The effects of topical diclofenac, flurbiprofen, and ketorolac on corneal sensitivity in normal cats

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Abstract

Surface ocular diseases are common, vision threatening, and painful ophthalmic conditions encountered in cats. When possible, specific therapies for the treatment of an underlying cause are administered. However, clinicians are often compelled to use symptomatic treatments such as topical glucocorticoids to reduce ocular inflammation and nonsteroidal anti-inflammatory drugs (NSAIDs) to control clinical signs associated with ocular pain. Although topical NSAIDs are commonly used in the treatment of a variety of surface ocular diseases, studies demonstrating the effects of topically applied NSAIDs in cats are limited. This study served to investigate the immediate and prolonged dosing effects of three different topical NSAIDs on corneal sensitivity in healthy domestic shorthair cats. Twelve normal, non-brachycephalic domestic shorthair cats were enrolled in this prospective, randomized, masked, crossover study. To determine the immediate dosing effects, one drop of the assigned treatment (0.1% diclofenac sodium ophthalmic solution, 0.5% ketorolac tromethamine ophthalmic solution, or 0.03% flurbiprofen sodium ophthalmic solution) or control (0.9% saline) solution was applied to both eyes every five minutes for five applications, and corneal sensitivity was measured using a Cochet-Bonnet esthesiometer every 15 minutes for one hour. To determine the prolonged dosing effects, one drop of the treatment or control solution was applied to both eyes every 12 hours for five days, and corneal sensitivity was measured at the end of the last treatment. A 2-day washout period occurred between each group for both treatment phases. Neither topical 0.1% diclofenac sodium, 0.5% ketorolac tromethamine, nor 0.03%

flurbiprofen sodium had any effect on corneal sensitivity immediately following repeated application or after prolonged twice-daily dosing in normal, non-brachycephalic cats.

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COX	Cyclooxygenase
CS	Corneal sensitivity
CTT	Corneal touch threshold
DSH	Domestic shorthair
NSAID	Nonsteroidal anti-inflammatory drug

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Chapter 1 Background Information and Hypothesis

Feline Surface Ocular Disease

The feline cornea is a densely innervated and highly sensitive tissue, with sensory nerves originating from the ophthalmic branch of the trigeminal ganglia (Chan-Ling 1989). In addition to providing sensory function, corneal nerves play an important role in maintaining the structure and function of the cornea, and in corneal wound repair. Consequently, damage to corneal nerves due to inflammation or trauma has the potential to lead to diminished corneal sensation and resultant corneal disease.

Surface ocular diseases affecting the cornea and conjunctiva are common, painful, and potentially vision threatening conditions in cats. Viral surface ocular disease attributable to feline herpesvirus-1 occurs with the greatest clinical frequency, often leading to conjunctivitis and corneal ulceration (Nasisse et al. 1989). When possible, specific therapies for treatment of the underlying disease (e.g., topical and systemic antiviral drugs) are administered. However, clinicians are often compelled to use symptomatic treatments to control clinical signs associated with ocular inflammation and discomfort. The most commonly used symptomatic treatments for ocular surface inflammation are topical anti-inflammatory medications (e.g., glucocorticoids and NSAIDs) and immunomodulatory medications (e.g., cyclosporine A and tacrolimus).

Ophthalmic NSAIDs

There is abundant evidence that topical glucocorticoids may exacerbate herpetic disease and should be avoided in most cases of surface ocular disease in cats and other species (Nasisse et al. 1989; Maggs 2005; Gould 2011; Haesaert 1986). When topical anti-inflammatory therapy is necessary, NSAIDs may be used preferentially over glucocorticoids in cases of corneal ulceration, trauma, or infection (Hendricks et al. 1990). Currently, there are no licensed topical NSAIDs for use in veterinary medicine, thus human products are used in an off-label manner. A variety of topical NSAIDs have been used to reduce ocular pain caused by corneal injury (Barba et al. 2000; Chen, Gallar, and Belmonte 1997), reduce localized inflammation during active periods of inflammation (Aragona et al. 2005; Szerenyi et al. 1994; Hendricks et al. 1990), for the treatment of anterior uveitis (Giuliano 2004; Gaynes and Fiscella 2002; Gaynes and Onyekwuluje 2008), and following surgical procedures in humans (Sun and Gimbel 1997; Narvaez, Kroll, and Guzek 2002) and various veterinary species including cats (Barba et al. 2000).

