ABSTRACT

THE OCULAR AND SYSTEMIC ADVERSE EFFECTS OF TOPICAL 0.1% DICLOFENAC IN HEALTHY CATS

Kimberly K. Hsu
University of Guelph, 2013

Advisor:
Stephanie Nykamp
Department of Clinical Studies

The objectives of this study were to characterize the ocular and systemic adverse effects, and systemic pharmacokinetics of topical 0.1% diclofenac. This was investigated in 8 healthy cats using a blinded, randomized, placebo-controlled, cross-over design. Drops were administered bilaterally 4 times daily for 7 days. Ocular, hepatic and renal variables were measured at various timepoints. Pharmacokinetic sampling occurred on Days 1 and 7.

Treated animals were 8 times more likely to develop conjunctival hyperemia than control animals (p=0.0161). Pharmacokinetic analysis showed that accumulation occurs with repeated dosing. Topical 0.1% diclofenac treatment did not have any significant effect on hepatic or renal function, other than reduction GFR in the second phase of the study (p=0.0013).

In conclusion, topical 0.1% diclofenac appears to be safe in healthy cats causing only mild ocular irritation. Careful patient selection may be indicated as systemically-absorbed diclofenac may be associated with reduction in GFR in volume-contracted states.
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I would like to thank my mom and dad for always believing in me, and my family and friends for their encouragement. I am forever grateful to my husband John for his support, patience and understanding.

Finally, I thank the Ontario Veterinary College Pet Trust Fund for their generous financial support.
DECLARATION OF WORK PERFORMED

I declare that with the exception of the items listed below, all of the work reported in this thesis was performed by me.

Ocular examination of all animals on arrival was performed by Dr. Chantale Pinard. Assistance performing the experiments and collecting data was provided by Karen Schwindt, Nicole Kudo, Dr. Chantale Pinard, Dr. Ron Johnson, Dr. Stephanie Nykamp as well as many graduate student, veterinarian, and veterinary technician volunteers.

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Statistical analysis was performed by Gabrielle Monteith with guidance from Dr. William Sears.

Dr. Butch Kukanich performed the pharmacokinetic analysis for the project and contributed to the description of this technique.
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<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BAB</td>
<td>Blood aqueous barrier</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete blood count</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>Maximal plasma concentration</td>
</tr>
<tr>
<td>CTT</td>
<td>Corneal touch threshold</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450 superfamily</td>
</tr>
<tr>
<td>DP</td>
<td>Prostanoid receptor where the most potent agonist is PGD$_2$</td>
</tr>
<tr>
<td>EP</td>
<td>Prostanoid receptor where the most potent agonist is PGE$_2$</td>
</tr>
<tr>
<td>FP</td>
<td>Prostanoid receptor where the most potent agonist is PGF$_{2\alpha}$</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>HPLC-MS</td>
<td>High-pressure liquid chromatography coupled with mass spectrometry</td>
</tr>
<tr>
<td>IOP</td>
<td>Intraocular Pressure</td>
</tr>
<tr>
<td>LT</td>
<td>Leukotriene</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>Neodymium:yttrium-aluminum-garnet</td>
</tr>
<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed cell volume</td>
</tr>
<tr>
<td>PD</td>
<td>Pupillary diameter</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>OIR</td>
<td>Ocular irritative response</td>
</tr>
<tr>
<td>PFIM</td>
<td>Pre-iridal fibrovascular membranes</td>
</tr>
<tr>
<td>RPF</td>
<td>Renal plasma flow</td>
</tr>
<tr>
<td>STT</td>
<td>Schirmer tear test</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>Time to $C_{\text{max}}$</td>
</tr>
<tr>
<td>TFBUT</td>
<td>Tear film break-up time</td>
</tr>
<tr>
<td>TP</td>
<td>Total protein</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Terminal elimination half-life</td>
</tr>
<tr>
<td>UA</td>
<td>Urinalysis</td>
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<tr>
<td>UPCR</td>
<td>Urine protein to creatinine ratio</td>
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CHAPTER 1: Introduction and Objectives

1.1 Introduction

Feline anterior uveitis is a common and potentially vision-threatening disease in the cat. Causes of feline anterior uveitis include traumatic, idiopathic, neoplastic, parasitic and infectious.\(^1\)\(^-\)\(^3\) Therapy of feline anterior uveitis includes removal of the underlying cause if possible, as well as anti-inflammatory therapy. As they are potent anti-inflammatory agents and are typically well tolerated in the cat, topical corticosteroids are the most commonly used anti-inflammatory agents in the treatment feline anterior uveitis. However, topical NSAIDs may be used with caution where topical corticosteroids are contraindicated, such as in the face of corneal ulceration, or as an adjunctive therapy to topical corticosteroids in severe cases of anterior uveitis. Depending on the severity and cause of anterior uveitis, the frequency of topical NSAID therapy may be as frequent as once every 2 hours\(^2\) and gradual tapering may be required to decrease the likelihood of recurrence.\(^1\)\(^-\)\(^3\) The efficacy of topical NSAIDs in reducing intraocular inflammation has been demonstrated,\(^4\) but studies confirming their ocular and systemic safety are lacking. At present, all topical NSAIDs used in veterinary medicine are off-label.

Adverse ocular effects with topical NSAID use in humans are rare.\(^5\)\(^-\)\(^{14}\) However, topical NSAIDs may delay corneal healing,\(^{15}\)\(^-\)\(^{19}\) and potentially blinding complications such as deep corneal ulceration and keratomalacia have been reported.\(^3,12,15,20\)\(^-\)\(^{23}\) In addition, topical NSAIDs have been associated with an increase in IOP in cats and in dogs where uveitis has been experimentally-induced.\(^1\)\(^-\)\(^4,24\)\(^-\)\(^{27}\)
Adverse systemic effects with topical NSAID use have not been reported in veterinary medicine and there are few studies that have quantified the amount of systemic NSAID absorption following topical application.\textsuperscript{1,2,28-39} However, though topical doses are small relative to systemic doses, topically applied ophthalmic medications can be absorbed via the conjunctiva and nasolacrimal mucosa.\textsuperscript{40,41} Studies in rabbits have shown that topical medications can achieve a systemic bioavailability ranging from 40 to 100\%.\textsuperscript{42} Systemic absorption of topical NSAIDs may be of concern in the cat, due to its limited ability to glucuronidate.\textsuperscript{43} Due to its delayed metabolism of NSAIDs, the cat has long been considered to be at higher risk than dogs for NSAID accumulation and toxicity including gastrointestinal, renal and hepatic effects.\textsuperscript{44}

1.2 Objectives

The objectives of this study were to determine if there are adverse effects associated with topical NSAIDs dosed according to an aggressive clinical regime in healthy cats. Diclofenac 0.1\% (Voltaren) was chosen because it is commonly used and readily available. Specifically, the objectives were to determine if:

1. Topical 0.1\% diclofenac is well tolerated and if it is associated with any adverse effects on corneal health, corneal sensation, aqueous tear production, tear film quality, PD, and IOP.
2. Detectable levels of diclofenac are achieved following topical application and if accumulation occurs with repeated dosing.
3. Topical 0.1\% diclofenac is associated with any adverse hepatic effects as measured by serum biochemistry panels, as well as to determine if topical 0.1\%
diclofenac is associated with any adverse renal effects as measured by UA, UPCR, and GFR.
1.3 References


CHAPTER 2: Literature Review

2.1 Feline Uveitis

Introduction to Feline Uveitis

Uveitis is a common ocular disease in domestic cats and 24% of feline patients presenting to the Ontario Veterinary College Health Science Centre Ophthalmology Service between 2001-2011 were diagnosed with this condition. There is a marked male predilection for feline uveitis and the mean age of affected cats has been reported to be approximately 9 years. Of affected cats, 32 to 48% have bilateral ocular involvement.

Uveitis is associated with exogenous or endogenous causes. Exogenous causes, such as corneal injury or trauma, are usually readily identified on ocular examination or identified via history. With trauma, uveitis may develop secondary to concussive, penetrating or perforating injury to the globe. Blunt trauma can be transmitted throughout the eye, and can result in concussive injury to the uvea and lens, as well as retinal detachment. Penetrating injury, such as with an ocular foreign body, can also affect multiple ocular structures including the uvea, lens, and posterior segment. Uveitis is a common complication of corneal injury or inflammation due to the oculo-pupillary reflex. The cornea is densely innervated, and corneal stimulation of the ophthalmic branch of the trigeminal nerve leads to constriction of the iris sphincter and ciliary muscle via stimulation of the parasympathetic fibers of the oculomotor nerve. This axonal reflex causes vasodilation and altered permeability of uveal blood vessels, as well as increased
leukocyte chemotaxis.\textsuperscript{4}

Endogenous causes of uveitis include idiopathic, neoplastic, lens-induced, parasitic, and infectious.\textsuperscript{5} A retrospective histopathological study showed that idiopathic uveitis, characterized by lymphocytic-plasmacytic inflammation, was the most common type of uveitis in the cat. In this study, lymphocytic-plasmacytic uveitis was diagnosed in 51/158 enucleated or eviscerated globes, with either diffuse (46/51) or a nodular (5/51) infiltration of the iris and ciliary body.\textsuperscript{6}

Lymphosarcoma is considered the most common neoplastic cause of feline uveitis, and is often associated with significant breakdown of the BAB.\textsuperscript{7,8} It was diagnosed in 33/158 globes in the histopathological study mentioned previously.\textsuperscript{6} Iris melanomas, the most common type of primary intraocular tumor in the cat, are typically associated with either no or mild ocular inflammation.\textsuperscript{7} Chronic uveitis occurs with feline ocular sarcoma, the second most frequently diagnosed primary intraocular tumor in the cat.\textsuperscript{8}

Lens-induced uveitis may occur secondary to cataract formation. With cataract formation, it is thought that small quantities of altered lens proteins escape from the lens, and induced an immune response. In veterinary ophthalmology, lens-induced uveitis is divided into two types: phacolytic uveitis and phacoclastic uveitis.\textsuperscript{9} Phacolytic uveitis is characterized by a lymphocytic-plasmacytic inflammation. Phacoclastic uveitis occurs following lens rupture, due to sudden release of large amounts of intact lens protein. Phacoclastic uveitis is characterized by a lymphocytic-plasmacytic as well as suppurative inflammation.\textsuperscript{10} Lens-induced uveitis in cats is considered to be less severe than lens-induced uveitis in dogs.\textsuperscript{3}
Infectious causes of uveitis include *Toxoplasma gondii*, feline leukemia virus, feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP), feline herpesvirus-1 (FHV-1), *Bartonella henselae*, and multiple mycoses.\(^{11-15}\) In a study of 93 cats with endogenous uveitis, 90% of samples were serologically positive for at least one infectious agent, with *T. gondii*, FIV, FeLV and feline coronoviruses showing a seroprevalence of 78.5%, 22.9%, 5.95%, and 27.0%, respectively.\(^{16}\) In another study, serologic evidence of infection by at least one infectious organism was found in 83.1% of samples.\(^{17}\)

Though infection may be an important cause of endogenous uveitis, establishing the causative relationship between infectious etiologies and uveitis has proven challenging given the poor positive predictive value of some diagnostic tests. Many cats without detectable ocular disease are seropositive for infectious organisms.\(^{18,19}\) Furthermore, while aqueous humor antibody and PCR testing may be performed, antibody production and PCR detection of the organism may not reflect ocular disease. It is possible that infectious organisms may induce ocular immune responses during the acute phases of infection without inducing ocular disease. Also, there may be induction of antibody production in chronically infected animals with non-specific stimulation.\(^{20}\) The number of cats with detectable concurrent systemic disease is significant but variable in the veterinary literature, ranging from 38 to 70%.\(^{2,3,16}\) It is however evident that despite complete systemic investigation, many cases of uveitis remain idiopathic.\(^{2,16}\)

As cats with uveitis can have concurrent or underlying ocular disease, a complete ocular exam should be performed in all cats with uveitis. The ocular exam should include slit lamp biomicroscopy, Schirmer Tear Test, fluorescein staining, tonometry and fundic
exam. Also, as cats with systemic diseases may first present to a veterinarian due to ocular signs, all patients receive a thorough physical examination and at minimum, a basic systemic work-up including CBC, serum biochemistry panel and urinalysis.\textsuperscript{8}

For a complete list of possible causes of feline uveitis, see Table 2.1.

**Breakdown of the BAB**

Uveitis is defined as inflammation of the uvea. The uvea, or vascular layer, is located between the outer fibrous layer (cornea and sclera) and inner neurosensory layer (retina) of the eye. The uvea is divided into the anterior uvea (iris and ciliary body) and the posterior uvea (choroid). The structures of the uvea serve many important functions: the iris changes pupillary diameter to regulate light entering the eye, the ciliary body produces aqueous humor, and the choroid provides nutrition to the outer retina.\textsuperscript{21} Uveitis can be further divided into anterior uveitis (iritis or iridocyclitis) and posterior uveitis (choroiditis).\textsuperscript{7,22,23}

Feline uveitis is most commonly detected as an anterior or an intermediate uveitis, with inflammation typically extending from the iris to the posterior aspect of the ciliary body, the pars plana.\textsuperscript{6,7} Anterior uveitis is most commonly treated with topical corticosteroids or NSAIDs. As the objective of this research project is to determine if there are any adverse effects associated with topical ophthalmic NSAIDs, specifically topical 0.1% diclofenac, this literature review will be limited to a discussion of anterior uveitis and topical NSAIDs. Following topical application, diclofenac permeates into ocular tissues, achieving high concentrations in aqueous humor and anterior uvea in humans.\textsuperscript{24-26} Although topical anti-inflammatory medications may be used to treat
anterior uveitis, systemic therapy is needed to target the posterior segment of the eye.\textsuperscript{22} Topical NSAIDs have been detected in the posterior segment, but do not reach sufficient concentrations to inhibit PG synthesis.\textsuperscript{27}

Anterior uveitis is associated with breakdown or increased permeability of the BAB. The BAB is made up of an endothelial layer at the level of iridal blood vessels and by an epithelial barrier at the level of the nonpigmented ciliary epithelium.\textsuperscript{28,29} When the BAB is intact, cell and protein movement is prevented across the vascular endothelial surface and across the nonpigmented epithelium of the ciliary body. In the healthy eye, aqueous humor protein concentrations are 200 times less than in plasma.\textsuperscript{22} With breakdown of the BAB, cells and proteins that would normally remain in the intravascular compartment and be confined by the tight junctions of the nonpigmented ciliary epithelium move into the anterior chamber. Increased protein and cell concentrations create turbidity within the anterior chamber, which is detectable on clinical examination as aqueous flare.\textsuperscript{22}

The following species are listed in order of decreasing BAB stability: chickens, ducks, rhesus monkeys, owl monkeys, cats, guinea pigs, and rabbits.\textsuperscript{30,31} The canine BAB is thought to be of intermediate stability.\textsuperscript{32} In general, an inverse relationship exists between the evolutionary development of visual acuity and the acute response to ocular injury.\textsuperscript{30}

Regardless of cause, uveitis is initiated by tissue injury.\textsuperscript{33} With injury, the OIR is initiated, which includes breakdown of the BAB, leukocyte infiltration, miosis, and changes in IOP.\textsuperscript{34} While the inflammatory response has evolved to be protective, the
unchecked sequelae of inflammation can be detrimental. Given the therapeutic implications, chemical mediators of uveitis are an important area of research. Despite extensive study, much remains to be understood about the exact role of individual mediators and species-specific response to inflammation. Ocular inflammation results from a complex cascade of events. Prostaglandins, LTs, platelet activating factor, interleukins, bradykinin, histamine and neuropeptides likely all play a role.\textsuperscript{32,35}

As NSAIDs are the focus of this research project, this literature review will focus on the role of their target enzyme, COX, and the products of COX, PGs. Prostaglandins are considered one of the main mediators of ocular inflammation.\textsuperscript{36-39} Following cell membrane damage, phospholipids are released from cell membranes and are hydrolyzed by phospholipase A\textsubscript{2}, which results in the formation of AA. Arachidonic acid is metabolized by either COX or lipoxygenase. COX activity results in the formation of PGG\textsubscript{2} and PGH\textsubscript{2}, which are then converted to PGI\textsubscript{2} by prostacyclin synthase, thromboxane A\textsubscript{2} (TxA\textsubscript{2}) by thromboxane synthase, and PGE\textsubscript{2}, PGF\textsubscript{2α}, and PGD\textsubscript{2} by their respective synthetase enzymes (Figure 2.1). Lipoxygenase activity results in the formation of LTs. The synthesis of PGs and their accumulation within the inflamed eye are thought to lead to breakdown of the BAB and the clinical signs associated with uveitis.\textsuperscript{40-42} The eye has a limited capacity to metabolize and inactivate PGs. With uveitis, PG 15-dehydrogenase, the enzyme responsible for inactivation of PGs, becomes overwhelmed, initiating the OIR.\textsuperscript{43} Excess PGs must be actively transported through the ciliary body into circulation for inactivation at a distant site, such as the lungs.\textsuperscript{43,44}
Clinical Manifestations of Uveitis

Non-specific signs of uveitis include blepharospasm, enophthalmos, lacrimation, and photophobia, which reflect ocular pain. Ciliary flush, or dilation of the deep, perilimbal or circumferential anterior ciliary vessels with increasing levels of PGs may occur, and is suggestive of intraocular disease. Corneal edema also occurs in uveitic patients due to reduction in corneal endothelial NaK-ATPase or epithelial NaCl pump activity. Aqueous flare, hypopyon, and hyphema are specific signs of uveitis, and reflect breakdown of the BAB. With increased permeability of uveal blood vessels, proteins, as well as leukocytes and red blood cells, will enter the anterior chamber. Similarly, keratic precipitates, or aggregates of inflammatory cells that are adherent to the corneal endothelium, also reflect BAB breakdown.

