection.

Blood samples were collected before administration of the first dose (time 0) during both phases to ensure that no circulating concentrations of diclofenac could be detected. Serial blood samples were collected for pharmacokinetic analysis after drop administration at 8 PM on days 1 and 7 of each phase of the study. Blood samples were collected 5, 15, 30, 60, 120, and 240 minutes after administration of the 8 PM drop administration. Timing of blood sample collection following the final dose of the day was chosen to maximize the likelihood for detecting diclofenac. Volume of each blood sample was 2 mL. After collection of each blood sample, 2 mL of saline (0.9% NaCl) solution was injected (1:1 replacement) to minimize volume depletion. Blood samples were collected into tubes containing sodium heparin and immediately
placed on ice; samples subsequently were centrifuged (3,000 \( \times \) g for 10 minutes). Plasma was harvested and stored at \(-80^\circ\text{C}\) until the conclusion of the study; all samples then were shipped on dry ice to a laboratory for diclofenac concentration determination via high-performance liquid chromatography coupled with mass spectrometry. For the 7 days of diclofenac administration, all samples (0, 5, 15, 30, 60, 120, and 240 minutes) were submitted for analysis. However, for the 7 days of administration of the control solution, only the samples at 0 (before drop administration) and 240 minutes were analyzed to ensure that there had been no accidental administration of diclofenac and to confirm satisfactory washout between phases.

Blood samples for a CBC and serum biochemical analysis were also collected 5 minutes after drop administration at 8 PM on day 7 of each phase; these samples were collected at the same time as the 5-minute pharmacokinetic sample. Free-catch urine samples for urinalysis and determination of the urine protein-to-creatinine concentration ratio were collected with plastic litter beads on day 7. All free-catch urine samples were collected between the hours of 8 AM and 8 PM. Prior to laboratory closing at 6 PM, urine samples were delivered immediately after collection to the laboratory for analysis to decrease the chance of cast dissolution before sediment examination. If a free-catch urine sample could not be obtained, cats were sedated with butorphanol (0.2 to 0.4 mg/kg, SC) and cystocentesis was performed at the completion of collection of the blood samples for pharmacokinetic analysis. Urine samples obtained via cystocentesis were refrigerated overnight and delivered to the laboratory the morning after collection. Measurement of GFR was performed the day after conclusion of each 7-day phase. To minimize the volume of blood collected, a CBC and serum biochemical analysis were not repeated before the second phase of the study if results obtained after the first phase were deemed to be within reference limits. At the end of phase 1, if there were notable abnormalities on a CBC, serum biochemical analysis, urinalysis, determination of urine protein-to-creatinine concentration ratio, or GFR measurement, then these tests were repeated before the start of phase 2 to ensure that values had returned to within reference limits.

Prior to GFR measurement and collection of blood samples for the pharmacokinetic analysis, PCV was measured to ensure that no cat was anemic. Total protein concentration was concurrently measured with a refractometer. Cats were not allowed to remain in the study unless they had a minimum PCV of 25% and had normal hydration during physical examination. A maximum of 22 mL of blood was collected within a 24-hour period when combining the volume collected for the CBC and serum biochemical analysis (2 mL), pharmacokinetic analysis (12 mL), and GFR determination (8 mL); cats had 6 or 11 days to recover before additional blood samples were collected, depending on the time during the study phase (day 1 vs 7). Assuming cats have a circulating blood volume of 47 to 65 mL/kg,\(^{33}\) 22 mL represented approximately 6.4% of the circulating blood volume in the study animals. Given that 1 week is the recommended minimum recovery time when 7.5% of the circulating blood volume is removed during a 24-hour period with no fluid replacement,\(^{33}\) recovery times were considered adequate for the cats in the study, especially considering that the amount of blood withdrawn was replaced 1:1 with saline solution. Oscillometric blood pressure,\(^{9}\) heart rate, and pulse quality were monitored during and after GFR measurement and collection of blood for the pharmacokinetic analysis.