Nonsteroidal anti-inflammatory drugs act by inhibiting COX, which mediate the breakdown of arachidonic acid, a precursor of pro-inflammatory prostaglandins and other metabolic products including eicosanoids and thromboxane (Giuliano 2004; Kim, Flach, and Jampol 2010; Ahuja et al. 2008; Gaynes and Onyekwuluje 2008). The COX enzyme exists in two prominent isoforms: COX-1 (constitutive) which is responsible for production of prostaglandins and required for normal tissue homeostasis, and COX-2

(inducible) which produces prostaglandins at sites of inflammation. Nonsteroidal anti-inflammatory drugs inhibit both isoforms of COX and are classified according to their ability to preferentially select for either COX-1 or COX-2 isoenzymes (Giuliano 2004; Gaynes and Fiscella 2002). In humans and various veterinary species, the inducible isoenzyme COX-2 has been associated with ocular inflammatory and pathological processes (Radi and Render 2008; Sellers, Silverman, and Khan 2004; Sim et al. 2018). Arachidonic acid metabolites contribute to the local inflammatory response and to the sensitization and excitation of various nociceptors (Aragona et al. 2000). Topical NSAIDs have been shown to decrease nociceptor excitation in clinically normal (i.e., non-inflamed) eyes of both cats (Barba et al. 2000; Chen, Gallar, and Belmonte 1997) and humans (Szerenyi et al. 1994; Aragona et al. 2000). However, different topically applied NSAIDs have shown variable efficacy in reducing ocular discomfort in humans (Acosta et al. 2005; Aragona et al. 2005; Narvaez, Kroll, and Guzek 2002; Sun and Gimbel 1997; Szerenyi et al. 1994).

In contrast to topical glucocorticoids, significant side effects of topical NSAIDs are uncommon, although ocular irritation with epiphora, blepharospasm, and conjunctival hyperemia may be noted (Gaynes and Fiscella 2002; Giuliano 2004; Seitz et al. 1996; Aragona et al. 2000). The effects of topical NSAIDs on corneal wound healing are ambiguous, with some studies finding no change (Szucs et al. 2000; Barba et al. 2000; Loya et al. 1994), and others suggesting that topical NSAIDs may be detrimental and prolong healing time (Hendrix, Ward, and Barnhill 2002; Tomas-Barberan and Fagerholm 1999).

Corneal analgesia following administration of topical NSAIDs can be attributed to a number of mechanisms. Through the inhibition of COX, NSAIDs reduce sensitization of nociceptors associated with prostaglandins in inflamed tissues, and thus the pain associated with inflammation. Corneal hypoesthesia may also occur due to mechanisms other than that of COX inhibition. Chen et al. demonstrated that topical NSAIDs decrease sensory influx from corneal polymodal nociceptors in anesthetized cats (Chen, Gallar, and Belmonte 1997). The authors theorized that this effect may be mediated by mechanisms other than COX inhibition alone, including blockade of neuronal ionic channels, or other mechanisms altering the excitability of polymodal nerves.

Although topical NSAIDs are commonly used in the treatment of a variety of surface ocular diseases, studies demonstrating their effects in cats are limited. Hsu et al. investigated the systemic absorption and adverse ocular and systemic effects of topically applied 0.1% diclofenac sodium in DSH cats, and found that there was no significant effect on CS in study cats receiving 0.1% diclofenac sodium when compared to a control group (Hsu et al. 2015). In that study, CS values were obtained immediately following administration of a single drop of 0.1% diclofenac sodium.

To the authors' knowledge, no studies have been conducted evaluating the immediate effects of topically applied 0.03% flurbiprofen sodium or 0.5% ketorolac tromethamine on CS in normal feline eyes. Additionally, no studies have evaluated the effects of prolonged dosing with any topically applied NSAIDs in cats. Therefore, the goals of this study were to investigate the

immediate and prolonged dosing effects of three different topical NSAIDs on CS in healthy DSH (non-brachycephalic) cats. The authors hypothesized there would be no effect on CS in normal cats with immediate or prolonged administration.

Chapter 2 Materials and Methods

Animals Studied

Twelve purpose-bred DSH cats owned by the Kansas State University Comparative Medicine Group were enrolled in a prospective, randomized, masked, crossover study. All protocols were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee. Prior to enrollment, each cat underwent an adnexal and anterior segment examination performed by a board-certified veterinary ophthalmologist to evaluate for active or previous surface ocular disease, which included a neuro-ophthalmic examination, fluorescein staining (fluorescein sodium, Akorn, Inc., Lake Forest, IL), rebound tonometry (Tono-Vet®, Icare Finland, Espoo, Finland), and slit-lamp biomicroscopy (SL-17, Kowa, Torrance, CA). All cats were housed in a climate-controlled environment with a 12-hour light-dark cycle, room temperature between 20°C and 23°C, and humidity between 54% and 57%. Cats were individually housed in separate enclosures for the immediate effects portion of the study, and group housed in their maintenance enclosures for the prolonged dosing effects portion of the study.