Miosis, or pupillary constriction, occurs due to the direct effects of PGs and other inflammatory mediators on the iris sphincter muscle. In the cat, miosis that occurs during uveitis is likely mediated via PG interaction with the FP subtype of prostanoid receptor. Experimentally, there is profound contraction of the feline iris sphincter when PGF\(_2\alpha\) is applied in vitro, or in vivo. In contrast, PGE\(_2\) is not as potent a miotic agent in the cat. With breakdown of the BAB, there is an initial immediate rise in IOP, likely due to uveal vasodilation, as well as increased ultrafiltration and extravasation of fluid into the eye. The rise in IOP seen with uveitis is also likely associated with increased protein and cellular infiltrates, which block the outflow of aqueous through the iridocorneal angle. Intracameral injections of PGE\(_1\) and PGE\(_2\) are associated with an increase in IOP.
in cats, suggesting that these two PGs may play a role in the initial elevation of IOP.\textsuperscript{50} 

Topically applied PGF\textsubscript{2\alpha} has been shown to cause transient ocular hypertension in cynomolgus monkeys and rabbits, but not in cats.\textsuperscript{51} The transient rise in IOP is typically followed by a more sustained fall in IOP. While it can be subtle, ocular hypotony is often one of the first signs clinically detected in uveitic eyes. Endogenous PGs produced by the uvea may trigger the increase in drainage via this pathway during the initial phases of the OIR, thus counteracting the acute rise in IOP.\textsuperscript{34} Prostaglandins are thought to cause ocular hypotension by causing relaxation of the ciliary body and increasing aqueous drainage via the uveoscleral outflow pathway.\textsuperscript{52}

Although the exact PGs that cause the decrease in IOP associated with uveitis are currently unknown, PGs have been experimentally administered to laboratory animals and changes in IOP and aqueous outflow examined. Prostaglandin F\textsubscript{2\alpha} has been shown to cause ocular hypotension in rabbits\textsuperscript{53} and monkeys\textsuperscript{54}, PGF\textsubscript{2\alpha} but likely does not have the same effect in the cat. In the cat, PGF\textsubscript{2\alpha} has been shown to decrease IOP,\textsuperscript{48,55} but commercially available PGF\textsubscript{2\alpha} analogues, including latanoprost, bimatoprost and unoprostone isopropyl, do not produce ocular hypotension in the cat, as they do in other species.\textsuperscript{56-58} The cat is unique in that it lacks FP receptors in its ciliary body and thus the effects of PGF\textsubscript{2\alpha} are likely mediated through EP receptors.\textsuperscript{59} It is thought that due to their high specificity for human FP receptors, human-labeled PGF\textsubscript{2\alpha} analogues cannot bind the feline EP receptor.\textsuperscript{60} In the cat, PGA\textsubscript{2} has been shown to be an effective
hypotensive agent. The hypotensive effects of PGA₂ have been experimentally demonstrated to be due to an increase in uveoscleral outflow and trabecular outflow facility. This effect may, however, be limited in the cat, a species where uveoscleral outflow only accounts for approximately 3% of aqueous drainage. With chronic uveitis, atrophy and fibrosis of the ciliary body may result in decreased aqueous production and chronic ocular hypotony. Over time, decreased aqueous humor production may result in phthisis bulbi, or shrinkage of the globe.

While studies to date have examined the eye’s response following application of PGs and demonstrated the presence of PG receptors in the eye, application of these findings to naturally-occurring uveitis remains challenging. Further research is needed to determine the relative quantities of different PGs in cases of experimentally-induced and naturally-occurring feline uveitis. Conclusions drawn from studies using different species must also be interpreted with caution. In particular, though rabbits are commonly used in the laboratory, the rabbit BAB is highly labile compared to other species, and rabbits show a profound PG-mediated OIR compared to other species. Species differences in ocular pharmacokinetics must also be considered as they may play a role in the intensity and duration of the OIR. For example, resistance to BAB breakdown in the chicken and duck may relate to the short half-life of the predominant COX products in these species, TxA₂ and prostacyclin, as well as the relatively fast flow of aqueous humor in the avian eye. Study design is also important in the evaluation of the effects of individual PGs on BAB breakdown. Cannulation of the eye to administer PGs likely causes BAB breakdown and release of endogenous PGs, thus likely exaggerating the response of the eye. In contrast, topical application of PGs avoids the inflammation caused by
cannulation of the eye, but likely result in decreased absorption of the PGs being examined.

**Sequelae of uveitis**

The sequelae of uveitis are potentially painful and vision threatening. They include cataract formation, lens luxation, synechiae, secondary glaucoma and phthisis bulbi.\textsuperscript{32}

Cataracts may occur following severe or chronic uveitis, due to altered lens metabolism with increased intraocular inflammatory mediators. Inflammatory mediators in contact with the lens may cause lens epithelial metaplasia, lens fiber degeneration, and lens fiber necrosis.\textsuperscript{63} Posterior synechiae can also lead to cataract formation,\textsuperscript{32} potentially due to the pigment deposition or growth of fibrovascular membranes onto the anterior lens capsule and alteration of lens metabolism. Intraocular inflammation may also lead to zonular breakdown and lens luxation. Uveitis is commonly associated with lens luxation; 30 of 44 eyes with lens luxation in a retrospective case series had concurrent uveitis.\textsuperscript{64}

Secondary glaucoma occurs due to obstruction of the iridocorneal angle by inflammatory cells and protein, iris bombe, circumferential peripheral anterior synechiae, and formation of PFIMs.\textsuperscript{32} As it is blinding and painful, secondary glaucoma is a common cause for enucleation with feline uveitis.\textsuperscript{6} In one study, over half of the cats with idiopathic anterior uveitis (20/37) developed secondary glaucoma.\textsuperscript{1} The increased likelihood of glaucoma with lymphocytic-plasmacytic uveitis may be associated with obstruction of the ciliary cleft and trabecular meshwork by lymphocytes.\textsuperscript{6}
Therapy of Feline Uveitis

Therapy of uveitis is aimed at decreasing inflammation and stabilizing the BAB. By doing so, the hope is to minimize sequelae, decrease pain and preserve vision. If an underlying cause for uveitis is identified, then therapy is aimed at eliminating that cause and thus the antigenic stimulus for inflammation. For example, broad-spectrum antibiotic therapy is used to prevent or treat bacterial infection in cases of ulcerative keratitis.3 Therapy of intraocular neoplasia is dependent on the type of neoplasia present. For example, the recommended treatment for feline ocular sarcoma is early removal of the globe, whereas lymphosarcoma is usually treated through a combination of systemic chemotherapy and topical corticosteroids.3 Where lens-induced uveitis is present, such as with cataracts or trauma to the lens with penetrating injuries, the lens is removed with extracapsular lensectomy or phacoemulsification.3 Systemic anti-fungal therapy, typically using fluconazole due to its ability to penetrate the central nervous system and eye, is initiated in cases of uveitis secondary to fungal infection.65 Oral clindamycin is used in cases of uveitis secondary to Toxoplasma gondii infection.23 Broad-spectrum antibiotic therapy is used to prevent or treat bacterial infection where corneal ulceration is present or in cases of traumatic anterior uveitis, particularly if penetration of the globe has occurred. Treatment for viral causes of uveitis, such as feline immunodeficiency virus, feline leukemia virus, and feline infectious peritonitis is remains supportive.23

Unfortunately, a specific cause of uveitis is often not identified, and a diagnosis of idiopathic uveitis is made. Thus, immunomodulatory drugs are the mainstay of uveitis treatment.7,22,37 Even when a diagnosis has been made and therapy for the underlying cause of uveitis is initiated, anti-inflammatory drugs are typically required to address the
inflammation that has been triggered. As the sequelae of uveitis are painful and potentially vision-threatening, prompt initiation of therapy is essential.

Corticosteroids are commonly administered as the primary anti-inflammatory therapy in the treatment of feline uveitis. Corticosteroids are highly potent anti-inflammatory agents and are generally well tolerated by cats.\textsuperscript{7,22} Systemic corticosteroid therapy is not initiated until infectious and certain neoplastic causes of uveitis have been ruled out. Despite their efficacy, corticosteroids are contraindicated when concurrent corneal ulceration is present because they delay corneal healing, exacerbate infection, and may lead to collagenolysis.\textsuperscript{32} Corticosteroids, particularly systemic corticosteroids, are also contraindicated in diabetic patients, patients with suspected lymphosarcoma, and uveitis secondary to suspected infectious disease. Given the large number of cats with concurrent systemic disease, treatment with NSAIDs may be indicated in a potential large proportion of uveitis cases.\textsuperscript{1,2}

Topical iridocycloplegic therapy is also indicated in the treatment of uveitis, to decrease the pain associated with spasm of the ciliary body and to dilate the iris. Dilation of the iris decreases the likelihood of posterior synechiae and cataract formation as there is less contact between the iris and the lens. The iridocycloplegic of choice is atropine 1%, an anti-cholinergic agent. The ointment form is recommended in cats, which frequently react to the bitter taste of the medication with hypersalivation or less commonly, vomiting. Due to obstruction of the iridocorneal angle with pupillary dilation, 1% tropicamide, a shorter acting mydriatic agent, may be used in cases where there is a risk of secondary glaucoma. \textsuperscript{7,8,22}
2.2 Pharmacodynamics and Pharmacokinetics of Topical NSAIDs

Mechanism of Action of NSAIDs

Nonsteroidal anti-inflammatory drugs act at the level of the COX enzyme, thus preventing synthesis of PGs. Most NSAIDs act by the competitive inhibition of COX enzymes, so their effects are reversible once the drug concentrations decrease. The exception to this rule is aspirin, which irreversibly acetylates a serine residue near the active site of the enzyme, in addition to competitively inhibiting the enzyme.\textsuperscript{66,67} By inhibition of COX, NSAIDs exert their therapeutic effect by decreasing PG synthesis. In the eye, PGs have been shown to produce miosis, increase vascular permeability and lead to breakdown of the BAB.\textsuperscript{68} Most NSAIDs inhibit both COX isoforms, COX-1 and COX-2, to varying degrees. This is in contrast to corticosteroids, which inhibit the inflammatory cascade at the level of phospholipase A\textsubscript{2}, thereby inhibiting synthesis of PGs and LTs.\textsuperscript{67,69} Corticosteroids exert additional anti-inflammatory actions including suppressing the action of lymphokines and reducing migration of macrophages and neutrophils.\textsuperscript{67}

In addition to their established effects on COX, NSAIDs may have other effects that enhance their anti-inflammatory activity. In particular, there is evidence suggesting that diclofenac may have a spectrum closer to that of corticosteroids, via inhibition of phospholipase A\textsubscript{2} or modification of AA uptake.\textsuperscript{70,71} Multiple NSAIDs, including diclofenac and carprofen, have also been shown to decrease production of pro-inflammatory cytokines such as IL-6.\textsuperscript{72-74} Inhibition of the leukocyte chemotaxis induced by Substance P is also possible with treatment with diclofenac and other NSAIDs.\textsuperscript{75,76}
NSAIDs may have additional anti-inflammatory action through reduction of mast cell degranulation,\textsuperscript{77} and through their ability to scavenge free radicals.\textsuperscript{78}

**Introduction to COX-1 vs. COX-2**

There are two main isoforms of COX, COX-1 and COX-2. COX-1 is constitutively expressed in the smooth endoplasmic reticulum of all cells, contributing to physiologic functions such as the maintenance of a healthy GI tract and renal system, platelet function, and the maintenance of blood flow to various organs. This is in contrast to the inducible isoenzyme COX-2, which is produced by activated macrophages and inflammatory cells. COX-2 expression is controlled by cytokines, growth factors, and mitogens. COX-2 likely initiates the synthesis of PGs involved with severe inflammation\textsuperscript{35,67,69} It is believed that COX-1 only functions when there are high concentrations of AA present, as with platelet aggregation or acute inflammation, whereas COX-2 can function when AA are low.\textsuperscript{52} A third isoform of COX, COX-3, has been identified but its function is currently unknown.\textsuperscript{79}

Research in dogs has supported the association of COX-2 with inflammatory and pathological ocular processes. COX-2 is upregulated in all layers of the cornea with keratitis.\textsuperscript{80} In glaucomatous globes, COX-2 is expressed in the cornea and aqueous outflow pathway.\textsuperscript{81} In canine globes with PFIMs, COX-2 expression is present in the blood vessels and spindle cells forming the PFIMs. In globes with PFIMs, COX-2 is also expressed in the cornea, ciliary body, lens, and retina.\textsuperscript{82}
Despite its association with inflammation, COX-2 may be constitutively expressed in many tissues including the kidney, reproductive system, central nervous system, as well as the eye. In healthy canine globes, COX-2 expression was limited to the ciliary body in one study,\(^8\) but found to be widely expressed in another study, with detection in the cornea, ciliary body, uveal and retinal vasculature.\(^2\) Although these differences could be explained by differences in staining techniques, failure to detect mild inflammation, and COX-1 cross-reactivity, it is likely that COX-2 plays a physiological role in eye. In healthy eyes, COX-2 has been demonstrated in the cornea of rabbits,\(^3\), the ciliary body of humans,\(^4\) the iris and ciliary body of rabbits,\(^5\) and the retina of mice, rats, and humans.\(^6\)

Furthermore, in the eye, COX-2 expression may not only be constitutive, but its loss may be associated with pathology. In humans, COX-2 expression is largely confined to the basolateral membranes of the non-pigmented epithelial cells of the ciliary body, and specific loss of COX-2 in this location occurs in patients with primary open angle glaucoma as well as steroid-induced glaucoma.\(^4\) Given the probable role of COX-2 loss in human glaucoma, gene therapies are being developed to try to restore COX-2 expression in human glaucoma patients. Using a feline experimental model, lentivirus-induced expression of COX-2 and FP receptors in the ciliary body epithelium and trabecular meshwork of normotensive eyes results in substantial IOP reductions.\(^7\)

Despite their use as a model for human glaucoma therapies, to the author’s knowledge, the ocular expression of COX-1 or COX-2 in health and disease states is currently unknown in cats, and should be characterized in future immunohistochemical or gene expression studies. Further research is also needed to determine the PG products and
physiological role played by each COX isoenzyme in the eye. A better understanding of species-differences in COX expression may also help to explain species differences in BAB stability.