**PHARMACOKINETIC ANALYSIS**

Plasma concentrations of diclofenac were determined with high-performance liquid chromatography\(^{9}\) with triple quadrupole mass spectrometry.\(^{9}\) Assay reference standards and test samples were processed in an identical manner. Plasma (0.2 mL) was added to 0.8 mL of acetonitrile in a microcentrifuge tube containing the internal standard, meclofenamic acid (62.5 ng/mL), to precipitate plasma proteins. Microcentrifuge tubes were mixed in a vortexer for 5 seconds and centrifuged for 5 minutes at 15,000 \( \times \) g; the supernatant then was transferred to a clean culture tube and evaporated to dryness under a stream of air at \(40^\circ\text{C}\) for 25 minutes. Test samples or reference standards were reconstituted with 0.2 mL of 10% methanol in deionized water, mixed in a vortexer, transferred to a microcentrifuge tube, and centrifuged for 5 minutes at 15,000 \( \times \) g to separate particulates. The supernatant was transferred to an injection vial; the injection volume was 0.05 mL. The mobile phase consisted of acetonitrile and 0.1% formic acid in deionized water. The mobile phase was initially at 85% from 0 to 0.5 minutes, followed by a linear gradient to 25% of the mobile phase at 4 minutes, followed by a linear gradient to 85% of the mobile phase at 5 minutes (total run time, 6.5 minutes). Separation was achieved with a phenyl column maintained at \(40^\circ\text{C}\). Qualifying and quantifying ions for diclofenac were \(m/z\) 296.12 and 215.00, respectively. Qualifying and quantifying ions for meclofenamic acid were \(m/z\) 296.12 and 245.00, respectively. Reference standard curves were constructed by use of blank feline plasma spiked with diclofenac to provide concentrations of 0, 10, 50, 100, 500, and 1,000 ng/mL; curves were accepted when the correlation coefficient was at least 0.99 and predicted concentrations were within 15% of the actual concentration.

Accuracy and coefficient of variation were determined on 5 replicates at each of 3 concentrations (10, 100, and 1,000 ng/mL). Accuracy of the assay was 99.8%, 100.1%, and 101.0% of the actual concentration at 10, 100, and 1,000 ng/mL, respectively. Coefficient of variation of the assay was 3.3%, 3.8%, and 3.6% at 10, 100, and 1,000 ng/mL, respectively.

Pharmacokinetic analysis of plasma diclofenac concentrations was performed via noncompartmental methods with pharmacokinetic functions for spread-
sheet software. Parameters generated or calculated included AUC from 0 to 240 minutes (which was determined with the linear trapezoidal rule) as well as Cmax and Tmax (both of which were determined directly from the data).

STATISTICAL ANALYSIS

Because scores of 2 to 4 were rare, signs of ocular irritation were considered as present or absent. The probability for each sign of ocular irritation was then calculated with a generalized linear mixed models procedure for binary measures. Effects in the model included cat, treatment, day, time of day, treatment period (first 7-day treatment period vs second 7-day treatment period following the washout period), and timing relative to drop administration (before vs after). Effects were removed if not significant. Duration of eye closure was analyzed with a general linear mixed model ANOVA.

All other ocular and systemic variables were analyzed with a general linear mixed model ANCOVA that included baseline values as a covariable. Baseline values were included as a covariable because preliminary statistical analysis revealed that baseline values had a significant effect on values obtained for some variables during the study. Variables measured at > 2 times were fitted to the best covariance structure to allow for analysis with repeated measures over time. Effects in the model included cat, treatment period, eye, and treatment depending on the variable, day, and time. All interactions of the effects in the model were accounted for and removed if not significant. Data were assessed for normality by means of a Shapiro-Wilk test and examination of the residuals. Data were logarithmically transformed if it improved normality. Post hoc tests included a Tukey or Dunnett adjustment, depending on the comparison. Standard errors were based on pooled SE for control and diclofenac treatments.

Baseline values were also compared with values obtained 1 day before the beginning of phase 2 of the study by use of an ANCOVA. Effects in the model included cat, treatment period, eye, and treatment depending on the variable, day, and time.

Pharmacokinetic parameters were assessed to detect differences between days 1 and 7. Differences in

AUC from 0 to 240 minutes and Cmax were assessed by means of a paired t test (the data were normally distributed with uniform variance). Differences in Tmax were assessed by means of a Wilcoxon signed rank test because the data were not distributed normally. For all statistical tests, values of \( P \leq 0.05 \) were considered significant.

Results

Results of physical and ocular examinations performed before the study were unremarkable for all cats. In 3 cats, superficial focal white corneal opacities were evident and were consistent with corneal scarring from previous injury. Because there were no signs of active inflammation or any other ocular abnormalities, these 3 cats were included in the study. All cats had negative results for fluorescein staining. Baseline TFBUT, STT, and IOP were consistent with published values. Baseline CTT was lower than published values but considered to be within reference limits given the large variability previously reported.