Treatments

Three commercially available ophthalmic NSAIDs and a 0.9% saline solution (sodium chloride injection 0.9%, Fresenius USA Manufacturing Inc., Walnut Creek, CA) control were used. The topical NSAIDs included 0.1% diclofenac sodium ophthalmic solution (Akorn, Inc., Lake Forest, IL), 0.5%

ketorolac tromethamine ophthalmic solution (Akorn, Inc., Lake Forest, IL), and 0.03% flurbiprofen sodium ophthalmic solution (Amici Pharmaceuticals, Melville, NY). The drugs and 0.9% saline solution were sterilely dispensed into eye dropper bottles designed to dispense a standard 0.05 milliliter drop and labeled ‘A’, ‘B’, ‘C’, and ‘D’ by the Kansas State University Veterinary Health Center Dispensary, with the investigators masked to the assignment of the drugs until the conclusion of data analysis.

The twelve study cats were given a 3-day acclimation period to the immediate phase housing environment and general handling for the study. During this acclimation period, each cat received a single drop of 0.9% saline solution applied to each eye twice daily and had CS measured in both eyes on the last day of the acclimation period, serving as a baseline for the immediate dosing effects portion of the study.

Following the acclimation period, the immediate effects portion of the study commenced. All cats were assigned to one of four groups (0.1% diclofenac sodium ophthalmic solution, 0.5% ketorolac tromethamine ophthalmic solution, 0.03% flurbiprofen sodium ophthalmic solution, and 0.9% saline solution) by use of an online randomizer tool (https://www.randomizer.org/). One drop of the assigned treatment was applied to both eyes every five minutes for a total of five applications. Fifteen minutes following instillation of the fifth round of drops, CS was measured for each eye, and measurements were repeated every 15 minutes for a total of four measurements. After a washout period of two days, a crossover was performed and subsequently repeated until each cat had received each of the

three drugs and the 0.9% saline solution control. During each washout period, CS was measured in both eyes once daily to confirm that it returned to baseline prior to the next treatment phase. The CS for each eye on the last day of the final washout period served as a baseline for the prolonged dosing effects portion of the study. A washout period of two days was selected based on the elimination half-life of diclofenac sodium, which has the longest half-life of the three NSAIDs used in this study (flurbiprofen sodium 1.55 hours in rabbits, ketorolac tromethamine 4.14 ± 1.18 hours in cats, and diclofenac sodium 5.9 ± 2.4 hours in cats) (Villa et al. 2015; Hsu et al. 2015; Tang-Liu, Liu, and Weinkam 1984).

Following completion of the immediate effects portion of the study, the prolonged effects portion of the study commenced. Cats were again randomly assigned to one of the four groups. One drop of the assigned treatment was applied to both eyes every 12 hours for a total of five days. Fifteen minutes following instillation of the last round of drops on the fifth day of treatment, CS was measured for each eye. After a washout period of two days, a crossover was performed and subsequently repeated until each cat had received each of the three drugs and the 0.9% saline solution control. During each washout period, CS was measured in both eyes once daily to confirm that it returned to baseline prior to the next treatment phase (Figure 2.1).