**COX-Selectivity of NSAIDs**

In order to maximize the therapeutic efficacy of NSAID therapy while avoiding adverse effects such as GI ulceration, clotting deficiency, and renal dysfunction, COX-2 selective or COX-1 sparing drugs have been the focus of recent research. This drug development strategy is based on the assumption that most adverse effects are associated with COX-1 inhibition. COX-2 versus COX-1 selectivity can be compared using COX inhibitory ratios, which are calculated by dividing the concentration of drug that will inhibit the COX-1 enzyme by a given percentage (typically 50% inhibition or IC$_{50}$) by the concentration of drug that will inhibit the COX-2 enzyme by the same percentage. Higher values thus represent greater COX-2 selectivity.

Despite some benefits, the advantages of selective COX-2 inhibition have been questioned. For example, in humans, while there has been a reduction in the NSAID-associated GI toxicity with COX-2 selective inhibitors, nephrotoxicity continues to be a problem. Rofecoxib, a COX-2 selective inhibitor, is also associated thromboembolic disease (heart attack and stroke) in human patients, leading to concerns about the cardiovascular safety of COX-2 inhibitors. Further research is needed in both cats and dogs to evaluate the safety of COX-2 selective NSAIDs.

Of the available commercial ophthalmic formulations, bromfenac is the most COX-2 selective. Based on in vitro assays, bromfenac is 3 to 18 times more potent in
inhibiting COX-2 than diclofenac, amfenac and ketorolac. Ketorolac is considered to be the most COX-1 selective of available ophthalmic NSAIDs. 69,90 To better understand COX inhibition in the eye, an in vitro cell assay system has recently been developed using bovine corneal endothelial cells and retinal pigment epithelial cells. 91 Despite an emphasis on selective inhibition of COX-2, the importance of this selectivity has not been established in the eye.

As veterinarians work with many different species, it is important to note that COX selectivity of NSAIDs may vary from species to species. For example, carprofen is relatively COX-2 selective in the dog (COX ratio: 6.5) and cat (ratio: 5.5), but non-selective in the horse (ratio: 1.9). 92 Although COX isoenzyme specificity is known for many NSAIDs in humans, limited information is available in the veterinary literature. In cats, whole blood assays have demonstrated that while robenacoxib (ratio 32.2) and carprofen are COX-2 selective, meloxicam (ratio: 2.7) and diclofenac (ratio: 3.9) are only slightly preferential for COX-2. Ketoprofen (ratio: 0.049) is COX-1 selective. 93-95

NSAID therapy should be selected with the species, tissue, and species-specific pharmacodynamics in mind. Further research is needed to assess COX isoenzyme expression in feline eyes in health and disease states to better evaluate the potential usefulness of more COX-2 specific NSAIDs.

Classification of NSAIDs and Available Commercial Ophthalmic Preparations

Six major classes of NSAIDs have been identified: salicylates, indole acetic acid derivatives, aryl acetic acid derivatives, aryl propionic acid derivatives, enolic acid derivatives, and fenamates. Although they are a chemically heterogenous group, all
NSAIDs inhibit eicosanoid formation, and lack the steroid nucleus derived from cholesterol present in corticosteroids.\textsuperscript{96} As topical NSAID formulations must be relatively water soluble for dissolution in solution and in tears, topical formulations as limited to the aryl acetic, indole acetic, and aryl propionic acid classes.\textsuperscript{97} Other NSAID classes, including the fenamates and salicylates tend to be poorly soluble.\textsuperscript{78} Current human-approved topical NSAIDs available in the United States include diclofenac, ketorolac, flurbiprofen, suprofen, bromfenac, and nepafenac. Indomethacin is available in Canada and Europe. Of the available commercial formulations, nepafenac is the only suspension.

In humans topical NSAIDs are approved for the control of allergic conjunctivitis, reduction of postoperative inflammation and photophobia following cataract surgery, and control of pain following refractive surgery. In addition, these agents are used before surgery to prevent intraoperative miosis and to decrease intraoperative inflammation.\textsuperscript{69}

In veterinary medicine, there are currently no licensed topical NSAIDs. Human topical products are used off-label for a number of inflammatory conditions including keratitis and uveitis. Like in humans, they are also used to prevent and treat inflammation following intraocular surgery.\textsuperscript{98-101} Topical NSAIDs are typically applied 2 to 4 times daily, depending on the amount of inflammation that is present. When used with a topical corticosteroid, topical NSAIDs allows for less frequent application of topical steroids and decreases the adverse effects associated with corticosteroid use.\textsuperscript{35} As noted previously, topical NSAIDs may be used preferentially instead of corticosteroids where corneal ulceration, trauma or infection are present.\textsuperscript{32} They may also be more prudent anti-inflammatory choices in patients with diabetes mellitus and suspected intraocular
Topical NSAIDs can also be used in the treatment of conjunctival and corneal inflammation, such as with herpetic keratoconjunctivitis. Topical NSAIDs should, however, be used with caution, because they have been associated with the development of keratomalacia in humans.

Diclofenac 0.1% is a phenylacetic acid derivative available in North America as Voltaren®. This medication is currently licensed for the treatment of post-operative inflammation and pain following cataract and photorefractive surgery in humans. This particular topical NSAID was chosen for this study because it is readily available and is one of the most commonly used topical NSAIDs in the author’s clinical practice. Its efficacy has also recently been demonstrated in an anterior chamber paracentesis model of feline uveitis, where it was more effective than another topical NSAID, 0.03% flurbiprofen, at reducing BAB. As Voltaren® will be used in the research project, the emphasis of this literature review will be on 0.1% diclofenac with a brief discussion of relevant literature about other topical NSAIDs.

Ocular pharmacokinetics of ophthalmic NSAIDs

Multiple studies have suggested that in treating anterior uveitis, the topical route is the route of choice. This is well illustrated by comparing aqueous humor concentrations of diclofenac administered via different routes. In one human experiment, the plasma concentration of diclofenac one minute after intravenous infusion was 13961 ± 4277 ng/mL while the diclofenac concentration in aqueous humor peaked at 21.7: ± 12.7 ng/mL after a lag phase of 60 minutes. By 120 minutes, aqueous humor levels had decreased to 4.4±0.4 ng/mL and by 300 minutes, 3.1 ± 1.3 ng/mL. In contrast, a single
pre-operative drop of topical 0.1% diclofenac produced aqueous concentrations of 22 ng/mL at 50 minutes and 52.6 ng/mL at 125 minutes in human cataract patients. In a second study, after a single drop, 0.1% diclofenac reached a peak average aqueous concentration of 82 ng/mL at 2.5 hours in human cataract patients. Aqueous humor concentrations of diclofenac remained above 20 ng/mL for over 4 hours and remained detectable for over 24 hours. The variation in diclofenac levels detected following topical application is unclear. However, delayed sample times in the second study could have allowed for increased absorption of diclofenac into the aqueous humor. Greater BAB breakdown could also have led to a more acidic environment and thus sequestration of diclofenac in the anterior chamber.

The ocular pharmacokinetics of 0.1% diclofenac have also been studied in an albino rabbit model of uveitis, which demonstrated that pharmacokinetics change depending on the target tissue and on the presence of inflammation. With uveitis, diclofenac reached peak concentrations at 30 minutes in the cornea and anterior uvea. Clearance of diclofenac from aqueous humor was very fast in uveitic eyes compared to control eyes. Possible mechanisms include increased uveal vasodilation and absorption of diclofenac, and increased aqueous turnover due to opening of the iridocorneal angle with miosis or increased uveoscleral outflow. Higher levels of diclofenac were found in the iris and ciliary body (AUC: 9.3 ug/mL/h) in this study as compared to the aqueous humor (AUC: 3.04 ug/mL/h), suggesting that therapeutic efficacy during uveitis may be associated with tissue levels of drug. Aqueous humor concentrations of diclofenac in rabbits were much higher than in humans, C_{max} in aqueous humor was 940ng/ml in uveitic rabbit eyes. Species differences in corneal thickness, drug binding to melanin,
and inflammatory response could help explain these differences.\textsuperscript{106-108} In addition, the use of general anesthesia in the rabbit experiment could also have played as a role as general anesthesia has been shown to decrease tear production.\textsuperscript{109}

Pharmaceutical research has been directed towards developing ophthalmic NSAIDs that are easily absorbed, non-toxic, and that have potent anti-inflammatory activity. Changes in biochemistry have enabled newer NSAID products to have better corneal penetration than older topical NSAIDs, which have more polar acidic structures. Nepafenac is delivered to the surface of the eye as a neutral prodrug, and is rapidly converted to the more potent NSAID amfenac by intraocular hydrolases. In rabbit corneas, nepafenac was shown to be 4-, 19-, and 28-times more permeable than of diclofenac, bromfenac, and ketorolac, with a rate constant of $6.4 \text{ cm/s} \times 10^{-5}$ versus 1.5, 0.34, and $0.23 \text{ cm/s} \times 10^{-5}$ for diclofenac, bromfenac, and ketorolac, respectively.\textsuperscript{110} Due to greater corneal penetration, aqueous humor concentrations of drug increase as well. After a single topical dose, 0.1\% nepafenac reached peak aqueous concentrations of 205.3 ng/mL in 30 minutes, compared to 0.4\% ketorolac, which peaked at 57.5 ng/mL in 60 minutes, despite having a 4-fold lower starting concentration.\textsuperscript{111}

In addition to aqueous humor concentrations, topical NSAIDs have been shown to achieve good concentrations in ocular tissues.\textsuperscript{26,112} Following topical application, flurbiprofen, ketorolac, indomethacin and diclofenac have been detected in the posterior segment.\textsuperscript{110,113} However, only nepafenac has been shown to effectively inhibit PG synthesis in vitreal and retinal inflammation.\textsuperscript{110,112,114} Thus, systemic anti-inflammatory therapy is indicated when treating posterior uveitis.\textsuperscript{35}
Bioavailability and systemic pharmacokinetics of topical ophthalmic NSAIDs

Although one goal of topical therapy is to limit systemic absorption, a significant proportion of topically applied medications may be systemically absorbed. Medications instilled into the conjunctival sac may rapidly enter systemic circulation through conjunctival absorption, drainage via the nasolacrimal system and absorption through the nasal mucosa. Topical medications may also be swallowed with absorption via the GI system. The nasal mucosa is considered the most important site for systemic uptake, with up to 80% of each drop administered draining via the nasolacrimal system.\textsuperscript{115} Punctal occlusion using gentle digital pressure is not commonly performed in veterinary medicine, but has been advocated in human medicine as one way of decreasing systemic absorption of topically applied ophthalmic drugs. Small molecular weight molecules can be rapidly absorbed systemically, often within the first 10 minutes.\textsuperscript{116,117} Drugs absorbed via the nasolacrimal and conjunctival mucosa do not undergo a hepatic first pass effect, and thus systemic bioavailability of topically applied ophthalmic medications may be high.\textsuperscript{115}

When excessive volumes of topical medications are administered, the excess volume cannot be accommodated by the ocular cul-de-sac, and is eliminated by nasolacrimal drainage. Thus, excessive volumes or administration of multiple eye drops result in more rapid decrease in precorneal tear levels or drug and increased systemic absorption.\textsuperscript{118} In humans, the maximum volume that the ocular cul-de-sac can accommodate is approximately 30uL.\textsuperscript{119} To the author’s knowledge, optimization of the volume of ophthalmic medications has not been studied in the cat.
Normal cat corneal dimensions are approximately 15-16mm vertically and 16-17mm horizontally with a thickness of 0.58mm centrally and peripherally.\textsuperscript{120} In contrast, the horizontal meridian of the human eye measures approximately 12mm in the horizontal meridian and the 11mm in the vertical meridian. The human cornea measures approximately 0.44-0.65mm centrally, and 0.70mm peripherally.\textsuperscript{121} Thus, the cat has a larger corneal surface area potentially available for absorption of topically applied medications into the eye. Of the medication that is absorbed through the cornea into the eye, the main route of elimination is via drainage of aqueous humor at the iridocorneal angle into the venous system.\textsuperscript{115} To the author’s knowledge, the impact of the cat’s large corneal diameter on drug absorption, ocular drug levels, and systemic drug levels is unknown.

Unfortunately, systemic drug levels achieved following topical administration of many ophthalmic formulations of drugs are unknown making determination of bioavailability difficult.\textsuperscript{117} This is likely due to the challenges of detecting minute plasma concentrations, the relatively small total doses administered when compared to doses achieved via oral or parenteral administration, and the general perception that topically applied drugs will not achieve sufficient plasma levels to cause any significant adverse systemic effects.

Of the data that does exist, results are often conflicting. For example, in humans, topical application of 3-16 drops of 0.1% diclofenac resulted in no detectable levels in plasma.\textsuperscript{9} In contrast, systemic bioavailability for a number of ophthalmic drugs in rabbits ranges from 40 to 100%.\textsuperscript{116} Pharmacokinetic studies in rabbits using the NSAID flurbiprofen have shown that systemic bioavailability is 74% with an ocular bioavailability
of 7-10% when the drug is topically applied.\textsuperscript{122} Studies using radiolabelled ketorolac have demonstrated an ocular bioavailability of only 4% and almost complete systemic bioavailability. The conjunctiva and highly vascularized tissues of the nasolacrimal tract were considered the primary sites of absorption in these studies.\textsuperscript{112}

Following topical application of a 30 µl drop containing 0.1% diclofenac in rabbits, a mean peak plasma concentration of 72.7 +/- 14.2 ng/mL was reached at 15 minutes, with plasma concentrations decreasing to 2.6 ng/ml +/- 0.5 ng/mL at 240 minutes.\textsuperscript{123} In contrast, in another study, much lower peak plasma levels, ranging from 6.1-37 ng/mL were observed in rabbits following application of 30 µl of 0.1% diclofenac in control rabbits, rabbits with experimentally-induced uveitis, and rabbits with experimentally-induced keratitis.\textsuperscript{26} In comparing these two rabbit studies, it is suspected that the later sampling points in the second study (30 min, 1, 3, 6, and 12 h after administration) may have led to true peak plasma levels being missed. The differences between studies may also be the result of the use of different assay methods with different sensitivities. Unfortunately, comparisons regarding the total amount of absorbed drug are not possible between studies, as AUC values were not reported the first study.