ADVERSE OCULAR EFFECTS

Mean ± SE probability that a cat would have at least 1 specific sign of irradiation was 4.34 ± 2.27% for the diclofenac treatment and 1.08 ± 0.60% for the control treatment; these values differed significantly (\( P = 0.032 \)). However, conjunctival hyperemia, the most common sign of ocular irritation, was the only sign for which diclofenac treatment was significantly (\( P = 0.016 \)) more likely to have a score than for the control treatment (Table 1). Two cats accounted for most of the instances in which multiple signs of ocular irritation were recorded, independent of their status in the control or diclofenac treatments. Mean ± SE amount of time that cats held their eyes closed after treatment with diclofenac was significantly (\( P = 0.006 \)) greater (33.1 ± 7.0 seconds) than that for cats after treatment with the control solution (2.0 ± 7.0 seconds).

A significant (\( P < 0.001 \)) baseline effect was identified for pupillary diameter, IOP, and STT. Thus, for example, cats with a large pupillary diameter prior to the study typically continued to have a large pupillary diameter throughout the study. Although

<table>
<thead>
<tr>
<th>Sign</th>
<th>Diclofenac</th>
<th>Control</th>
<th>OR (95% CI)</th>
<th>( P ) value*</th>
<th>No. of diclofenac-treated cats with sign</th>
<th>No. of control-treated cats with sign</th>
<th>No. of diclofenac-treated cats with sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival hyperemia</td>
<td>3.18 ± 16.93</td>
<td>5.32 ± 4.11</td>
<td>8 (1.72–37.03)</td>
<td>0.016</td>
<td>7</td>
<td>5</td>
<td>2†</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td>2.22 ± 1.50</td>
<td>0.37 ± 0.29</td>
<td>—</td>
<td>0.070</td>
<td>5</td>
<td>3</td>
<td>2†</td>
</tr>
<tr>
<td>Chemosis</td>
<td>0.52 ± 0.11</td>
<td>0.42 ± 0.09</td>
<td>—</td>
<td>0.484</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nictitans prolapse</td>
<td>0.50 ± 0.13</td>
<td>0.43 ± 0.11</td>
<td>—</td>
<td>0.680</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ocular discharge</td>
<td>0.90 ± 0.46</td>
<td>0.69 ± 0.36</td>
<td>—</td>
<td>0.818</td>
<td>3</td>
<td>3</td>
<td>2†</td>
</tr>
</tbody>
</table>

*Values were considered significant at \( P \leq 0.05 \). †The same 2 cats had blepharospasm, conjunctival hyperemia, and ocular discharge for both treatments.

- = Not determined.
Table 2—Values of selected ocular variables for 8 healthy cats at baseline and after topical administration of 0.1% diclofenac or a control solution.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline*</th>
<th>Control</th>
<th>Diclofenac</th>
<th>P value† Published values</th>
</tr>
</thead>
</table>
| STT (mm/min)      | 13.5 ± 1.5| 14.2 ± 0.7| 12.6 ± 0.7 | 0.094 | 10.8 ± 0.8
t|          |          |          |             |                     |
| IOP (mm Hg)       | 17.8 ± 0.9| 18.2 ± 0.6| 18.2 ± 0.6 | 0.971 | 10.4 ± 0.8
|                  |          |          |            |             |                     |
| CTT (g/mm²)       | 1.08 ± 0.05| 1.07 ± 0.11| 1.15 ± 0.10 | 0.575 | 1.79 ± 2.3
|                  |          |          |            |             |                     |
| Pupillary diameter (mm) | 8.4 (7.6–9.6) | 7.0 (5.9–8.4) | 7.5 (6.3–9.0) | 0.165 | NA
|                  |          |          |            |             |                     |
| TFBUT (s)         | 13.0 (12.1–13.9) | 11.2 (10.5–12.0) | 11.1 (10.4–11.9) | 0.669 | 16.7 ± 4.5
|                  |          |          |            |             |                     |

Values reported are mean ± SE or mean (95% CI). Pupillary diameter and TFBUT are reported as 95% CIs because of logarithmic transformation of data and asymmetry of the confidence limits.

*Baseline ocular data were collected on 3 days within a 2-week period before the beginning of the study. †Represents a comparison between diclofenac and control treatments, whereby baseline data are included as a covariable in the statistical model; values were considered significant at P < 0.05.

NA = Not applicable.

Table 3—Mean (95% CI) pupillary diameter (mm) at selected times after topical administration of 0.1% diclofenac or control solution 4 times/d for 7 days to 8 healthy cats.

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Control</th>
<th>Diclofenac</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7 PM</td>
<td>6.4 (5.4–7.5)</td>
<td>7.6 (6.4–9.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>7 AM</td>
<td>6.9 (5.8–8.2)</td>
<td>7.8 (6.6–9.2)</td>
<td>0.030</td>
</tr>
<tr>
<td>4</td>
<td>3 PM</td>
<td>7.1 (6.0–8.5)</td>
<td>7.5 (6.3–8.9)</td>
<td>0.012</td>
</tr>
<tr>
<td>6</td>
<td>7 PM</td>
<td>6.5 (5.5–7.7)</td>
<td>7.2 (6.0–8.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

First day of administration was designated as day 1. See Table 1 for remainder of key.