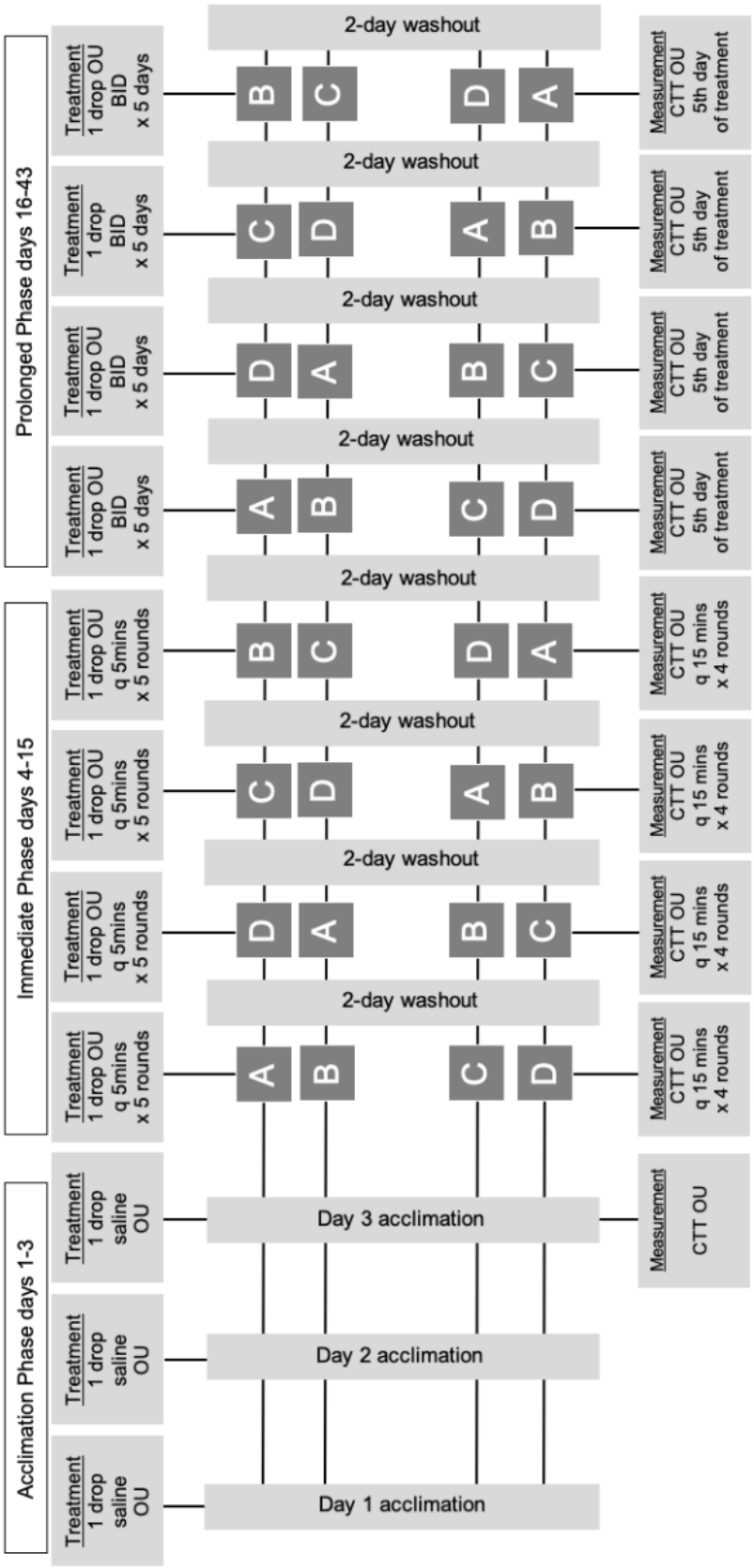

Figure 2.1 Study design schematic for randomized crossover study

Corneal Sensitivity Measurement

Corneal sensitivity measurements were obtained using a Cochet-Bonnet esthesiometer (Luneau Ophtalmologie, Chartres Cedex, France). Starting at a length of 60 millimeters, the 0.012-millimeter nylon filament was advanced perpendicularly toward the axial cornea until a slight deflection of the filament was noted after corneal contact (Figure 2.2).

Figure 2.2 Photograph of the left eye of a domestic shorthair cat. A Cochet-Bonnet esthesiometer is used to measure corneal sensitivity.

If a blink reflex was not noted on at least three of five attempts, the filament length was shortened in 5-millimeter decrements and the procedure was repeated. Corneal sensitivity was recorded as the shortest length of filament in millimeters that induced a blink response on three of five attempts (i.e., a shorter

filament length correlates with a less sensitive cornea). Corneal sensitivity was then converted to CTT in grams per millimeter squared (g/mm^2) based on a conversion table supplied by the manufacturer. Only the axial cornea was evaluated, as it has previously been shown to be the most sensitive region (Blocker and Van Der Woerdt 2001). Data were collected in a longitudinal fashion, in which change over time for each drug was assessed and time 0 was baseline for each subject. In an effort to minimize environmental (e.g., lighting conditions, temperature, humidity) and procedural variability on corneal esthesiometry, all measurements were performed in the same examination room by a single investigator. Minimal and gentle manual restraint was used for all study subjects. Additionally, to eliminate concerns for different mechanical properties (e.g., rigidity and flexibility) between older filaments and those recently manufactured, a new filament was obtained and utilized throughout the study (Lum and Murphy 2018). During all measurements, ambient temperature and humidity were recorded with a commercial thermometer and hygrometer (Extech, Waltham, MA).