Interestingly, greater plasma levels of diclofenac were achieved in animals with keratitis (AUC: 0.88 µg/ml/h) as compared to control animals (AUC: 0.115 µg/ml/h).\textsuperscript{26} Increased systemic absorption could have occurred due to concurrent conjunctival inflammation. Mild uveitis breakdown of the BAB could also have occurred secondary to keratitis. However, this explanation is less likely, as animals with experimentally-induced uveitis had decreased plasma decreased drug levels (AUC: 0.109 µg/ml/h).\textsuperscript{26}
Although there are very few studies to examine metabolism of topical NSAIDs, it is likely that given the significant systemic absorption, topical NSAIDs are metabolized by the same mechanisms as systemic NSAIDs. In one study, ketorolac was cleared very slowly from the anterior chamber following topical administration in rabbits (11 µl/min) but was cleared approximately 500 times more rapidly once it entered the systemic circulation.\textsuperscript{112} Despite significant absorption into the bloodstream and systemic metabolism, plasma half-lives of NSAIDs following topical administration may be prolonged, perhaps because removal of drug into the systemic circulation from the eye is rate limiting. The ocular elimination half-life of flurbiprofen in rabbits corresponds to the turnover rate of aqueous humor, suggesting that aqueous humor drainage is the major route of elimination for flurbiprofen from the globe.\textsuperscript{122} With ketorolac, the mean plasma half-life following topical administration was 6.9 hours as compared to the much shorter half-life of 1.1 hours following intravenous administration.\textsuperscript{112}

As the treatment of uveitis necessitates application of topical NSAIDs for days to weeks at minimum, further research is needed to evaluate drug accumulation and pharmacokinetics following multiple doses of topical NSAID. As feline uveitis is often a bilateral disease, future studies should consider bilateral application of topical NSAIDs. The effect of BAB breakdown on systemic absorption also requires further investigation. Studies employing a robust number of sampling time points are needed to ensure that peak plasma levels are not missed, while providing key information on the persistence of drug in the body. As feline uveitis tends to occur in middle-aged to older animals and is frequently accompanied by systemic disease,\textsuperscript{1,27} the effect of age and concurrent disease states on pharmacokinetics should also be considered.
Pharmacokinetics of systemically administered diclofenac

As detailed pharmacokinetic studies on ophthalmic diclofenac are lacking, information derived from the study of oral diclofenac in humans and animal models is presented. Similarities likely exist because a significant proportion of topically applied medications is absorbed via the GI tract, in addition to the conjunctiva and nasolacrimal system. When administered orally, diclofenac is absorbed quickly and completely. Complete absorption has been demonstrated in the rat, dog, rhesus monkey, and human. In the rat, the absorption half-life is less than 2 minutes. It is hypothesized that diclofenac may alter jejunal permeability, enhancing absorption. C$_{max}$ following oral administration is reached within 10 to 40 minutes. Diclofenac has been shown to be 99.7% bound to serum protein, of which 99-99.4% is bound to serum albumin in humans. Diclofenac may undergo first pass metabolism in the human, with only approximately 50-60% of the drug reaching systemic circulation. Enterohepatic recycling occurs in dogs and in rats, which can have a significant influence on half-life. In humans, the volume of distribution is between 0.1 – 0.2 L/kg. The small volume of distribution likely reflects a high degree of plasma protein binding. Metabolism and elimination of diclofenac shows high species variability. In the bile of dogs and rats, glucuronide conjugates have been identified. These metabolites are reverted to diclofenac by hydrolysis, allowing for enterohepatic circulation. Similar conjugates have not been identified in human bile. Studies have shown that the dog produces unique conjugates in bile and in urine that have not been identified in any other species. Excretion of diclofenac in rats is primarily biliary, whereas in the rhesus monkey and human, renal excretion is the predominant route of elimination. Elimination of diclofenac occurs
rapidly, with 90% of drug clearance occurring within 3-4 hours.\textsuperscript{105} The terminal half-life of diclofenac is short, ranging from 1.2 – 1.8h following an oral dose.\textsuperscript{105,127} Given the short half-life of the parent drug, it is postulated that one or more metabolites may persist in the body longer than the parent drug, allowing for a longer clinical effect.\textsuperscript{127} Two of diclofenac’s metabolites have shown limited anti-inflammatory activity.\textsuperscript{129} Because it is an organic acid, diclofenac, like other NSAIDs, may accumulate at the site of inflammation, which is beneficial in the treatment of inflammatory disease.\textsuperscript{78} As hepatic first pass metabolism is bypassed with absorption through the conjunctiva or nasal mucosa, first pass metabolism would be bypassed with topical application of diclofenac, potentially allowing for a higher bioavailability.

\textbf{2.3 The Ocular Effects of Topical NSAIDs}

\textbf{Effects on the conjunctiva and skin}

In humans, a transient burning sensation and conjunctival hyperemia has been reported following application of multiple topical NSAIDs including diclofenac, indomethacin, ketorolac and flurbiprofen.\textsuperscript{130,131} Clinically, veterinary patients receiving topical NSAIDs will shake their heads and rub their face, likely in reaction to this sensation.

Most of the irritation and stinging associated with topical NSAIDs has been attributed to inherent properties of the acidic free NSAID compound, which is why many NSAIDs are formulated as a salt. Formulation as a salt increases the aqueous solubility of topical NSAIDs and decreases irritation associated with the free drug.\textsuperscript{67}
A contact dermatitis has been reported with topical NSAID use and is characterized by pruritus, edema and erythema of the bulbar conjunctiva and eyelids following topical NSAID use. Reactions can take weeks to months to develop due to the development of delayed hypersensitivity reactions, but in susceptible individuals with previous exposure may be immediate.\textsuperscript{132}

In addition, the preservatives and additives in various solutions may also play a role. For example, patients receiving preservative-free diclofenac had a significantly faster decrease in conjunctival hyperemia as compared to those receiving preserved diclofenac.\textsuperscript{133} Sorbic acid and edetate disodium, which are preservative agents, as well as Cremophor EL, a surfactant, are all components of the commercially available 0.1% diclofenac sodium solution, Voltaren\textsuperscript{®}. All three of these compounds have both been associated with an allergic conjunctivitis or dermatitis.\textsuperscript{a134,135}

\textbf{Effect on Tear Film}

Research on human dry eye syndrome has demonstrated that PGE\textsubscript{1}, an eicosanoid with anti-inflammatory properties, may be an important stimulator of aqueous tear production.\textsuperscript{136} In human dry eye patients, n-3 fatty acid supplementation has been attempted to suppress the biosynthesis of AA-derived eicosanoids such as PGE\textsubscript{2} and promote the synthesis of eicosanoids from other fatty acid precursors such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).\textsuperscript{137} To the author’s knowledge, the role of PGs in lacrimal secretion has not yet been characterized in the cat, and species differences have not been identified.

Also, to the author’s knowledge, NSAIDs have not been associated with altered
tear production or tear quality in either people or animals. NSAIDs are currently used as an adjunctive treatment in human cases of dry eye.\textsuperscript{138}

Topical diclofenac has been reported to decrease substance P levels in human tears. Decreased substance P has been associated with delayed wound healing and has been associated with numerous keratopathies in humans.\textsuperscript{131,139}

**Effects on the Cornea**

*Effects on Corneal Pain and Corneal Sensitivity*

Clinically, topical NSAIDs have been shown to decrease corneal pain following photorefractive keratectomy (PRK) and cataract surgery. Human patients receiving topical NSAID therapy reported lower pain scores, and were less likely to require rescue therapy using oral pain medications.\textsuperscript{140-143} When compared to indomethacin, post-operative PRK patients had lower pain scores when treated with diclofenac, possibly due to the anesthetic effect associated with this medication.\textsuperscript{144}

Experimentally, corneal sensitivity, as measured using a Cochet-Bonnet esthesiometer, is decreased following treatment using topical diclofenac and ketorolac in healthy human test subjects.\textsuperscript{130,145} The desensitizing effect increases with repeat administration of topical NSAID, and returns to baseline within 30 minutes to 60 minutes of drop discontinuation.\textsuperscript{130} Another study demonstrated that topical diclofenac results in a level of anesthesia very close to that of oxybuprocaine, but failed to demonstrate any anesthetic effect associated with ketorolac, indomethacin or flurbiprofen.\textsuperscript{131} However, rabbits receiving diclofenac following corneal excimer laser ablation did not experience a
significant decrease in corneal sensitivity.\textsuperscript{146} In humans, the effects of topical NSAIDs on corneal sensitivity was significantly greater and longer lasting in non-white adults as opposed to white adults,\textsuperscript{130} suggesting that both inter and intra-species differences may exist.

Corneal analgesia can be attributed to a number of mechanisms. Through inhibition of COX, NSAIDs reduce sensitization of nociceptors associated with PGs in damaged tissues, and thus the pain associated with inflammation.\textsuperscript{140} It has also been proposed that diclofenac may downregulate sensitized nociceptors by stimulating the nitric-oxide guanosine monophosphate pathway, which counteracts the excitation by inflammatory mediators.\textsuperscript{147} In addition to their indirect effects on nociceptors, NSAIDs may also have direct effects on corneal sensory nerves. In anesthetized cats, diclofenac and multiple other topical NSAIDs have been shown to decrease the frequency of impulses generated by corneal polymodal nociceptors following chemical irritation by a CO$_2$ stimulus.\textsuperscript{148,149} In rabbits, diclofenac attenuates neural activity in the cornea after exciser laser ablation.\textsuperscript{150} Diclofenac may also have a local anesthetic effect through direct blockage of cation channels and further alteration of corneal nerve excitability. The diclofenac molecule is thought to interact directly with key ligands within sodium channels through its phenyl groups.\textsuperscript{145} In cultured mice trigeminal ganglion neurons, diclofenac significantly suppressed sodium currents,\textsuperscript{149} and experimentally, has been shown to decrease the responsiveness of all functional subtypes of corneal sensory fibres.\textsuperscript{148} Diclofenac and flurbiprofen may also block acid-sensing ion channels, thus decreasing pain associated with acidosis in inflamed tissues.\textsuperscript{151} These and other mechanisms are likely responsible for the decreased corneal sensitivity and enhanced
comfort observed with topical NSAID use in people and animals.

Although decreased corneal sensitization may be therapeutic, it may play a role in the formation of deep or perforating corneal ulcers in humans.\textsuperscript{152} In a small case series, decreased corneal sensitivity was documented in 2/5 patients. Decreased sensation to pain may have delayed the seeking of medical attention.\textsuperscript{152} In veterinary medicine, where patients rely on their owners to notice signs of pain such as blepharospasm, the corneal anesthesia effect may further delay treatment.

\textit{Effects on Corneal Health and Wound Healing}

Topical NSAIDs are often chosen over corticosteroids when there are concerns about corneal wound healing. This is supported by review of the human literature; there are few reports of topical NSAIDs causing corneal pathology. However, reported lesions in humans include persistent epithelial defects, superficial punctate keratitis, and subepithelial infiltrates.\textsuperscript{153-155} It is suspected that superficial punctate keratitis is associated with decreased corneal sensation, and is reversible with discontinuation of the NSAID therapy. Despite the association with topical NSAID use, patients who develop superficial punctate keratitis often have pre-existing corneal or conjunctival ocular disease, which may confound the diagnosis of an NSAID-induced keratitis.\textsuperscript{153} In humans, the prevalence of diclofenac-associated keratitis is approximately 1\%.\textsuperscript{153}

The effects of topical NSAIDs on corneal wound healing are unclear, with some studies finding no effect, and others suggesting that topical NSAIDs may be detrimental. In multiple human studies, topical NSAIDs reduced pain without causing increased healing times with corneal abrasions\textsuperscript{156} and photorefractive keratectomy.\textsuperscript{140,144,157} In
rabbits, diclofenac can be used up to 8 times daily without causing any delay in epithelial wound healing.\textsuperscript{146} Similarly, nepafenac did not cause any ocular irritation or delay corneal wound healing when dosed 4 times daily for 27 days in rabbits following corneal incision.\textsuperscript{158}

However, in human patients, topical diclofenac significantly delayed epithelial healing when compared to the placebo in another study when used following photorefractive keratectomy. In this study, diclofenac delayed healing to a greater degree than topical dexamethasone.\textsuperscript{159} Though the effect may not be detected clinically, in vitro studies suggest that subclinical effects may occur with topical NSAID use. Epithelial cells of patients treated three times daily with diclofenac following phacoemulsification surgery showed mild elongation of epithelial cells and an increase in permeability following surgery.\textsuperscript{155} Similarly, in cultures of canine corneal epithelial cells, suprofen caused a dose dependent rounding, shrinkage and detachment of cells from the culture plate. Suprofen also caused a dose dependent delay in defect closure.\textsuperscript{160} The clinical relevance of these findings is not known, but strongly suggests that corneal health should be monitored in all patients receiving topical NSAID therapy, especially those with concurrent corneal disease or a predisposition to poor wound healing.

Although uncommon, topical NSAID use has been associated with the development of deep, melting, or perforating ulcers. This has been reported in a small number of human cases in association with generic diclofenac, brand-name diclofenac, brand-name ketorolac and brand-name bromfenac.\textsuperscript{161} Most of the cases were post-operative cataract cases, but cases following refractive surgery have also been reported.\textsuperscript{162} The higher incidence of corneal malacia associated with generic diclofenac
(termed diclofenac sodium ophthalmic solution or DSOS) ultimately led to this product being removed from the market. However, the exact role of topical NSAID use in these cases remains unclear, and is complicated by many confounding factors. Many patients who develop this complication also have a history of recent intraocular surgery, concurrent corticosteroid use, and corneal surface disease. Other confounding factors include advanced age and diabetes, which may contribute to poor healing. This potentially severe consequence of topical NSAID use has not been reported in the veterinary literature, but has been seen anecdotally. If NSAID-associated corneal melting does occur with veterinary patients, an even greater number of confounding factors exist making the establishment of cause-effect even more difficult. Additional confounding factors in veterinary medicine include poor patient hygiene, poor owner compliance to treatment regimes, self-trauma, and conformational abnormalities such as lagophthalmos and trichiasis.

Proposed factors that contribute to NSAID-induced corneal ulceration and malacia include a direct cytotoxic effect, epithelial hypoxia, and induction of MMP activity on the cornea. In particular, the role of corneal microenvironment on the activity of NSAIDs is a potentially important but often overlooked contributor to corneal inflammation. In particular, the presence of corneal hypoxia, such as with tear film abnormalities, has been shown to suppress COX-1 activity, and enhance pro-inflammatory pathways mediated by CYP450 and lipoxygenase. In addition, indomethacin and flurbiprofen have both been shown to induce COX-2. High doses of diclofenac have also been shown to induce a novel form of COX-2, which is sensitive to acetaminophen, but not associated with increased levels of PGE2. Further research is
necessary to establish the relationship between NSAIDs, corneal hypoxia, altered inflammatory pathways, and adverse corneal events.

In addition to effects on COX and CYP activity, topical NSAIDs may promote MMP activity. Increased levels of corneal MMP-9 activity were found in human corneal melting patients treated with generic diclofenac 0.1%. While MMPs likely play an important role in corneal healing, they may also promote breakdown of the collagen-rich corneal stroma. At present, however, it is unclear as to whether or not increased MMP-9 is a result of diclofenac therapy or simply secondary to corneal pathology.

In using topical NSAIDs, the effects of other components within the solutions must also be considered. Cremophor EL and sorbic acid, additives found in Voltaren®, both exhibit cytotoxic effects in rabbit epithelial cell cultures. Benzalkonium chloride, present in commercial solutions of ketorolac, bromfenac, and nepafenac, causes structural changes to corneal epithelium, decreases tear production and shortens tear-film break-up time. Also, a tocopherol (Vitamin E) compound was used as a solubiliser in generic diclofenac 0.1%. While Vitamin E derived compounds are considered beneficial due to their anti-oxidant properties, tocopherol has been shown to induce apoptosis of mouse mammary cells and inhibit retinal pigment epithelial cell proliferation. The possible role of tocopherol in corneal healing and in the corneal melting observed with generic diclofenac remains to be elucidated.

**Effects on Blood Ocular Barrier**

To study the effects of BAB breakdown, various experimental models of uveitis have been used in veterinary research including anterior chamber paracentesis,
laser capsulotomy, topical pilocarpine application and corneal surgery. Intravitreal Lipopolysaccharide (LPS) injection has also been used to simulate infectious uveitis. BAB breakdown can be measured experimentally using various techniques, including slit lamp biomicroscopy, microprotein assays, fluorophotometry, laser flaremetry, and inflammatory mediator quantification.