Figure 1—Mean ± SD plasma concentration of diclofenac at selected times after topical ocular administration 4 times/d for 7 days to 9 healthy cats. First day of administration was day 1 (black circles), and last day of administration was day 7 (white circles).

**SYSTEMIC ABSORPTION**

No detectable concentrations of diclofenac (<10 ng/mL) were found in samples collected before the start of either phase. No detectable concentrations of diclofenac were found in any cats during control treatment, except for 1 cat that had accidentally been given a single dose of diclofenac at 4 PM on day 1 of phase 1. In this cat, the plasma concentration of diclofenac at 240 minutes was 24.3 ng/mL. Diclofenac concentration in this cat was below the limit of detection by day 7.

Plasma concentrations of diclofenac were detected following the last drop on day 1 and 7 of the study (Figure 1). The Cmax, Tmax, and AUC from 0 to 240 minutes were determined for each cat and summarized (Table 4). The Cmax and AUC from 0 to 240 minutes on day 7 were significantly greater than values on day 1.

**SYSTEMIC ADVERSE EFFECTS**

For all cats, results of general physical examinations at the time of arrival and throughout the study were unremarkable, with no signs of dehydration,
Variables and Reference Ranges

Table 5—Mean ± SE values of selected serum biochemical and urinalysis variables for 9 healthy cats at baseline and after topical administration of 0.1% diclofenac or control solution.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range</th>
<th>Baseline*</th>
<th>Control</th>
<th>Diclofenac</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/L)</td>
<td>30–44</td>
<td>37.4 ± 1.0</td>
<td>33.7 ± 0.1</td>
<td>33.1 ± 0.1</td>
<td>0.076</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>27–48</td>
<td>29.1 ± 1.5</td>
<td>30.2 ± 0.8</td>
<td>29.3 ± 0.8</td>
<td>0.264</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>46–84</td>
<td>66.0 ± 2.0</td>
<td>65.9 ± 2.1</td>
<td>65.7 ± 2.1</td>
<td>0.791</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.6–7.7</td>
<td>4.74 ± 0.49</td>
<td>4.71 ± 0.47</td>
<td>4.69 ± 0.47</td>
<td>0.971</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>16–113</td>
<td>50.2 ± 4.5</td>
<td>41.0 ± 1.6</td>
<td>38.4 ± 1.6</td>
<td>0.155</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>31–105</td>
<td>66.9 ± 6.3</td>
<td>94.3 ± 8.1</td>
<td>83.7 ± 8.1</td>
<td>0.511</td>
</tr>
<tr>
<td>γ-glutamyl transpeptidase (U/L)</td>
<td>0–6</td>
<td>0.22 ± 0.15</td>
<td>0.23 ± 0.21</td>
<td>0.33 ± 0.21</td>
<td>0.652</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>6–12</td>
<td>8.00 ± 0.40</td>
<td>8.93 ± 0.33</td>
<td>8.77 ± 0.33</td>
<td>0.328</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>50–190</td>
<td>97.2 ± 4.1</td>
<td>96.6 ± 3.1</td>
<td>95.0 ± 3.1</td>
<td>0.497</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>0.80–2.29</td>
<td>2.27 ± 0.09</td>
<td>2.20 ± 0.07</td>
<td>1.87 ± 0.01</td>
<td>0.747</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.6–5.2</td>
<td>4.70 ± 0.10</td>
<td>4.68 ± 0.10</td>
<td>4.73 ± 0.10</td>
<td>0.771</td>
</tr>
<tr>
<td>Urine specific gravity</td>
<td>&gt; 1.035</td>
<td>1.046 ± 0.003</td>
<td>1.047 ± 0.000</td>
<td>1.051 ± 0.000</td>
<td>0.784</td>
</tr>
<tr>
<td>Urine protein-to-creatinine ratio</td>
<td>&lt; 0.4</td>
<td>0.44 ± 0.06</td>
<td>0.43 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>0.439</td>
</tr>
</tbody>
</table>

*Baseline data were obtained approximately 2 weeks before the start of the study.
†See Table 2 for remainder of key.