Statistical Analysis

Statistical analyses were performed to compare the esthesiometry data obtained in relation to time (in minutes and hours) and each drug used. To test for normality, an Anderson-Darling test was applied. Multiple time periods in each eye were not normally distributed, therefore a nonparametric repeated measurements ANOVA (Friedman's Test) with a Tukey's multiple comparisons

posthoc was used to compare CS over time in each eye within each treatment group, and to compare CS between baseline and each day of the washout period. Because the data were not normally distributed, CS were reported as median (min-max) values. $P < 0.05$ was considered significant for all comparisons.

Chapter 3 Results

Mean age was 3.3 ± 1.3 years, with 10 castrated males and two spayed females. Each eye was treated as an individual variable. There were no significant differences between right and left eyes at any time points during the immediate ($p \geq 0.66$) and prolonged ($p \geq 0.82$) dosing phases. Baseline median (min-max) CTT for all subjects evaluated during the immediate dosing portion of the study was 0.70 (0.55-0.8) g/mm^2 (filament length 45.0 (40-50) mm) for right eyes, and 0.70 (0.55-0.8) g/mm^2 (filament length 45.0 (40-50) mm) for left eyes. Baseline median (min-max) CTT for all subjects evaluated during the prolonged dosing portion of the study was 0.70 (0.5-1.0) g/mm^2 (filament length 45.0 (35-55) mm) for right eyes, and 0.75 (0.5-1.0) g/mm^2 (filament length 42.5 (35-55) mm) for left eyes.

Table 3.1 shows CS represented by the median (min-max) Cochet-Bonnet filament length (mm) and CTT (g/mm^2) for each evaluation time and drug. After multiple instillations and during each washout day, none of the study drugs or the 0.9% saline control solution had any significant effect on CS when compared to baseline measurements ($p \geq 0.09$).

Table 3.2 shows CS represented by the median (min-max) Cochet-Bonnet filament length (mm) and CTT (g/mm^2) for each evaluation time and drug. After repeated twice-daily instillations and during each washout day, none of the study drugs or the 0.9% saline control solution had any significant effect on CS when compared to baseline measurements ($p \geq 0.05$).

Treatment	Eye	Variable	Baseline	Time after instillation of last dose (min)				*P* value*
				15	30	45	60	
0.9% Saline solution	OD	Filament length (mm)	45.0 (40-50)	45.0 (35-60)	45.0 (35-60)	45.0 (35-55)	45.0 (35-50)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.70 (0.4-1.0)	0.70 (0.4-1.0)	0.70 (0.5-1.0)	0.70 (0.55-1.0)	0.09
	OS	Filament length (mm)	45.0 (40-50)	45.0 (35-60)	40.0 (35-55)	45.0 (30-50)	42.5 (35-50)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.70 (0.4-1.0)	0.80 (0.5-1.0)	0.70 (0.55-1.4)	0.75 (0.55-1.8)	0.11
0.1% Diclofenac sodium	OD	Filament length (mm)	45.0 (40-50)	45.0 (30-55)	45.0 (35-55)	45.0 (35-55)	45.0 (35-55)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.70 (0.5-1.4)	0.70 (0.5-1.0)	0.70 (0.5-1.0)	0.70 (0.5-1.0)	0.09
	OS	Filament length (mm)	45.0 (40-50)	42.5 (35-55)	45.0 (30-55)	45.0 (40-55)	45.0 (30-55)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.75 (0.5-1.0)	0.70 (0.5-1.4)	0.70 (0.5-0.8)	0.70 (0.5-1.4)	0.17
0.5% Ketorolac tromethamine	OD	Filament length (mm)	45.0 (40-50)	40.0 (35-55)	42.5 (30-55)	45.0 (30-55)	42.5 (35-55)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.80 (0.5-1.0)	0.75 (0.5-1.4)	0.70 (0.5-1.4)	0.75 (0.5-1.0)	0.89
	OS	Filament length (mm)	45.0 (40-50)	40.0 (35-60)	40.0 (35-55)	45.0 (35-55)	42.5 (35-50)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.80 (0.4-1.0)	0.80 (0.5-1.0)	0.70 (0.5-1.0)	0.75 (0.55-1.0)	0.62
0.03% Flurbiprofen sodium	OD	Filament length (mm)	45.0 (40-50)	45.0 (35-50)	45.0 (30-50)	45.0 (35-55)	45.0 (30-50)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.70 (0.55-1.0)	0.70 (0.55-1.4)	0.70 (0.5-1.0)	0.70 (0.55-1.4)	0.16
	OS	Filament length (mm)	45.0 (40-50)	40.0 (35-50)	40.0 (30-50)	42.5 (35-50)	45.0 (30-50)	—
		CTT (g/mm^2)	0.70 (0.55-0.8)	0.80 (0.55-1.0)	0.80 (0.55-1.4)	0.75 (0.55-1.0)	0.70 (0.55-1.4)	0.21

Table 3.1 Median (min-max) Cochet-Bonnet filament length (mm) and CTT (g/mm^2) for the immediate dosing phase at times 0 min (baseline), 15 min, 30 min, 45 min, and 60 min for cats treated topically with 0.9% sodium chloride control, 0.1% diclofenac, 0.5% ketorolac, and 0.03% flurbiprofen.