Although there are many studies demonstrating the efficacy of NSAIDs in dogs, few studies have been performed in cats. In a recent study, anterior chamber paracentesis was used to induce BAB breakdown in cats, quantified by laser flaremetry. Topical diclofenac as well as topical prednisolone helped decrease aqueous flare, whereas topical flurbiprofen and dexamethasone did not. Oral administration of prednisolone and meloxicam were effective in decreasing intraocular inflammation in another experiment, while prednisone and acetylsalicyclic acid were ineffective. Prior to these studies, only one other study had examined the effectiveness of anti-inflammatory therapy in cats. In dogs, multiple studies have demonstrated the BAB-stabilizing effects of topical diclofenac, flurbiprofen, suprofen, and indomethacin. The BAB-stabilizing effects of numerous systemic NSAIDs have also been demonstrated in the dog. These include flunixin, phenybutazone, tolfenamic acid, and carprofen.

Though topical and systemic NSAID therapy have been well characterized for the treatment of canine uveitis, more studies are required to determine which medications are best suited to treating feline uveitis. Though paracentesis and laser capsulotomy have been extensively used in veterinary research to induce uveitis, their usefulness may be
limited in the study of feline uveitis. One model of paracentesis in dogs showed that BAB breakdown, as demonstrated by fluorophotometry, is maximal approximately one day following the procedure, but declines in the 3-4 days following paracentesis. As feline idiopathic uveitis tends to have a chronic, waxing and waning course, these models of acute BAB breakdown may be less appropriate. Intravitreal LPS injection is a model of uveitis recently described in the cat. In this model, clinical signs of uveitis were observed up to 45 days following injection. Interestingly, 7 days after injection of LPS, the leukocyte population had shifted from neutrophils to lymphocytes. Given the importance of lymphocytic-plasmacytic uveitis, LPS injection may be a suitable research model for feline uveitis. As this model currently leads to retinal inflammation with loss of photoreceptors, anterior chamber injection as well as modifications to the dose of LPS administered should be investigated.

Release of inflammatory mediators, particularly PGs, is thought to one of the principal players that mediate the BAB breakdown. PGE₂ has been measured in multiple canine studies of experimentally-induced canine uveitis and anti-inflammatory efficacy. Based on experimental models, LTs are thought to be likely less important mediators of canine uveitis. Similar studies measuring inflammatory mediators following BAB breakdown are lacking in cat. Studies are needed to best mimic natural occurring disease and also better understand which mediators play in a role feline uveitis and thus which therapies are best suited to treating feline uveitis.
Effects on Pupil Size

Many of the studies in the human literature have thus investigated the role of topical NSAIDs in maintaining an adequate pupil size. In human and veterinary medicine, miosis during cataract surgery due to the stimulation of PG release with surgical manipulation or trauma is a well-recognized phenomenon. Constriction of the pupil makes cataract removal difficult, and increases the risk of postoperative inflammation and complications. Flurbiprofen 0.03% and suprofen 1% were the first medications of this class labeled for use as intraoperative inhibitors of miosis during cataract surgery but all commercially available topical NSAIDs share this benefit.\textsuperscript{69,161}

In the veterinary literature, topical flurbiprofen,\textsuperscript{36,180} intravenous flunixin,\textsuperscript{36} and subcutaneous tolfenamic acid\textsuperscript{37} have been shown to maintain mydriasis following induction of uveitis in the dog. However, in canine healthy eyes, topical flurbiprofen did not have any effect on pupil size.\textsuperscript{195} This suggests that NSAIDs may only have an effect on iridal tone when excessive PGs are present. Research examining the effects of topical NSAID application on pupil size is lacking in the cat and should be examined in future studies.

In some individuals who received pre-operative NSAIDs, atonic mydriasis is an unusual post-operative complication. Patients with atonic mydriasis have an enlarged, non-responsive pupil that does not constrict following application of pilocarpine, but does dilate with application of mydriatics. The exact mechanism of this rare complication is unknown, and is thought to involve damage to the iris sphincter. In addition to topical NSAIDs, surgical trauma and toxicity to viscoelastics, and other medications used during
cataract surgery may play a role.\textsuperscript{67,196,197}

**Effects on IOP**

While it is generally thought that PGs do not influence aqueous production, they play an important physiological role in aqueous outflow. Prostaglandin receptors are found in both the conventional and uveoscleral aqueous outflow pathways. PGs are thought to modulate resistance in both pathways by stimulating degradation of the extracellular matrix through their effects on MMPs.\textsuperscript{52} Modification of intraocular PGs may thus lead to changes in aqueous outflow and consequently IOP. Indeed, PGF\textsubscript{2\alpha} analogues are important anti-glaucoma agents where the decrease in IOP is likely due to increases in uveoscleral outflow. Latanoprost and travoprost are important anti-glaucoma agents in humans and in dogs but not in the cat, where effects are transient.\textsuperscript{52,56-58,60,67}

The effects of non-specific PG inhibition on aqueous humor dynamics were demonstrated in a laser capsulotomy model of canine uveitis. In this model, eyes were cannulated to measure aqueous flow. Topical flurbiprofen caused a decrease in aqueous outflow as compared to control eyes. Interestingly, the decrease in aqueous outflow was more pronounced in inflamed eyes as compared to control eyes, potentially due to the additive effects of flurbiprofen and blockage of outflow by inflammatory debris.\textsuperscript{198}

In cats, topical 0.03\% flurbiprofen and 0.1\% diclofenac both resulted in an increase in IOP following paracentesis. The increase in IOP was mild, ranging from 1.7-2.7 mmHg between 4 and 26 hours following paracentesis. In contrast, there was no difference between topical 1\% prednisolone acetate and 0.1\% dexamethasone treated
eyes as compared to control eyes. A similar effect has been observed in canine studies.\textsuperscript{176,181}

Despite the documented increase in IOP in multiple canine studies, an increase in IOP following NSAID treatment in uveitic eyes has not been consistently documented (Table 2.2). This may be due to variation in the timing of IOP measurements. In a few studies with short timelines, IOP may not have had sufficient time to rise past baseline. Small sample sizes may also have contributed to the lack of significance in veterinary studies. Inconsistency in findings may also relate to differences in the methodology used to induce BAB breakdown between studies.

Again, given the paucity of literature in the cat, further studies are needed to investigate the effects of NSAID therapy on IOP. As species differences in the response to PGF\textsubscript{2\alpha} illustrate, the cat’s response to PG alteration may be very different from that of the dog. Unlike the dog and human, cats lack FP receptors in their ciliary body, and thus the effects of PGs on the feline ciliary body (and thus uveoscleral outflow) are predominantly mediated by EP receptors.\textsuperscript{59,199} Expression of PG receptors in aqueous outflow pathways may thus also be different. Fluorophotometry allows for non-invasive and accurate measurement of aqueous humor flow rate. It has been used in normal cats and in those treated with anti-glaucoma medications.\textsuperscript{200,201} It could potentially be used to determine the effects of topical NSAID application on aqueous humor flow rates in both healthy and inflamed feline eyes.

Interestingly, corticosteroids, but not NSAIDs, are associated with ocular hypertension and glaucoma in humans. In 4-6\% of the human population, IOP may rise
more than 15mmHg following topical steroid treatment. Similar IOP elevations have also been documented Beagles with primary open angle glaucoma.\textsuperscript{202} As with NSAID-associated ocular hypertension, corticosteroid-associated hypertension is due to decreased aqueous outflow. As with NSAIDs, corticosteroids may be associated with decreased levels of ocular PGs. Corticosteroids may decrease PGs through their action on phospholipase A\textsubscript{2} or more likely through inhibition of basal expression of COX-2.\textsuperscript{84,203} However, additional impedance to aqueous outflow is likely due to corticosteroid-associated induction or alteration in myocilin, an important trabecular meshwork protein. Mutations in this protein have also been associated with human open angle glaucoma in the absence of corticosteroid use.\textsuperscript{204} In order to study this important adverse effect, animal models have been developed. After 5-7 days of topical dexamethasone or prednisolone application, ocular hypertension has been shown to develop in otherwise healthy feline eyes.\textsuperscript{205,206} The mechanism underlying feline corticosteroid-induced hypertension is currently unknown.\textsuperscript{205} Studies are needed to determine if the decreased aqueous outflow seen in these cats is associated with decreased PG synthesis, as is likely the case with NSAIDs, or due to other mechanisms, such as altered myocilin.

**Effects on the Lens and Posterior Segment of the Eye**

To the author’s knowledge NSAIDs have not been associated with adverse effects of the lens or posterior segment of the eye. Topical and systemic corticosteroids, but not NSAIDs, have been associated with an increased incidence of posterior subcapsular cataracts in human patients. Oddly, topical corticosteroid use was also associated with the development of cataracts in 28% to 50% of cats in the feline model of corticosteroid-induced glaucoma.\textsuperscript{206} This effect has not be clinically documented.\textsuperscript{207} To date, altered PG
synthesis has not been incriminated as the cause for these cataracts. It is postulated that formation of corticosteroid-induced cataracts may involve steroid binding to lens protein, reduced glutathione synthesis, and altered anterior lens epithelial cell function.²⁰⁴

### 2.4 Possible Systemic Effects of Topical NSAIDs

There have been no reports of systemic adverse effects in humans or veterinary patients with topical NSAIDs, with the exception of asthmatic attacks in a select group of human patients. However, given the systemic absorption and high bioavailability of topical medications, a risk of adverse systemic effects with topical NSAID does exist. This may be especially true in cats, a species with delayed NSAID clearance and limited ability for hepatic glucuronidation. As there are no known reports of adverse effects following topical NSAID use in cats, an overview of the known systemic effects of topical NSAID administration in humans will be presented. This will be followed by a brief review of the published literature on systemic NSAID use in cats. It is expected that given sufficient systemic absorption and accumulation, adverse effects associated with topical NSAID use are similar to those seen with systemic NSAID use.

**Asthmatic Attacks in Humans**

In the human literature, isolated cases of asthma attacks following topical NSAID application have been reported with indomethacin²⁰⁸,²⁰⁹ and diclofenac.²¹⁰ A history of pre-existing mild to moderate asthma was documented in most cases, and in all cases, symptoms of asthma resolved once topical NSAIDs were discontinued. In one patient with diclofenac-induced asthma, nasolacrimal punctual occlusion prevented attacks with further diclofenac use.²¹⁰
The proposed mechanism in NSAID-induced asthma is COX inhibition within the respiratory tract, leading to shunting of AA from the COX to the lipoxygenase pathway. Lipoygenase activity results in the production of multiple LTs, such as LTE₄ and LTD₄. Accumulation of LTs causes spasm of non-vascular smooth muscles within the bronchi.²⁰⁸⁻²¹⁰ NSAID-induced asthma is most commonly associated with oral ingestion of aspirin, however any non-selective COX-inhibitor may trigger these attacks.²⁰,²¹¹

Despite the small number of cases, topical NSAID-induced asthma in human patients illustrates that even with a very small quantity of absorbed NSAID, severe systemic signs may develop. Topical NSAIDs should be used with caution in feline patients with asthma. In humans, NSAID sensitivity often develops in middle-aged patients, aged 30 years or older, with a history of chronic rhinitis, sinusitis, and nasal polyps, suggesting that repeat systemic exposure may play a role in sensitizing the body to NSAIDs, a condition that is known as “Samter’s Triad”.²¹²,²¹³ NSAID hypersensitivity also may also manifest clinically through the development of conjunctivitis, rhinitis, urticaria, angioedema and anaphylaxis.²¹²,²¹⁴ To the author’s knowledge, NSAID-associated asthma attacks have not been reported in cats.

Systemic NSAIDs in Cats

It has long been recognized that NSAIDs need be used with caution in cats due to their limited capacity for hepatic glucuronidation.³⁵ Due to decreased metabolism of NSAIDs, accumulation and subsequent toxicity is more likely in cats.³⁸⁹ This is illustrated by the much longer half-life of drugs that are eliminated via glucuronidation in the cat, such as carprofen and acetylsalicylic acid. For example, the half-life of carprofen in the
cat is 19 hours following a dose of 4 mg/kg given either intravenously or subcutaneously as compared to the dog, which shows a shorter half-life (5 – 8.6 hours). Results of studies on carprofen and acetylsalicylic acid also show a greater individual variation in the pharmacokinetics of NSAIDs in cats. Similarly, ibuprofen toxicity in cats occurs at approximately half the dose required to cause toxicosis in dogs.

Despite their inability to glucuronidate, cats may be able to utilize alternative pathways to metabolize certain NSAIDs. For example, drugs metabolized via oxidation, such as meloxicam and piroxicam, have a similar half-life in the cat as in the dog. Flunixin and ketoprofen are glucuronidated in dogs, but are not more slowly eliminated in cats, suggesting alternative metabolic pathways may exist. Alternative pathways, such as organic ion transport into bile and thioesterification may compensate for the decreased glucuronidation in cats. The relative importance of glucuronidation versus other pathways of drug elimination remains to be determined in both dogs and cats.

Unfortunately, there is much less information available regarding adverse drug effects in cats than in dogs, which is likely due, in part, to less licensed products available for cats. In dogs, 64% of NSAID-related adverse drug experiences (ADEs) reported to the US Federal Drug Administration are related to the GI tract, 21% are related to the renal system, and 14% are related to the hepatic system. Between 2005 and 2010, 1244 feline cases of NSAID toxicity were reported to the ASPCA Animal Poison Control Centre, as compared to the 15823 canine cases. At present, the total incidence of adverse effects following systemic and topical NSAID use in veterinary medicine is unknown.
Compared to dogs, there are very few licensed systemic NSAIDs in cats, and most licensed products can only be administered perioperatively once or for a period of days. Meloxicam is licensed in the USA (perioperatively 0.3 mg/kg SQ once), Canada (perioperatively - 0.2 mg/kg SQ once followed by 0.05 mg/kg PO for up to 2 days; acute musculoskeletal disorders – 0.1 mg/kg PO once followed by 0.05 mg/kg for up to 4 days), Australia and Europe (0.05 mg/kg PO chronic use approved). Carprofen is licensed in Europe (4 mg/kg SQ or IV once). Ketoprofen is licensed in Canada, Europe, Australia (2 mg/kg SQ followed by 1 mg/kg PO for up to 4 days, or 2 mg/kg SQ for up to 3 days in severe cases). Tolfenamic acid is licensed in Canada (4 mg/kg for up to 3-5 days), Australia and Europe (2 mg/kg SQ or PO for up to 3 days). Robenacoxib is licensed in Canada and Europe (1-2.4 mg/kg for up to 6 days) and the US (6 mg or 12 mg depending on weight of cat for up to 3 days)\textsuperscript{89,224,225}

In general, further studies are needed in cats receiving clinically relevant doses of NSAIDs for prolonged periods of time. At present, many of the studies in the literature investigate short-term use of NSAIDs, likely due to the limited period that NSAIDs are labeled for in cats. In general, prior to initiating systemic NSAID therapy, the veterinarian should ensure that the patient is normotensive and well hydrated. Patients should also have normal renal and hepatic function, normal hemostatic function, no GI signs, and should not be receiving concurrent systemic corticosteroids.\textsuperscript{226}
Gastrointestinal Effects