Results of baseline urinalysis were unremarkable for all cats. Urine protein-to-creatinine concentration ratio, determined from urine samples collected via cystocentesis, was borderline abnormal in 4 cats (0.4) and high in 3 cats (0.5 to 0.7). Urine protein-to-creatinine concentration ratios are typically < 0.2, whereas 0.2 to 0.4 is considered borderline abnormal, and postmortem examination or for the CBC and serum biochemical analysis performed the day before the cat was found dead. The cause of death was unknown but was suspected to be associated with a thromboembolic episode following placement of the catheter.
During the control treatment, elevations in ALT activity (2.53 ± 0.22 U/L; reference range, 31 to 105 U/L) were detected in 2 of 9 cats during diclofenac treatment and 3 of 9 cats during the control treatment. Mild hypoglycemia (4.0 to 4.2 mmol/L) was detected in 1 of 9 cats during diclofenac treatment and 4 of 9 cats during the control treatment. All cats receiving diclofenac or the control treatment had a decrease in GFR after phase 2, in which they were maintained or increased during phase 2, in which these cats received the control solution. In contrast, all cats receiving the control solution during phase 1 had a decrease in GFR after phase 2, in which they received diclofenac (Table 6).

**Discussion**

The goal of the study reported here was to characterize adverse ocular, renal, or hepatic effects associated with topical ophthalmic use of 0.1% diclofenac administered 4 times/d for 7 days to healthy cats. Systemic absorption of diclofenac was also examined. On the basis of the results of this study, mild conjunctival hyperemia was the only clinically relevant adverse ocular effect after topical application of 0.1% diclofenac to eyes of healthy cats. Systemic concentrations of diclofenac were detected, with systemic accumulation after multiple doses. In 1 cat, there were detectable diclofenac concentrations after only 1 drop in each eye. No adverse hepatic effects were detected. There were no adverse renal effects detected in phase 1, but they became apparent in phase 2, which suggested a potential risk of renal toxicosis with topical use of 0.1% diclofenac in presumably volume-contracted animals. Cats in the present study were clinically normal, and volume contraction was assumed to be attributable to collection of serial blood samples; renal effects may be more pronounced in clinically volume-contracted or dehydrated animals.

Mild conjunctival inflammation in the cats of the present study is consistent with reports in human patients, whereby transient conjunctival hyperemia and a stinging sensation are the most common adverse effects observed in humans topically treated with NSAIDs. Conjunctivitis (of unknown origin) was also detected in a recent study of cats in which the blood-aqueous barrier-stabilizing effects of 0.1% diclofenac and other topicaly administered anti-inflammatories were evaluated. Irritation associated with topical administration of NSAIDs such as 0.1% diclofenac may be attributed to the acidic nature of the free NSAID compound or to additives such as sorbic acid, edetate.

### Table 6
Mean ± SE GFR (mL/kg/min) for 9 healthy cats at baseline and after topical administration of 0.1% diclofenac and a control solution.

<table>
<thead>
<tr>
<th>GFR</th>
<th>Baseline*</th>
<th>Control</th>
<th>Diclofenac</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3.07 ± 0.22</td>
<td>3.12 ± 0.11</td>
<td>2.53 ± 0.22</td>
<td>0.288</td>
</tr>
<tr>
<td>Phase 1‡</td>
<td>NA</td>
<td>2.67 ± 0.16</td>
<td>2.75 ± 0.14</td>
<td>0.832</td>
</tr>
<tr>
<td>Phase 2‡</td>
<td>NA</td>
<td>2.75 ± 0.16</td>
<td>2.31 ± 0.16</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Baseline GFR was determined 1 day before phase 1 through plasma clearance of technetium Tc 99m pentatate; blood samples were collected via jugular catheters. ‡Cats received one of the treatments 4 times/d for 7 days (phase 1), which was followed by a 12-day washout period and then administration of the other treatment for another 7 days (phase 2).

See Table 2 for remainder of key.
The cause for ocular irritation associated with the control solution is unclear but may have been associated with repeated manipulation of the eye or reaction to components of the drop.

The CTT was evaluated with a Cochet-Bonnet aesthesiometer, and no evidence of diclofenac-induced change in corneal sensitivity was found in the present study. This is in contrast to studies\(^{11,12,14}\) of humans in which topical administration of 0.1% diclofenac and other NSAIDs decreased ocular pain following photo- refractive keratectomy in affected patients and decreased corneal sensitivity in healthy subjects. Numerous mechanisms have been proposed for diclofenac’s analgesic effect, including direct blockade of cation channels and alteration of corneal nerve excitability.\(^{44}\) Although a treatment effect was not identified in the present study, an effect cannot be ruled out. In contrast to the design for this study, many human studies\(^{11,12,14}\) have involved measurement of corneal sensitivity soon after multiple rounds of closely spaced diclofenac administration, which potentially allowed for an accumulation effect. Limitations in the sensitivity of the Cochet-Bonnet aesthesiometer, which is shortened in 0.5-cm increments, and the stress caused by the procedure may also have masked differences between treatments.