Treatment	Eye	Variable	Baseline	Time after instillation of last dose (h)			*P* value*
				0.25	24	48	
0.9% Saline solution	OD	Filament length (mm)	45.0 (35-55)	42.5 (35-55)	40.0 (35-55)	42.5 (35-55)	—
		CTT (g/mm^2)	0.70 (0.5-1.0)	0.75 (0.5-1.0)	0.80 (0.5-1.0)	0.75 (0.5-1.0)	0.25
	OS	Filament length (mm)	42.5 (35-55)	40.0 (30-55)	42.5 (30-50)	45 (30-55)	—
		CTT (g/mm^2)	0.75 (0.5-1.0)	0.80 (0.5-1.4)	0.77 (0.55-1.4)	0.70 (0.5-1.4)	0.44
0.1% Diclofenac sodium	OD	Filament length (mm)	45.0 (35-55)	45.0 (35-55)	40.0 (35-50)	40.0 (35-55)	—
		CTT (g/mm^2)	0.70 (0.5-1.0)	0.70 (0.4-1.0)	0.80 (0.55-1.0)	0.80 (0.5-1.0)	0.06
	OS	Filament length (mm)	42.5 (35-55)	45.0 (30-60)	45.0 (35-55)	40.0 (35-55)	—
		CTT (g/mm^2)	0.75 (0.5-1.0)	0.70 (0.4-1.4)	0.70 (0.5-1.0)	0.80 (0.5-1.0)	0.47
0.5% Ketorolac tromethamine	OD	Filament length (mm)	45.0 (35-55)	40.0 (35-55)	42.5 (35-55)	40.0 (35-55)	—
		CTT (g/mm^2)	0.70 (0.5-1.0)	0.80 (0.5-1.0)	0.75 (0.5-1.0)	0.80 (0.5-1.0)	0.71
	OS	Filament length (mm)	42.5 (35-55)	42.5 (35-55)	45.0 (35-55)	45.0 (30-50)	—
		CTT (g/mm^2)	0.75 (0.5-1.0)	0.75 (0.5-1.0)	0.70 (0.5-1.0)	0.70 (0.55-1.4)	0.80
0.03% Flurbiprofen sodium	OD	Filament length (mm)	45.0 (35-55)	42.5 (35-55)	45.0 (35-60)	40.0 (35-55)	—
		CTT (g/mm^2)	0.70 (0.5-1.0)	0.75 (0.5-1.0)	0.70 (0.4-1.0)	0.80 (0.5-1.0)	0.05
	OS	Filament length (mm)	42.5 (35-55)	45.0 (40-55)	42.5 (30-60)	40.0 (35-55)	—
		CTT (g/mm^2)	0.75 (0.5-1.0)	0.70 (0.5-0.8)	0.75 (0.4-1.4)	0.80 (0.5-1.0)	0.26

Table 3.2 Median (min-max) Cochet-Bonnet filament length (mm) and CTT (g/mm^2) for the prolonged dosing phase at times 0 min (baseline), immediately following administration of the last round of drops (0.25 hrs), 24 hours following administration of the last rounds of drops (24 hrs), and 48 hours following administration of the last round of drops (48 hrs) for cats treated topically with 0.9% sodium chloride control, 0.1% diclofenac, 0.5% ketorolac, and 0.03% flurbiprofen.

Chapter 4 Discussion

Although previous similar studies in dogs (Cantarella et al. 2017) and humans (Aragona et al. 2005; Aragona et al. 2000; Seitz et al. 1996; Singer, Kennedy, and Wittpenn 2015; Sun and Gimbel 1997; Szerenyi et al. 1994; Narvaez, Kroll, and Guzek 2002) have demonstrated the analgesic potential of topical diclofenac, ketorolac, and flurbiprofen, the findings of the present study suggest that repeated immediate and prolonged twice-daily application of these NSAIDs does not cause hypoesthesia in healthy feline corneas. The median baseline CTT (both immediate and prolonged dosing phases) for subjects in this study was lower than other published values for cats, but still considered within reference range due to the large variability in previously published studies (Chan-Ling 1989; Hsu et al. 2015; Blocker and Van Der Woerdt 2001; Binder and Herring 2006).