In most species, NSAID-associated GI adverse effects are thought to be the most common and significant ADEs compared to other organ toxicities. GI adverse effects include vomiting, diarrhea, gastric erosions and gastric ulcerations. Although not completely understood, NSAID-induced GI signs are likely due to PG-mediated inhibition of mucosal protective mechanisms. Inhibition of COX-1 is thought to cause decreased mucus formation, decreased bicarbonate production, and adverse vascular effects, which predispose the GI mucosa to ulceration. Although the importance of COX-2 in cats remains to be determined, COX-2 inhibition has been shown to delay ulcer healing in other species.\textsuperscript{227} Deep GI ulceration has been seen with aspirin,\textsuperscript{228,229} indomethacin,\textsuperscript{230} and carprofen\textsuperscript{231} administration in cats. An increased risk of toxicity is seen with prolonged use of NSAIDs, excessive doses, as well as concurrent administration of other NSAIDs.\textsuperscript{89,231}

Recent studies have, however, suggested that NSAIDs could potentially be safely used in cats without GI toxicity, especially in healthy animals and at low doses. No GI lesions were detected via endoscopy 8 hours following a single 4 mg/kg SQ carprofen injection in cats\textsuperscript{217} or following 6 days of carprofen treatment at decreasing doses.\textsuperscript{232} Administration of robenacoxib at several times its labeled dose, at up to 10 mg/kg for up to 42 days, resulted mild GI toxicity, with only a small percentage of animals developing soft stools.\textsuperscript{225} Long-term oral meloxicam in cats with osteoarthritis at a daily dose of 0.01- 0.03 mg/kg for an average of 5.8 months resulted in only 2/46 (4%) of cats developing signs of GI upset.\textsuperscript{233} The excellent long term safety and tolerability associated with meloxicam in cats may relate to its metabolism via oxidation.
Despite reports of good GI safety, adverse GI events have also been reported in the feline NSAID literature. In a retrospective study of 73 cats receiving piroxicam for various neoplasms, 16.4% of cats experienced vomiting and 2.7% of cats experienced diarrhea.\textsuperscript{234} However, concurrent disease, chemotherapy and radiation therapy acted as confounding factors so the effects of piroxicam could not be isolated. Despite the high frequency of GI adverse effects, clinical signs were mild and neither NSAID therapy nor chemotherapy had to be discontinued in any patients as a result of GI toxicity. In another retrospective study of 57 cats receiving an average of 0.03 mg/kg/day of meloxicam, 18.2% of cats developed GI signs. However, the median duration of treatment prior to time to onset of ADEs was very long, with a median of 448 days.\textsuperscript{235}

Experiments conducted during safety trials for meloxicam illustrate the potential dangers of high dosages and prolonged NSAID use.\textsuperscript{6} Cats given 0.3 mg/kg SQ or 0.6 mg/kg of meloxicam for 8 days developed inappetance, lethargy, vomiting, and diarrhea. On Day 9 of treatment, one cat in the 0.3 mg/kg group died and another cat in the 0.6 mg/kg group was moribund. Although the cause of death was not definitively determined, pyloric and duodenal ulcerations were reported on necropsy, suggesting that GI disease likely played a role. As is expected, high doses (up to 1.5 mg/kg or 5x the recommended dose) administered for 3 days resulted in vomiting and loose stools in experimental cats. Fecal blood was reported in 10/24 animals.

Additional research is needed to establish the GI safety of NSAIDs in cats. Sensitivity to subclinical GI lesions may be improved through the use of endoscopy, although this may not be feasible in populations of client owned animals.
Renal Effects

Renal PG levels are relatively low in normovolemic individuals. However, during hypovolemia and dehydration, PGs are necessary to counteract the vasoconstriction associated with stimulation of the sympathetic nervous system, norepinephrine release or activation of the renin-angiotensin system.\textsuperscript{236,237}

Unlike in other tissues, COX-2 likely plays an important role in the kidney. As such, increased use of COX-2 selective NSAIDs has not lead to increased renal safety in humans.\textsuperscript{238}

Constitutive COX-2 expression has been demonstrated in other species, and may be upregulated by volume depletion.\textsuperscript{239} In particular, medullary COX-2 expression appears to be upregulated with dehydration or a hypertonic environment.\textsuperscript{240} When a hypertonic environment is present, PGE\(_2\) is thought to play a cytoprotective role and decrease sodium reabsorption at the thick ascending limb of the Loop of Henle. By decreasing PGE\(_2\) and increasing sodium reabsorption, NSAIDs can lead to edema and weight gain in human patients.\textsuperscript{241,242} Cortical COX-2 synthesizes PGs that lead to release of renin and activation of the renin-angiotensin-aldosterone system in situations of volume depletion or decreased salt intake. Activation of this system leads to increased tubular reabsorption of sodium and maintenance of intravascular homeostasis.\textsuperscript{243} In addition to sodium, PGs are also involved in the regulation of potassium excretion at the level of the kidney. Prostaglandin I\(_2\) has been shown to cause renin release, which increases aldosterone secretion and potassium secretion by the distal nephron. Thus, NSAID administration can result in hyperkalemia, which can range from mild to
potentially lethal. Decreases in circulating blood flow stimulate the release of PGs, particularly PGI\(_2\). These PGs help to maintain renal blood flow by counteracting vasoconstrictors such as norepinephrine and angiotensin II. Decreased renal blood flow can be associated with reduced GFR, which can be transient, or if severe, acute tubular necrosis.  

In a recent retrospective study, 21/48 cats presenting in acute renal failure had a history of recent nimesulide, tolfenamic acid or ketoprofen ingestion. Vomiting, polyuria, polydipsia, and dehydration were commonly reported on admission. While most of the cats received only one dose of NSAID, their hydration status and renal health prior to NSAID administration was largely unknown. Furthermore, one of the two cases linked to ketoprofen toxicity had received 15 times the recommended dose. In contrast, in cats receiving long-term treatment for osteoarthritis or neoplasia, there was no significant increase in renal parameters on serum biochemistry panels or clinical signs of renal insufficiency. Despite the apparent renal safety of meloxicam and piroxicam in these studies, a few cats showed progression of pre-existing renal disease or developed azotemia. In these cases, it was difficult to tell if the development or worsening of renal disease was associated with NSAID use or with age, dehydration, or natural progression of disease. Similarly, in a retrospective study, 6/12 cats treated with meloxicam and having available serum biochemistries showed an increase in creatinine, although a significant treatment effect was not found. These studies also illustrate the difficulty in determining renal function using only serum biochemistry panels and urinalysis, which are not altered until the later stages of renal disease.
Studies using more sensitive and accurate tests for renal function have recently been performed. In one study, a 0.2 mg/kg oral loading dose of meloxicam followed by a maintenance dose of 0.1 mg/kg for 4 days in healthy cats did not result in any significant change in GFR as measured by iohexol clearance.\textsuperscript{251} Similarly, renal scintigraphy performed up to 45 days following a 14 day course of tolfenamic acid or vedaprofen in healthy cats also did not demonstrate any effects on renal function.\textsuperscript{252} There was no evidence of renal toxicity in cats treated for up to 6 weeks with robenacoxib based on serum biochemistry panels, urinalyses or postmortem gross and histopathological examination.\textsuperscript{225}

As with gastrointestinal ADEs, high doses of NSAID are associated with a higher incidence of renal ADEs. Two of six cats given meloxicam subcutaneously at 5x the recommended subcutaneous dose (1.5 mg/kg) showed increased blood urea nitrogen and creatinine. On post-mortem, multiple renal lesions were noted including dilated medullary and cortical tubules, renal inflammation, renal interstitial fibrosis. Five of six cats receiving 5x the recommended dose developed papillary necrosis.\textsuperscript{b}

The effect of NSAIDs on the renal system requires further study. In particular, further studies are needed to determine the effects of prolonged NSAID administration, age, and concurrent disease states on renal function. Diagnostics that should be utilized include serum biochemistry panel (for blood urea nitrogen, creatinine, and electrolytes), urinalysis, urine protein:creatinine ratio, and abdominal ultrasound.\textsuperscript{253,254} Highly sensitive indicators of renal function, such as GFR, are should be utilized if possible so that early renal disease can be detected. As systemic NSAIDs tend to increase renal
sodium reabsorption by decreasing tubular PGE₂, fractional excretion of sodium could also be used to determine the effect of NSAIDs on the kidney.²⁵⁵,²⁵⁶

**Hepatic Effects**

At present, there are no known literature reports of hepatic toxicity following use of NSAIDs in cats. An idiosyncratic hepatocellular toxicosis has been reported following carprofen ingestion in dogs.²⁵⁷ Interestingly, one of the hypotheses for the idiosyncratic toxicity is haptenization of hepatic proteins by glucuronide metabolites. If this is true across species, then cats may be more resistant to this type of hepatotoxicity.²⁵⁸ There was no evidence of hepatic toxicity in cats receiving long-term meloxicam or piroxicam therapy.²³³–²³⁵ There was no evidence of hepatic toxicity in cats treated for up to 6 weeks with robenacoxib based on serum biochemistry panels, urinalyses or postmortem gross and histopathological examination.²²⁵ AST elevations were noted with perioperative meloxicam and carprofen administration in cats undergoing ovariohysterectomy. However, no other hepatic parameters were abnormal and the significance of this elevation is not clear as AST is not specific to the liver.²⁵⁹ Acetaminophen, which can cause hepatic toxicity in dogs and humans, causes methemoglobinemia and Heinz body anemia in cats but does not typically cause hepatic damage.⁸⁹

The effect of NSAIDs on the liver requires further study. The use of multiple diagnostic tests reflecting hepatic function and health (i.e. serum biochemistry, bile acid stimulation, abdominal ultrasound, etc.) may help to better evaluate hepatic function following NSAID use.
**Clotting Function**

NSAIDs can alter hemostasis through their effects on platelets or vascular endothelium.\(^{89}\) Platelet aggregation is dependent upon production of thromboxane A\(_2\) by COX-1 in platelets. In contrast, inhibition of the platelet plug is mediated by PGI\(_2\), which is produced by COX-2 in endothelial cells.\(^{260}\) Clinically, the anticoagulant effect associated with COX-1 inhibition has been exploited in the treatment and prevention of thromboembolic disease using aspirin in cats. Although COX-1 inhibition can be beneficial in prothrombotic disease states, it is may be associated with increased risk of hemorrhage. Intraoperative hemorrhage was documented in one cat receiving meloxicam and in one cat receiving carprofen in a group of 80 cats undergoing ovariohysterectomy.\(^{259}\) However, in both of these cases, definitive establishment of NSAID therapy as the cause for hemorrhage could not be made. In safety trials for meloxicam, 1/6 cats receiving 5x the recommended dose developed prolongation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).\(^b\)

As cats with cardiac disease are at a high risk of developing thromboembolic disease,\(^{261}\) the risks of COX-2 inhibition and the development of thrombi should be examined.

More data is needed to properly evaluate the risks of hemorrhage and thromboembolic complications with NSAID use in cats. Clotting function can be evaluated through more traditional tests such as PTT and APTT. More sensitive tests of NSAID induced platelet-dysfunction might include thromboelastography, platelet
aggregometry and platelet thromboxane B₂. These tests have been used to evaluate the effects of COX-2 inhibitors in the dog.²⁶²-²⁶⁴

2.5 Summary

Anterior uveitis is a common and potentially vision-threatening condition in the cat. In order to avoid the sequelae of uveitis, prompt and aggressive anti-inflammatory therapy is indicated.¹,⁷,²² Topical NSAIDs are potent anti-inflammatories that are used in the treatment of feline uveitis, particularly in cases where topical corticosteroids are contraindicated.²²,³⁵ Topical administration of NSAIDs also allows for high concentrations of NSAIDs to be achieved locally, while minimizing systemic exposure to NSAIDs. Although topical NSAIDs have been shown to be effective in canine and feline models of uveitis,¹⁷¹,¹⁷³,¹⁷⁶,¹⁸⁰,¹⁹¹ little is currently known regarding possible ocular or systemic adverse effects, particularly after repeated administrations. Corneal lesions, ranging from very mild to vision threatening, have been documented in the humans.¹⁵²,¹⁵³

Mild elevations in IOP were observed following topical NSAID administration use in a recent feline study.¹⁷³ To the author’s knowledge, there are currently no published studies in cats that have examined the effects of topical NSAIDs on tear production or quality, corneal health, corneal sensitivity. Furthermore, the effect of topical NSAIDs on IOP needs to be further investigated in cats, as glaucoma is a serious complication of feline uveitis.

Although one goal of topical therapy is to limit systemic absorption, a proportion of topically applied medications is systemically absorbed, primarily through the conjunctiva and nasal mucosa.¹¹⁶,¹¹⁷ Due to their limited capacity for hepatic
glucuronidation and metabolism of NSAIDs, cats may be at increased risk for systemic accumulation and adverse effects, even with topical administration. To the author’s knowledge, there have been no studies to date that have characterized the systemic absorption of topical NSAIDs in cats, or their effects on the GI, renal or hepatic systems. Evaluation of the ocular and systemic adverse effects of topical NSAIDs is necessary to determine whether this route of administration and class of drug can be used safely to treat uveitis in cats.

Given the lack of published information regarding possible ocular and systemic adverse effects of topical NSAIDs in cats, particularly with frequent dosing long-term, research is needed to establish whether or not these medications can be safely used in the context of feline uveitis. Thus, the goal of this research project is to use available clinical tests to look for any ocular or systemic toxicity associated with topical NSAID use. Diclofenac 0.1% has been selected for this research project given its effectiveness in an experimentally-induced model of feline uveitis, good availability and high frequency of use. Daily slit lamp biomicroscopy and fluorescein staining will be used to examine possible effects of 0.1% diclofenac on corneal and conjunctival health, and Cochet-Bonnet esthesiometry will be used to evaluate for changes in corneal sensitivity. Aqueous tear production and TFBUT will be used to evaluate for any NSAID-induced changes in tear quality or quantity. Rebound tonometry performed multiple times a day will be used to further investigate the possibility of IOP elevation with topical NSAID use.

At present, the systemic pharmacokinetics and toxicity of topical NSAIDs in cats have not been characterized. Therefore, a basic pharmacokinetic study will be performed to quantify the amount of drug absorption into systemic absorption, and to determine if
there is an accumulation effect due to decreased metabolism. Serum biochemistry panels and urinalysis will also be performed to evaluate for the possibility of renal and hepatic toxicity with topical NSAID use. Renal function will also be evaluated through GFR to increase the likelihood that early or mild changes in renal function will be detected.