In human and veterinary medicine, NSAIDs are used to prevent intraoperative or experimentally induced inflammation and miosis.\(^{7,45}\) There is profound contraction of the iris sphincter of cats when prostaglandin F\(_2\alpha\) is applied in vitro to muscle strip preparations\(^{46}\) or for in vivo assessment of pupil diameter.\(^{47}\) In the present study, there was no significant treatment effect on pupillary diameter when all time points were considered together. However, there was a significant treatment effect on pupil diameter when all time points were considered together. Furthermore, the true peak concentration may have been missed. It is unlikely that the differences in pupil diameter would be of clinical consequence, although it is possible that NSAIDs, by decreasing endogenous concentrations of prostaglandins in the eyes, resulted in relative mydriasis.

No difference in IOP was found between the control and diclofenac treatments at any time point throughout the study. To the authors’ knowledge, only 1 feline study\(^{48}\) has been conducted to examine the effects of topical application of NSAIDs on IOP. In that study,\(^{16}\) mild increases in IOP in eyes treated with 0.03% flurbiprofen and 0.1% diclofenac were detected between 4 and 26 hours after induction of uveitis by means of anterior chamber paracentesis. The primary difference between the study reported here and that study\(^{16}\) is likely the absence of active uveitis in cats of the present study. The initial increase in IOP with breakdown of the blood-aqueous barrier can be attributed to uveal vasodilation and increased ultrafiltration and extravasation of fluid with blockage of the irido- corneal angle by protein and inflammatory cells.\(^{8}\) Differences in aqueous humor outflow and IOP between treatments may not have been detected in the present study because of the limited sensitivity of the measuring instrument, amount of time elapsed between drop administration and IOP measurement, and effects of circadian rhythm.\(^{40}\)

Despite its apparent safety in healthy feline eyes, patients receiving diclofenac topically should be carefully monitored, particularly those with concurrent corneal disease, such as corneal sequestra and feline herpesvirus–associated keratitis, considering that diclofenac has been associated with delayed epithelial healing and altered epithelial cell morphology.\(^{48}\) Although extremely rare and likely multifactorial, topical application of diclofenac has been associated with development of deep, melting, or perforating ulcers in human patients.\(^{10}\)

Plasma concentrations of diclofenac in the present study were determined with high-performance liquid chromatography coupled with mass spectrometry. The authors are aware of only 2 other studies\(^{49,50}\) in which investigators evaluated plasma concentrations of diclofenac following topical application. Both of those studies\(^{49,50}\) involved single-dose pharmacokinetic analysis of topical administration of 0.1% diclofenac to rabbits (0.02 mg/kg\(^{50}\) and 0.008 mg/kg\(^{50}\)). Despite species differences and the multiple-dosing regimen used in the present study, mean ± SD Cmax (36.8 ± 24.4 ng/mL and 87.6 ± 23.3 ng/mL on days 1 and 7, respectively) and Tmax (26.3 ± 15.5 minutes and 24.6 ± 15.5 minutes for days 1 and 7, respectively) determined for cats of the present study were similar to those obtained for rabbits (mean ± SE Cmax, 72.7 ± 14.2 ng/mL; Tmax, 15 minutes) in one of those studies.\(^{49}\) In contrast, the Cmax determined for the cats of the present study was considerably higher than the Cmax determined for rabbits in the other study\(^{50}\) (6.1 ng/mL). Considering that sample collection did not begin until 30 minutes after administration of eye drops in that study,\(^{48}\) it is suspected that the true peak concentration may have been missed. Furthermore, Tmax in that study\(^{50}\) (82.3 minutes) was also significantly later than for cats of the present study, possibly because plasma diclofenac concentrations did not fit a biexponential curve, which made curve fitting and estimation of Tmax a challenge. Despite differences in Cmax and Tmax, the mean ± SD AUC from 0 to 240 minutes determined for the cats reported here (day 1, 10,953 ± 3,502 min•ng/mL; day 7, 16,502 ± 4,568 min•ng/mL), particularly after day 1, was similar to that determined for rabbits (6,540 min•ng/mL),\(^{50}\) which suggested that total exposure was similar between the studies.

Systemic drug accumulation occurs when a drug is incompletely eliminated from the body before the next dose is administered. Accumulation continues with successive doses until a steady state is reached, whereby the rate of drug input equals the rate of elimination.\(^{31}\) Plasma accumulation of diclofenac was detected in the present study, with plasma concentrations and AUC of diclofenac on day 7 being signifi-
cently greater than on day 1. The primary reason for drug accumulation was suspected to be the relatively short dosing interval (every 4 hours), compared with the plasma half-life.22 Further studies would be necessary to determine the plasma half-life and to better characterize plasma accumulation after topical administration of diclofenac to cats. Additional minor contributors to accumulation include hypoproteinemia, hypovolemia, and a reduced GFR.