Our study produced similar findings when compared to a 2017 study by Dorbandt et al., in which neither 0.1% diclofenac sodium nor 0.03% flurbiprofen sodium had any effect on CS after multiple drops or twice daily dosing for 30 days in clinically normal canine eyes (Dorbandt et al. 2017). Another 2017 study of non-brachycephalic dogs concluded that CS was decreased by topical 0.1% diclofenac sodium at 75- and 90-minutes following application, while 0.03% flurbiprofen sodium resulted in an immediate increase in CS between 15- and 30-minutes following application, and that application of 0.5% ketorolac tromethamine had no effects on CS (Cantarella et al. 2017). Some of the results of the aforementioned canine studies as well as our current feline study contrast with

what has been shown in humans, where repeated administration of numerous topical NSAIDs including 0.1% diclofenac sodium, 0.5% ketorolac tromethamine, 0.3% nepafenac, and 0.07% bromfenac have been observed to cause an immediate (but transient) decrease in CS in clinically normal eyes (Aragona et al. 2000; Szerenyi et al. 1994; Seitz et al. 1996; Singer, Kennedy, and Wittpenn 2015; Sun and Gimbel 1997; Narvaez, Kroll, and Guzek 2002; Acosta et al. 2005; Aragona et al. 2005). The conflicting results observed between felines, canines and humans may ultimately reflect species-specific differences in neurochemistry and corneal receptor prevalence and density (i.e., proportion and distribution of mechanonociceptors and polymodal nociceptors).

Limited information is available about COX expression in the ocular tissues of cats, although arachidonic acid metabolites (e.g., prostaglandins and thromboxane) have been detected in ocular tissues of healthy cats (Kulkarni, Fleisher, and Srinivasan 1984). A 2018 study evaluating COX-2 expression in normal and uveitic feline eyes demonstrated that COX-2 was not constituently expressed in healthy corneas, but that it was detected in nearly half of the corneas of uveitic eyes, suggesting that it may only be present when induced during active states of inflammation (Sim et al. 2018). These findings compliment a study by Sellers et al. which revealed that COX-2 expression was up-regulated in all layers of the canine cornea during active episodes of keratitis, but was not present in non-diseased canine corneas (Sellers, Silverman, and Khan 2004). This information may serve to explain in part why the administration of various topical NSAIDs in the present study did not produce any effects on CS, as all cats

evaluated were ophthalmologically normal. Although all ophthalmic NSAIDs are considered non-selective COX inhibitors, the relative ratio of COX-1:COX-2 inhibitory potential of each drug differs markedly (Gaynes and Onyekwuluje 2008; Waterbury, Silliman, and Jolas 2006; Masferrer and Kulkarni 1997). Of the topical NSAIDs selected for use in the current study, 0.5% ketorolac tromethamine is the most COX-1 selective and 0.1% diclofenac sodium is the most COX-2 selective (Waterbury, Silliman, and Jolas 2006; Gaynes and Onyekwuluje 2008). Additional research is needed to evaluate COX isoenzyme expression in both healthy and diseased feline eyes, to better evaluate the potential therapeutic usefulness of topically applied COX isoenzyme-specific NSAIDs.

Although no change in CS was demonstrated in the current study, results could differ in diseased feline eyes. Future studies evaluating the influence of topical NSAID administration on actively inflamed eyes (e.g., herpetic keratitis with and without ulceration) may show clinical analgesic benefit. A 2005 evaluation of human patients with corneal epithelial defects secondary to Sjogren's syndrome (an autoimmune disorder with recognized inflammatory effects on the cornea and conjunctiva) demonstrated that dosing with topical 0.1% indomethacin or 0.1% diclofenac three times daily resulted in a significant reduction in CS and ocular discomfort scores (Aragona et al. 2005). To the authors' knowledge, there are no studies evaluating the antinociceptive effect of topically applied NSAIDs on cats with active, naturally occurring surface ocular disease.