2.6 Footnotes

a. Ontario Veterinary College Health Sciences Centre Medical Records
b. Voltaren Ophtha product monograph, Novartis Pharmaceuticals Canada Inc, Dorval, Quebec, Canada
c. Metacam (meloxicam) 5 mg/ml injectable product monograph, Boehringer Ingelheim, St. Joseph, MO, USA
2.7 Tables

Table 2.1: Causes of feline uveitis\(^3\)

<table>
<thead>
<tr>
<th>Category</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traumatic</td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td></td>
</tr>
<tr>
<td>Neoplastic</td>
<td>Diffuse iris melanoma</td>
</tr>
<tr>
<td></td>
<td>Primary ocular sarcomas</td>
</tr>
<tr>
<td></td>
<td>Primary ciliary body adenomas</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>Metastatic uveal neoplasms (mostly adenocarcinomas)</td>
</tr>
<tr>
<td>Lens-induced</td>
<td>Cataract-induced</td>
</tr>
<tr>
<td>Parasitic</td>
<td>Toxoplasma gondii</td>
</tr>
<tr>
<td></td>
<td>Ophthalmomyiasis</td>
</tr>
<tr>
<td>Infectious</td>
<td>Viral Causes</td>
</tr>
<tr>
<td></td>
<td>Feline immunodeficiency virus (FIV)</td>
</tr>
<tr>
<td></td>
<td>Feline leukemia virus (FeLV)</td>
</tr>
<tr>
<td></td>
<td>Feline infectious peritonitis (FIP)</td>
</tr>
<tr>
<td></td>
<td>Bartonella henselae</td>
</tr>
<tr>
<td>Fungal Causes</td>
<td>Cryptococcus neoformans</td>
</tr>
<tr>
<td></td>
<td>Coccidioides immitis</td>
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<tr>
<td></td>
<td>Blastomyces dermatitidis</td>
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<tr>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td></td>
<td>Histoplasma capsulatum</td>
</tr>
<tr>
<td></td>
<td>Periarteritis</td>
</tr>
</tbody>
</table>
Table 2.2: Summary of veterinary ophthalmology experiments examining the relationship between NSAIDs and IOP (Time 0 = inflammatory stimulus)

<table>
<thead>
<tr>
<th>NSAID / Route / Species</th>
<th>Uveitis Model</th>
<th>NSAID Pretreatment</th>
<th>Timing of NSAID administration after uveitis induction</th>
<th>Time of IOP measurements</th>
<th>Differences in IOP between treated and control eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flurbiprofen, Diclofenac (topical) Cat&lt;sup&gt;173&lt;/sup&gt;</td>
<td>Anterior chamber paracentesis</td>
<td>Immediately prior to paracentesis</td>
<td>0, 6, 10, 24h</td>
<td>4, 8, 26h (2-4h following drops)</td>
<td>Flurbiprofen: 4, 8h Diclofenac: 8, 26h</td>
</tr>
<tr>
<td>Flurbiprofen (topical), Flunixin IV Dog&lt;sup&gt;176&lt;/sup&gt;</td>
<td>Nd:YAG capsulotomy</td>
<td>Flurbiprofen: 120, 90, 60, 30 min prior to laser Flunixin 30 min prior to laser</td>
<td>None</td>
<td>5, 30, 60, 120 min (35-125min following drop)</td>
<td>Flurbiprofen: 30, 60, 90, 120 Flunixin: 60, 90, 120min</td>
</tr>
<tr>
<td>Suprofen, Diclofenac, Flurbiprofen (topical) Dog&lt;sup&gt;188&lt;/sup&gt;</td>
<td>2% pilocarpine TID for 2d</td>
<td>n/a</td>
<td>Administered with pilocarpine</td>
<td>Start, 7h and 31h after start of pilocarpine treatment</td>
<td>Flurbiprofen: 7, 31h, Diclofenac and Suprofen: 31h</td>
</tr>
<tr>
<td>Flurbiprofen (topical) Dog&lt;sup&gt;265&lt;/sup&gt;</td>
<td>Nd:YAG laser capsulotomy</td>
<td>QID for 48h</td>
<td>None</td>
<td>5, 15, 45, 60min</td>
<td>None found</td>
</tr>
<tr>
<td>Flunixin IV Dog&lt;sup&gt;36&lt;/sup&gt;</td>
<td>Nd:YAG laser capsulotomy</td>
<td>30min prior to laser</td>
<td>None</td>
<td>5, 30, 60min</td>
<td>None found</td>
</tr>
<tr>
<td>Aspirin PO Dog&lt;sup&gt;167&lt;/sup&gt;</td>
<td>Corneal incision</td>
<td>q8h for 40h</td>
<td>None</td>
<td>q24h for 14d</td>
<td>None found</td>
</tr>
</tbody>
</table>
Figure 2.1: Synthesis of PGs from AA

Cell Membrane Damage

Release of Membrane Phospholipid

Phospholipase A₂

Corticosteroids

NSAIDs

Arachidonic Acid

Lipoxygenase

Cyclooxygenase

PGH₂

Hydroperoxidase

PGG₂

PGD₂

Txₐ₂

PGI₁

PGE₂

PGF₂α

PGE synthase

PGD synthase

Txₐ synthase

PGI synthase

PGE 9-ketoreductase


2.9 References


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the ocular irritative response in dogs. Veterinary & comparative ophthalmology 1995;5:42.
CHAPTER 3: The Ocular Adverse Effects of Topical 0.1% Diclofenac in Healthy Cats

3.1 Abstract:

Objective: To determine if there are any adverse ocular effects associated with an aggressive dosing regime of 0.1% diclofenac in healthy animals.

Animals: 8 healthy male cats.

Procedures: A blinded, randomized, placebo-controlled cross-over study design was used. Either topical 0.1% diclofenac (n = 4 cats) or an artificial tear solution (n = 4 cats) was administered in both eyes 4 times daily (8am, 12pm, 4pm, 8pm) for 7 days. There was a 12-day wash-out period before cats were crossed-over. Slit lamp biomicroscopy, STT, fluorescein staining, TFBUT, rebound tonometry, and measurement of PD were performed on a daily basis prior to administration of eye drops. Signs of ocular irritation (conjunctival hyperemia, blepharospasm, chemosis, nictitans prolapse, and ocular discharge), length of eye closure, and non-specific signs of irritation (licking, face rubbing, sneezing, and head shaking) were also noted when drops were administered. Corneal touch threshold was measured prior to and at the conclusion of each phase of the experiment.

Results: There was no significant difference between treated and control animals for STT, TFBUT, IOP, PD, and CTT. No abnormalities were detected on examination of the
anterior segment using slit lamp biomicroscopy and all eyes remained fluorescein negative. Treated animals were 8 times more likely to develop conjunctival hyperemia than control animals (p = 0.0161).

**Conclusions and Clinical Relevance:** In healthy cats, topical 0.1% diclofenac is well tolerated and has few adverse effects other than mild signs of ocular irritation.

### 3.2 Introduction:

Anterior uveitis is a common condition in domestic cats. Between 2001 and 2011, 58 (24%) of 237 cats presenting to the Ontario Veterinary College Health Science Centre Ophthalmology Service were diagnosed with uveitis. Exogenous causes include corneal injury or trauma, and endogenous causes include idiopathic, neoplastic, lens-induced, and infectious. The number of cats with detectable concurrent systemic disease is significant but variable in the veterinary literature, ranging between 38 and 70%. It is, however, evident that despite complete systemic investigation, many cases of feline anterior uveitis remain idiopathic. In a retrospective histopathological study, idiopathic uveitis, characterized by lymphocytic-plasmacytic inflammation, was the most common type of uveitis in the cat, occurring in 32% of enucleated or eviscerated globes.

Sequela of anterior uveitis include corneal endothelial damage leading to permanent corneal edema, cataract formation, lens luxation, posterior synechiae, secondary glaucoma, and phthisis bulbi. As it is a painful and potentially vision-threatening condition, prompt and aggressive anti-inflammatory therapy is indicated. Topical corticosteroids are commonly administered as the primary therapy in the treatment of feline anterior uveitis, as they are potent anti-inflammatory agents and are
generally well tolerated by cats.\textsuperscript{20,22,23} Topical NSAIDs are potent anti-inflammatories that may be used in the treatment of feline uveitis, particularly in cases where topical corticosteroids are contraindicated, such as cases of corneal ulceration, perforation, laceration or infection.\textsuperscript{20,24}

NSAIDs exert their therapeutic effect by inhibiting COX, which catalyzes the release of PGs from AA. Prostaglandins are considered to be important mediators of ocular inflammation; they have been shown to produce miosis, increase vascular permeability and lead to breakdown of the BAB.\textsuperscript{20,25} However, while COX activity and PG formation are associated with inflammation and pathology, they are also involved in maintenance and physiologic functions.\textsuperscript{23,24,26} Thus decrease in PG levels with NSAID use may be associated with adverse effects.

In the eye, through their effects on matrix metalloproteinases in aqueous outflow pathways, the trabecular outflow pathway and the uveoscleral outflow pathway, PGs play an important physiological role in regulating IOP.\textsuperscript{27,28} Prostaglandins may also play a role in regulating ocular blood flow.\textsuperscript{29} In the cat, FP receptor agonists have been shown to increase blood flow to the anterior sclera and iris.\textsuperscript{29} Results of previous studies have demonstrated the presence of EP\textsubscript{1}, EP\textsubscript{2} and DP prostanoid receptors in the ciliary body,\textsuperscript{30-32} as well as EP\textsubscript{1}, EP\textsubscript{2} and FP receptors in the iris sphincter muscle of the feline eye.\textsuperscript{32,33} Recent studies in healthy human, mouse and pig eyes have, however, demonstrated that prostanoid receptors are present in virtually every ocular tissue including the conjunctiva, cornea, sclera, iris, ciliary body, lens, choroid and retina.\textsuperscript{34-36} The significance of the widespread distribution of prostanoid receptors in the eye remains to be determined and these findings suggest that PGs play an important role in the eye in both health and in
Although topical NSAIDs have been shown to be effective in reducing BAB breakdown in canine and feline models of uveitis, little is currently known regarding potential ocular adverse effects, particularly when aggressive dosing regimes are used long-term. Given the likely importance of PGs in multiple ocular tissues, the safety of this class of medication must be established. To the author’s knowledge, there are currently no published studies in cats that have examined the effects of topical NSAIDs on tear production, tear film quality, corneal health or corneal sensitivity. In humans, topical NSAID use has been associated with conjunctival hyperemia, decreased corneal sensation, as well as a corneal lesions.

The objective of this study was to establish if topical 0.1% diclofenac can be safely used in cats according to an aggressive clinical regime. It was hypothesized that topical diclofenac would be associated with signs of mild ocular irritation, decreased corneal sensitivity, and mild increase in IOP but would not have any significant effect on any of the other parameters examined.

3.3 Materials and Methods:

Animals:

Eight intact male purpose-bred, barrier-raised domestic shorthair cats of approximately one year of age (range 8 – 14 months) were included in the study. Sample size was determined following a power analysis based on IOP differences detected in a previous study as IOP was the only outcome of interest for which there was published
numerical data. Cats were purchased from a commercial supplier. Cats weighed between 5.02 and 6.63 kg. Prior to inclusion, all cats received a general physical examination. Complete blood count, serum biochemistry, UA and UPCR were also performed. Ocular examination including neuro-ophthalmic exam (palpebral reflex, dazzle reflex, menace response, pupillary light reflexes), slit lamp biomicroscopy, STT, fluorescein staining, rebound tonometry and indirect ophthalmoscopy following administration of tropicamide 1% was performed prior to the study.

Cats were exposed to an automated 12-hour light-dark cycle (light phase from 7am to 7pm, dark phase from 7pm to 7am). Cats were acclimatized to handling and ocular procedures for one month prior to the start of the experiment. This study was approved by the University of Guelph Animal Care and Use Committee.

**Experimental Procedures:**

Baseline data was collected on 3 separate days within a 2-week period prior to the beginning the experiment. A blinded placebo-controlled crossover design was used. The study consisted of two 7-day treatment periods (Phases 1 and 2), with a 12-day washout period in between periods. Prior to beginning Phase 1 of the experiment, cats were randomly assigned to control (n=4) and treatment (n=4) groups by drawing names out of a hat. During each 7-day phase, cats in the treatment group received one drop (50ul) of 0.1% diclofenac ophthalmic solution and control cats received one drop (50ul) of an artificial tear in each eye at 8am, 12pm, 4pm, and 8pm. Ocular exams and tests were continued once daily at 8am for the first 3 days of the wash-out period. On the final day of the washout period, all ocular tests were repeated to ensure that no abnormalities were
present and that lingering treatment effects were unlikely prior to proceeding to the next phase. Following the washout period, cats were crossed over: cats initially receiving 0.1% diclofenac received the control treatment, and cats initially receiving control treatment received the 0.1% diclofenac drops. In order to maintain masking of the principal investigator (KKH), drops were administered from identical sterile syringes by a research technician, summer student or another investigator (CLP).

**Ocular Tests:**

All ocular tests were performed in the same environment by one investigator (KKH). Ocular tests included slit lamp biomicroscopy, STT, fluorescein staining, TFBUT, rebound tonometry, and measurement of PD. Schirmer tear test, fluorescein staining and TFBUT were measured once a day during the experiment. Intraocular pressure, PD, and ocular examination via slit lamp biomicroscopy were performed four times a day during the experiment (Table 3.1). For all ocular tests, cats were manually restrained without the use of sedation or topical anesthesia. Prior to and following each drug administration, eyes were examined using a slit lamp and scored according to a modified Hackett-MacDonald scoring system, using a 0-4 scale to score blepharospasm, ocular discharge, conjunctival hyperemia, conjunctival chemosis, and third eyelid prolapse. A score of zero represented an absence of signs and a score of 4 represented maximal severity of signs. In addition, non-specific signs of ocular irritation, including licking, face rubbing, sneezing and head shaking were marked as present or absent following application of eye drops. Following application of the topical drop to both eyes, the time that the eyes remained closed was timed as an indicator of ocular irritation.
or discomfort. Once the eyes were fully open, each cat was then re-examined using a slit lamp and scored according the scale outlined above.

Corneal touch threshold was performed three days prior to the start of each treatment phase and at 8:30pm on Day 7 following administration of the last drop.

Horizontal PD was measured using Jameson calipers held adjacent to the cornea. Measurements were always performed in the same room, where lighting was maintained between 172-196 luxes (16-18 candles).

A rebound tonometer was used to measure IOP. The same instrument, calibrated and used according to the manufacturer’s recommendations, was used throughout the study. The first IOP reading with no error (mean of 6 separate values) was recorded.

Aqueous tear production was measured using a standardized Schirmer tear strip, placed into the ventral conjunctival sac for one minute.

Tear film break-up time was performed by placing a drop of fluorescein in the dorsolateral conjunctival sac, and holding the eyelids closed for a 3 to 4 seconds. Eyelids were then opened, and a slit lamp set to 16x magnification with cobalt blue filtration was used to observe the dorsolateral cornea. Tear film break-up time was measured as the time from eyelid opening to the first signs of tear film break-up. This was seen as a dark spot within the fluorescent yellow tear film. Three readings were performed for each cat, and the average of the three readings was taken. TFBUT was evaluated using previously described methods.
Following measurement of TFBUT, the cornea was rinsed with a sterile eye wash solution and examined for any uptake of fluorescein stain using the slit lamp. A stopwatch was used to measure time for both STT and TFBUT.

Corneal touch threshold (CTT) was measured as previously described using a Cochet-Bonnet esthesiometer with a 0.12 mm cross-sectional diameter nylon monofilament. To determine corneal sensitivity, the filament of the esthesiometer was slowly advanced towards the globe and applied perpendicularly to the central cornea. Pressure was increased until a slight deviation of the filament was noted. Corneal sensitivity was recorded as the length of the esthesiometer filament that induced a blink reflex on at least 3 out of 5 stimulations for each specific filament length. An initial filament length of 5.0 cm was used and if no blink reflex was elicited, then the filament length was decreased in 0.5 cm increments until a blink reflex was detected on at least 3 out of 5 stimulations at a given filament length. Measures of filament length were converted to units of force (g/mm²) according to the chart provided by the manufacturer.

**Statistical Analysis:**

All parameters (STT, IOP, PD, fluorescein staining, TFBUT, CTT,) were analyzed with a general linear mixed model that included baseline values as a covariable (ANCOVA) Baseline values were included as a covariable because preliminary statistical analysis showed that baseline values for STT, IOP and PD had a significant effect on experimental values obtained during the experiment. Parameters measured at more than 2 time points were fitted to the best covariance structure to allow for making repeated measures over time. Effects in the model included cat, period, eye, and
treatment depending on the parameter, day, and time. All interactions of the aforementioned effects (ie. cat, period, eye, etc.) in the model were accounted for and removed if not significant. Data was assessed for normality using a Shapiro-Wilk test and examination of the residuals. Data was logarithmically transformed if it improved normality. Post hoc tests used included a Tukey or Dunnett’s adjustment depending on the comparison. Standard errors presented are based on pooled standard error for placebo and treatment animals.

Baseline values were also compared to values obtained one day prior to beginning the Phase 2 of the experiment using an ANCOVA. Again, effects in the model included cat, period, eye, and treatment depending on the parameter, day, and time.