The major finding for the serum biochemical analysis was hypoproteinemia, although there were no significant (P = 0.215) differences between the control and diclofenac treatments. Hypoproteinemia was likely a result of repeated blood sampling and replacement of blood with saline solution. Given that diclofenac is highly protein bound,53 it might be anticipated that decreased protein concentrations could lead to increased concentrations of unbound active drug, which would increase the potential for toxic effects, such as a reduction in GFR. However, it is unlikely that hypoproteinemia contributed to the suspected adverse renal effects, given that the concentration of albumin, the plasma protein to which diclofenac is 99% to 99.4% bound, remained within reference limits54 throughout the study for both the control and diclofenac treatments. In addition, it is possible that withdrawal of large volumes of blood led to hypovolemia with redistribution of blood away from organs of elimination (eg, the liver).

Reduction of GFR in diclofenac-treated cats during phase 2 of the study was presumably attributable to induction of a volume-contracted state from repeated blood sampling, with cumulative effects becoming apparent only later in the study. Although volume contraction was not proven, it is suspected that saline solution replacement did not maintain euvoolemia. The effect of a reduction in GFR on the elimination of diclofenac may also play a role in accumulation, although the route of elimination of diclofenac in cats remains unknown. If diclofenac is renally eliminated in cats, reduction in GFR may cause prolonged elimination, accumulation, and development of a cycle of increased diclofenac concentrations and reduced GFR. In human patients with renal insufficiency, initial accumulation of diclofenac metabolites occurs as a result of a decrease in GFR but is of little clinical concern because diclofenac and its metabolites are likely eliminated via alternate compensatory mechanisms (eg, biliary excretion) with no net decrease in elimination or accumulation over time.55 In addition to their role in the elimination of drugs, the kidneys may also play a limited role in metabolism. For example, in humans, the renal parenchyma contributes substantially to metabolism of acetaminophen.56 Thus, although much remains to be clarified with regard to the pharmacokinetics of diclofenac in cats, renal compromise may have led to accumulation of diclofenac as a result of reduced elimination or metabolism.

Through inhibition of prostaglandin synthesis, NSAIDs blunt the ability of the kidneys to counteract decreased renal perfusion in volume-contracted states. In euvoolemia, prostaglandins do not contribute substantially to renal perfusion and thus NSAIDs do not have a major effect on renal function.57-59 In healthy euvolemic cats, there were no changes in GFR following a 5-day course of meloxicam.20 Similarly, although studies in cats are lacking, systemic diclofenac administration to young and elderly euvolemic human subjects perioperatively or over several weeks had no effect on GFR.59 In contrast, volume contraction is associated with stimulation of the renin-angiotensin-aldosterone system and an increase in sympathetic outflow, both of which promote renal vasoconstriction.57-58 In the face of increased systemic concentrations of vasopressors, prostaglandins play an important role in maintaining renal perfusion by stimulating compensatory renal vasodilation at the level of the renal medullary and cortical arterioles.60,61 In cats, prostaglandins E1 and E2 have potent dilatory activity in the renal vascular bed,62 and prostaglandins E3 and A5 can counteract vasoconstriction secondary to sympathetic stimulus and increased angiotensin concentrations.62

On the basis of results of serum biochemical analyses, there was no evidence for hepatic toxicosis associated with topical administration of 0.1% diclofenac in the present study. Similarly, studies of long-term meloxicam, piroxicam, or robenacoxib use in cats did not reveal adverse hepatic effects.54,55,60,64 and perioperative administration of meloxicam and carprofen was associated with elevations in aspartate aminotransferase activity but no other alterations in hepatic variables.65 Further studies with a larger sample size are needed in view of the fact that the incidence of hepatic toxicosis is expected to be low. In humans, it is estimated that there are between 1 and 8 cases of hepatotoxicosis for every 100,000 NSAID prescriptions.66,67 Although NSAID-associated hepatotoxicosis is incompletely understood, it is attributed to 2 main mechanisms in human patients: hypersensitivity reaction and alteration of hepatic metabolism.68