The benefit of ameliorating pain associated with surface ocular inflammation by decreasing CS should not overshadow the potential risk of delayed corneal wound healing or alterations in neurotrophic influence, which could lead to the development of a neurotrophic epitheliopathy (Gaynes and Fiscella 2002). Hendrix et al. examined the effects of commonly used topical anti-inflammatory medications and their associated preservatives on the morphologic characteristics and migration ability of canine corneal epithelial cells *in vitro*. Suprofen was found to cause no changes in morphologic characteristics at the lowest concentrations evaluated, but at higher levels there was a concentration-dependent degree of epithelial cell rounding and shrinking. Additionally, thimerosal (a preservative used in some topical NSAIDs including flurbiprofen and suprofen) was found to impede the migration of corneal epithelial cells (Hendrix, Ward, and Barnhill 2002). The effects of topical anti-inflammatory drugs on wound healing in experimentally induced corneal and limbal wounds in cats has also been evaluated (Barba et al. 2000). Cats with limbal and clear corneal incisions that were treated with 0.5% ketorolac tromethamine ophthalmic solution three times daily demonstrated equivalent wound healing compared to cats that were not treated with a topical anti-inflammatory medication (Barba et al. 2000).

Systemic nonsteroidal anti-inflammatory drugs should be used cautiously in cats due to their low capacity for hepatic glucuronidation, which is the major mechanism of metabolism and excretion for these drugs (Giuliano 2004; Court 2013). Hsu et al. demonstrated that administration of 0.1% diclofenac ophthalmic

solution applied to both eyes four times daily was well tolerated in healthy cats, with only mild signs of ocular irritation including transient blepharospasm and conjunctival hyperemia. In that study, detectable systemic concentrations of diclofenac were achieved with accumulation over the week-long treatment phase (Hsu et al. 2015). Lanuza et al. also evaluated the systemic absorption of 0.1% diclofenac ophthalmic solution as well as 0.03% flurbiprofen ophthalmic solution, and found that domestic shorthair cats treated four times daily to both eyes for two weeks had detectable systemic concentrations of both topical NSAIDs (Lanuza et al. 2016). According to the results of the Hsu et al. 2015 study, systemic absorption of diclofenac may be associated with reduced glomerular filtration rate particularly in volume-depleted cats, therefore, clinicians are encouraged to use topical diclofenac cautiously, especially with respect to chronic dosing in systemically ill cats (Hsu et al. 2015).

A limitation of the current study was the small number of animals evaluated. It is possible that an increase in the number of test subjects may have demonstrated different results. Purpose-bred DSH cats owned by the Kansas State University Comparative Medicine Group were used, and as such, the number of study subjects was limited. Another limitation was the lack of a true control group. Although the 0.9% saline solution served as a control, a more appropriate control solution may have been one containing a preservative, as it has been previously shown that preservative agents have significant effects on CS (Cantarella et al. 2017). Selecting a control solution containing a single preservative agent would have been complicated by the fact that all three

commercially available topical NSAIDs selected for use in this study contain different preservatives; 0.1% diclofenac sodium contains boric acid, 0.5% ketorolac tromethamine contains benzalkonium chloride, and 0.03% flurbiprofen sodium contains thimerosal. Additionally, twice-daily dosing (as employed during the prolonged dosing effects phase of the current study) may be insufficient to induce changes to CS in cats, given the comparatively large size of the feline cornea when compared to the human cornea. The dimensions of a normal feline cornea are approximately 15-16mm vertically and 16-17mm horizontally (Carrington and Woodward 1986). In contrast, the horizontal length of the human cornea measures approximately 12mm and the vertical height measures 11mm (Sridhar 2018). Therefore, cats have a significantly larger corneal surface area available for absorption of topically applied medications. Finally, corneal esthesiometry has inherent limitations and observer subjectivity. In order to minimize procedural variability on corneal esthesiometry, all measurements were performed by the same investigator for the duration of the study.

Chapter 5 Conclusions

The results of the current study demonstrate that the CS of healthy DSH cats is not affected by immediately repeated or prolonged twice-daily application of 0.1% diclofenac sodium, 0.5% ketorolac tromethamine, or 0.03% flurbiprofen sodium. Given the potential differences in COX expression between normal and diseased feline eyes, future studies evaluating the effects of topical NSAIDs on cats with surface ocular inflammation are warranted.

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Footnotes

Fluorescein sodium, Akorn, Inc., Lake Forest, IL

Tono-Vet®, Icare Finland, Espoo, Finland

SL-17, Kowa, Torrance, CA

Sodium chloride injection 0.9%, Fresenius USA Manufacturing Inc., Walnut Creek, CA

0.1% Diclofenac sodium ophthalmic solution, Akorn, Inc., Lake Forest, IL

0.5% Ketorolac tromethamine ophthalmic solution, Akorn, Inc., Lake Forest, IL

0.03% Flurbiprofen sodium, Amici Pharmaceuticals, Melville, NY

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