A major limitation of the present study was the likely induction of an anemic, hypoproteinemic, and volume-contracted state, despite an effort to limit the number of blood samples. Separation of the pharmacokinetic and GFR analyses or use of a parallel rather than crossover design would have permitted a greater number of blood samples to be collected during the pharmacokinetic analysis, which would have allowed for a more robust analysis of drug disposition, especially the terminal slope and accurate determination of terminal half-life. Alternatively, a longer washout period could have been used. In the present study, blood volumes collected and washout periods observed adhered to the guidelines published by the European Federation of Pharmaceutical Industries Associations and the European Centre for the Validation of Alternative Methods for multiple sampling.69 In addition, collected blood was replaced 1:1 with saline solution, a regimen chosen to minimize risk of hypovolemia while minimizing dilution of the blood volume. Use of
more stringent minimums for PCV and inclusion of a minimum for total protein concentration before being allowed to continue in additional phases of the study could also decrease the likelihood of hypovolemia. Additional limitations of the present study included the small number of cats, timing of sample collection, and site of sample collection. In addition to separation of the pharmacokinetic analysis and GFR determination, separation of the ocular examination from the systemic measurements would have been beneficial. Although similar manipulations were performed on all cats, it is likely that ocular irritation scores, STT, TFBUT, and IOP were affected by sedative agents used for placement of catheters in jugular veins, Elizabethan collars, neck bandages, and a lack of normal grooming.

In the study reported here, topical application of 0.1% diclofenac (4 times/d for 7 days) was tolerated well by healthy cats. Mild conjunctival hyperemia was the only adverse ocular effect worthy of mention. Repeated topical administration of 0.1% diclofenac resulted in systemic drug accumulation. Although the concentrations of diclofenac attained were low, they may have led to a reduction in GFR, likely in the face of a volume-contracted or hypovolemic state. Volume contraction is hypothesized to have occurred secondary to serial blood sampling. Thus, results of this study suggested that topical administration of 0.1% diclofenac should be used with caution in patients in volume-contracted states. Patients that may be at risk for adverse renal effects include those affected by hypovolemia, dehydration, cardiac disease, hepatic disease, renal disease, and hypotension.37–50 Considering that anterior uveitis in cats can be secondary to systemic diseases such as infection and neoplasia,1,5,17 careful patient selection may be necessary. No detectable hepatic effects were associated with topical administration of diclofenac in this study. Future studies are needed for more accurate determination of pharmacokinetics and to determine the effects of topically administered NSAIDs without the confounding effects of hypovolemia.

Acknowledgments

This manuscript represents a portion of a thesis submitted by Dr. Hsu to the Ontario Veterinary College Department of Clinical Studies as partial fulfillment of the requirements for a Master of Science degree. Supported by the Ontario Veterinary College Pet Trust Fund.

The authors thank Gabrielle Monteith and Dr. William Sears for assistance with statistical analysis. Dr. Jerome Del Castillo for assistance with pharmacokinetic analysis design, and Karen Schwindt for assistance with data collection.

Footnotes

a. Liberty Research, Waverly, NY.
b. Animal Health Laboratories, Ontario Veterinary College, Guelph, ON, Canada.
c. Kowa SL-15, Kowa, Tokyo, Japan.
d. STT strips, Alcon Canada, Mississauga, ON, Canada.
e. Fluoresets, Chauvin Pharmaceuticals Ltd, Aubenas, France.
f. TonoVet, Tiolat Ltd, Helsinki, Finland.
g. Heine Omega 2c, Heine Optotechnik, Herrsching, Germany.
h. Mydriacyl 1%, Alcon Canada, Mississauga, ON, Canada.
i. Voltaren Ophtha, Novartis Pharmaceuticals Canada Inc, Dorval, QC, Canada.
j. Tears Natured II, Alcon Canada, Mississauga, ON, Canada.
k. Timex Ironman Triathlon women’s watch, Timex Canada, Markham, ON, Canada.
l. Cochot-Bonnet aesthesiometer, Luneau Ophthalmologie, Chartres, France.
m. NeoMedical V-Cath PICC, NeoMedical Inc, Fremont, Calif.
n. Vetalar, Bioniche Animal Health Canada Inc, Belleville, ON, Canada.
p. Acetpromazine 2 mg/mL, Pharmacy, Ontario Veterinary College, Guelph, ON, Canada.
q. Diprivana, Astra Zeneca, Mississauga, ON, Canada.
r. 95% DTPA, Bristol-Myers Squibb Imaging, Montreal, QC, Canada.
s. Nosorb, Carco Inc, Cape Coral, Fl.
u. Cardell veterinary monitor, model 9401, Midmark, Tampa, Fl.
w. Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, Md.
x. API 2000, Applied Biosystems, Foster City, Calif.
z. Usansky, Desai, Tang-Liu, Irvine, Calif.
ab. SigmaPlot, version 12, Systat Software Inc, San Jose, Calif.

References