As scores of 2-4 were rare, signs of ocular irritation were considered to be present or absent. The probability for each sign of ocular irritation (conjunctival hyperemia, blepharospasm, chemosis, ocular discharge, licking behavior, sneezing, head shaking and face rubbing) was then calculated using a GLIMMIX procedure for binary measures. Effects in the model included cat, treatment, day, time of day, period, and timing relative to drop administration (pre vs. post). Effects were removed if not significant. Time of eye closure was analyzed using a general mixed linear model (ANOVA).

A p value of <0.05 was considered to be significant.
3.4 Results:

**Pre-experimental Screening:**

General physical examination performed prior to the experiment was unremarkable in all animals. Prior to the beginning of the experiment, ocular examinations on all cats were unremarkable. No abnormalities were found on neuro-ophthalmic examinations. There were no signs of ocular irritation identified in any cat. All cats were fluorescein negative. Baseline TFBUT was consistent with values previously published.\(^{50}\) Baseline STT values were also within the range of values previously published in clinically normal cats.\(^{51-54}\) Baseline CTT was lower than values previously published, but considered to be within the normal range given the large variability in previous experiments.\(^{55,56}\) Baseline IOP was similar to those previously reported using the Tonovet.\(^{57}\) In three animals, superficial focal white corneal opacities were present, and were consistent with corneal scarring from previous injury. As there were no signs of active inflammation or any other ocular abnormalities, these animals were included in the study. Fundic exam via indirect ophthalmoscopy was within normal limits for all cats.

**Experimental Results:**

**Ocular Irritation Scoring:**

Although scoring of ocular irritation was performed on a 0-4 scale, in all cases, signs of ocular irritation were very mild, with a score of 1 being most frequently
assigned, with the occasional 2 being assigned. Thus, in light of these results, signs were considered either present (1) or absent (0) for statistical analysis.

Slit lamp examinations to evaluate ocular irritation were performed before (pre-scores) and after administration (post-scores) each set of eye drops. Given the very small number of positive scores, to improve power and the validity of our model, pre-scores and post-scores were combined for statistical analysis. Animals treated with topical 0.1% diclofenac were more likely to exhibit signs of irritation (Table 3.2), however conjunctival hyperemia was the only sign where treated animals were statistically more likely to have a positive score than control animals (p = 0.0161). Conjunctival hyperemia was also the most common sign of ocular irritation to be recorded; conjunctival hyperemia was present in 31.08 ± 16.93% (mean ± SE) of exams performed on treated animals and 5.32 ± 4.1% of exams performed on control animals. Conjunctival hyperemia was 8 (1.72-37.03)(odds ratio) (95% confidence interval) times more likely in treated animals than in control animals. Treated animals were not significantly more likely than control animals to develop blepharospasm (p = 0.0696), chemosis (p = 0.4839), nictitans prolapse (p = 0.6798) or ocular discharge (p = 0.8181).

Regardless of treatment, most animals were noted to have one sign of ocular irritation at least once during the experiment. (Table 3.3) The probability of exhibiting at least one sign of irritation was 4.34 ± 2.27% in treated animals and 1.08 ± 0.60% in control animals (p = 0.0321) with an odds ratio of 4.15 (1.19-14.49). Two animals accounted for most of the instances where multiple signs of ocular irritation were documented (Table 3.3) independent of their status as control or treated. All of the signs of ocular irritation (blepharospasm, conjunctival hyperemia, chemosis, nictitans prolapse
and ocular discharge) were observed in these two animals. Two or more signs of ocular irritation were only noted in 2 additional animals on 4 exams. Due to the small number of animals with multiple signs of ocular irritation, statistical analysis was not possible. One of the animals prone to developing multiple signs of ocular irritation was one of the three animals with pre-existing corneal opacities.

Non-specific signs of ocular irritation were also evaluated, including length of eye closure following administration of drops, licking, rubbing, sneezing and head shaking. Treated animals also tended to hold their eyes closed for longer (33.1 ± 7.0 s) than control animals (2.0 ± 7.0 s) (p = 0.0061). Treated animals were also 6.90 (1.31-37.03) times more likely to exhibit licking behavior than control animals (p = 0.0294). There were no significant differences between treated and control animals for other non-specific signs of ocular irritation including face rubbing (p = 0.9192), sneezing (p = 0.7719), or head shaking (p=0.1968). (Table 3.4)

**Schirmer Tear Test (STT):**

A significant baseline effect was identified for STT (p < 0.0001). That is, there was a significant correlation between individual STT values at baseline and individual STT values obtained during the experiment. For example, individuals with relatively low STT values prior to the experiment tended to continue to have relatively low STT values throughout the experiment. No significant treatment effect was found for STT (p = 0.0939). (Table 3.5)
**Corneal Touch Threshold (CTT):**

No significant treatment effect was identified for CTT (p = 0.3305). (Table 3.5)

**Fluorescein Staining:**

Fluorescein staining was negative for both treated and placebo animals at all time points and no corneal abnormalities were noted throughout the experiment. In the three cats with pre-existing corneal opacities, there was no change to these lesions.

**Tear Film Break-up Time (TFBUT):**

A baseline effect was suspected for TFBUT but this effect was not statistically significant (p = 0.0779). No significant treatment effect was found for TFBUT (p = 0.6685) following logarithmic transformation. A significant difference was noted between the right and left eye for TFBUT (p = 0.0387), but differences between right eye (11.5, 10.9 – 12.7s) (mean, 95% confidence interval) and left eye (10.9, 10.3 – 11.5) were not considered clinically significant. No significant treatment effect was found for TFBUT (p = 0.6685) following logarithmic transformation. (Table 3.5)

**Pupillary Diameter (PD):**

A significant baseline effect was identified for PD (p = 0.0009); individuals with large PD prior to the experiment tended to continue to have large PD throughout the experiment. Pupillary diameter was largest at 7am (9.0, 7.7-10.2 mm) (mean, range 95% confidence interval) and 11am (9.0, 7.9-10.2 mm), decreased at 3pm (8.0, 6.6-9.4 mm), and smallest at 7pm (7.5, 6.1-8.8 mm) (Figure 3.1). Like at baseline, PD decreased
through the day during the experiment; it was largest at 7am (7.6, 6.6-8.7 mm) and
smallest at 8pm (7.0, 6.1-8.1 mm). (Figure 3.1) A significant time effect was found (p =
0.0008). Although a treatment effect was not noted when all time points were considered
together (p = 0.1653) (Table 3.5), a significant treatment effect was found at 7pm
(p=0.00039) on Day 2, 7am (p=0.0296) on Day 3, 3pm (p=0.012) on Day 4, and 7pm
(p<0.0001) on Day 6 (Table 3.6).

Intraocular Pressure (IOP):

At baseline, a time of day effect was noted for IOP, suggestive of a circadian
rhythm.\textsuperscript{58} Intraocular pressure remained within normal range at all times, but was highest
at 7am (19.2 ± 1.3 mmHg) (mean ± SE) and 11am (18.0 ± 1.7 mmHg), lowest at 3pm
(16.4 ± 1.2 mmHg) and increased at 7pm (17.0 ± 1.1 mmHg) (Figure I). A significant
baseline effect was identified for IOP (p = 0.0002). Thus, animals with low IOP values at
baseline tended to continue to have low IOP values throughout the experiment and vice
versa. Throughout the experiment, though its pattern differed from baseline, a time of day
effect was noted for IOP (p = 0.007). Intraocular pressure oscillated throughout the day; it
was lowest at 7am (17.9 ± 0.6 mmHg) and 3pm (17.8 ± 0.6 mmHg) and highest at 11am
(18.3 ± 0.6 mmHg) and 7pm (18.7 ± 0.6 mmHg) (Figure 3.2). No treatment effect was
noted at any time.

Wash-out data:

Values did not return to baseline prior to Phase 2 of the experiment for CTT,
TFBUT or PD. Corneal touch threshold was higher (3.2 ± 0.2 cm), TFBUT was shorter
(11.5, 10.7-12.4 s), and PD was smaller (6.9, 5.9-8.0 mm) than prior to Phase 1.
However, no treatment effect was detected suggesting that the changes were an effect of manipulation rather than medication. Also, despite the differences, all values were considered to be within the normal range following the wash-out period prior to the beginning of the second phase.

3.5 Discussion:

In this study, topical 0.1% diclofenac was applied bilaterally 4 times daily for 7 days in clinically normal eyes to simulate an aggressive clinical regime administered on an outpatient basis. In hospitalized patients with severe uveitis, topical anti-inflammatory can be used as often as every 2 hours with doses continued through the night.\textsuperscript{22} In contrast, when owners are administering medications on an outpatient basis, the frequency of application typically does not exceed 4 times daily and most doses are given during daylight hours. Thus, in our experiment, 0.1% diclofenac was administered 4 times between 8 and 8pm. Topical 0.1% diclofenac (Voltaren) was chosen for this study because it is commercially available and is one of the most commonly used topical NSAIDs. It’s efficacy has also recently been demonstrated in an anterior chamber paracentesis model of feline uveitis.\textsuperscript{41} Following topical application, diclofenac permeates into ocular tissues, achieving high concentrations in the aqueous humor and anterior uvea in rabbits and in humans.\textsuperscript{59-61}

In this study, possible effects on corneal health, CTT, STT, TFBUT, IOP, and PD were evaluated. Signs of ocular irritation, such as conjunctival hyperemia and blepharospasm, were also examined. At this time, little is currently known regarding possible ocular adverse effects of topical NSAIDs, particularly after repeated long-term
administration. By performing this study, our goal was to establish the safety of this commonly used anti-inflammatory medication.

In our study, treated animals were 8 times more likely to exhibit conjunctival hyperemia than control animals (p = 0.0161), and treated animals also held their eyes closed for longer than control animals following administration of eye drops (p = 0.0061). These ocular and behavioral findings are consistent with reports in human patients, where a transient conjunctival hyperemia and stinging sensation are the most common adverse effects observed in people treated with topical NSAIDs. Most of the irritation associated with topical NSAIDs such as 0.1% diclofenac is attributed to the acidic nature of the free NSAID compound. In addition to the active ingredient, 0.1% diclofenac, Voltaren also contains multiple additives that may contribute to local irritation. Sorbic acid and edetate disodium, which are preservative agents, as well as Cremophor EL, a surfactant, are all components of Voltaren. In human patients, all three of these compounds have both been associated with an allergic conjunctivitis or dermatitis. To illustrate the possible contribution of these additives, human patients receiving preservative-free diclofenac had a significantly faster decrease in conjunctival hyperemia than those receiving preserved diclofenac. In addition to local conjunctival irritation, licking of the lips may suggest that movement of the drop into the oropharynx is associated with a bad taste or irritation of the oral mucosa.

To evaluate for persistence of ocular irritation from previous administration of drops, slit lamp biomicroscopy was performed prior to drop administration. Though there was insufficient power for statistical analysis, treated cats showed signs of ocular irritation on 2.5 times more exams than control cats (Table 3.3), suggesting that in
addition to an immediate reaction following administration, certain individuals may experience a more sustained reaction to topical 0.1% diclofenac. Conjunctivitis was documented in a recent feline study where the BAB-stabilizing effects of topical 0.1% diclofenac and other anti-inflammatories were evaluated. However, the cause of conjunctivitis was not determined and cats with conjunctivitis were removed from the study. In our study, treated cats were more likely to show signs of ocular irritation than placebo cats; however, signs of ocular irritation were also documented in the control group (Tables 3.2 and 3.3). The cause for ocular irritation associated with the placebo, Tears Naturale II, a lubricating drop, is unclear. To the author’s knowledge, Tears Naturale II is generally well tolerated. In particular, the preservative agent used in this product, Polyquaternium-1, shows little to no cytotoxicity and is associated with good patient comfort in humans. Despite careful handling, it is possible that the signs of ocular irritation seen in both groups were associated with repeated manipulation of the eye. It is also likely that certain individuals are more sensitive than others to either manipulation of the eye or components of ophthalmic formulations. In our study, multiple signs of ocular irritation were rarely documented in a single exam. Multiple signs of irritation occurred primarily in two animals, where multiple signs of ocular irritation were documented in both phases of the study. As one of these two animals had corneal opacities consistent with prior corneal trauma, it is possible that animals with prior ocular inflammation may be more reactive to the irritative properties of 0.1% diclofenac.

Due to repeat exams on a small sample population by one investigator, it is likely that the ocular irritation scoring was not free of bias, particularly since many of our research cats could be identified by distinct coloration or markings. To decrease
identification of individual cats by the investigator evaluating ocular irritation, future experiments should consider the use of photography for scoring. Standardized photos of the eyelids and conjunctiva could be taken for each individual and presented to the evaluator, allowing for better blinding and direct comparison of different animals. Independent scoring by multiple investigators would allow evaluation of interobserver variation. Representative photos of each grade from 0 to 4 could be provided to all observers, allowing for more consistent assignment of scores.

Mean aqueous tear production, as measured by the STT, and tear film stability, as measured by TFBUT, remained within normal limits for both groups throughout the study. There was no significant difference in either STT or TFBUT between treatment and placebo groups. To the author’s knowledge, there are no reports in the literature describing changes to STT or TFBUT with topical NSAID use.

Corneal touch threshold was evaluated through the use of a Cochet-Bonnet esthesiometer, and no evidence of diclofenac-induced change in corneal sensitivity was found in our study. Clinically, topical 0.1% diclofenac and other NSAIDs have been shown to decrease ocular pain following photorefractive keratectomy in humans. Experimentally, topical 0.1% diclofenac administration has been associated with decreased corneal sensitivity as measured by Cochet-Bonnet esthesiometry in healthy human subjects suggesting that in addition to its anti-inflammatory effect, diclofenac may also have an analgesic or anesthetic effect. Numerous mechanisms have been proposed for diclofenac’s analgesic effect, including direct blockage of cation channels and alteration of corneal nerve excitability. Stimulation of the cornea with a Cochet-
Bonnet esthesiometer causes a fast, sudden excitation of A-delta mechano- and polymodal nociceptors as well as a slower excitation of C-polymodal nociceptors.\textsuperscript{73}

Although a treatment effect was not identified in this study, an effect cannot be ruled out. In our experiment, CTT measurements were performed prior to each 7-day phase and after the last treatment, and a single 50ul drop was administered at 8am, 12pm, 4pm, and 8pm during the experiment. In contrast, in human studies, esthesiometry was performed after multiple rounds of closely-spaced diclofenac administration, potentially allowing for an accumulation effect.\textsuperscript{42,43,72} In one study, a treatment effect was not found when two drops of topical diclofenac were administered at the same time, but was evident following multiple doses of diclofenac spaced 5 minutes apart. Furthermore, a direct comparison of human and veterinary esthesiometry studies is difficult to make because human subjects are asked to verbally indicate when a touch is felt,\textsuperscript{42,43} whereas in veterinary species, a blink response must be used to determine corneal touch threshold. Challenges in establishing CTT may help to explain the variability of normal values reported in veterinary medicine.\textsuperscript{55,56} Experimentally, single sensory nerve fiber units have been isolated in cats under general anesthesia. In these studies, the effect of diclofenac on polymodal nociceptors, but not mechano-nociceptors, have been evaluated.\textsuperscript{73-75} Corneal polymodal nociceptors respond to mechanical force, as well as inflammatory mediators, chemical irritants, and extreme temperatures. They make up approximately 70\% of corneal sensory fibers. Mechano-nociceptors respond exclusively to mechanical force, and make up approximately 20\% of corneal sensory fibers. In these studies, topical diclofenac as well as other NSAIDs have been shown to reduce the response of corneal polymodal nociceptors to chemical stimulation, with little to no effect on mechanical
Based on these results, it is possible that species differences exist, and corneal sensitivity to mechanical stimulus in cats may not be affected by NSAID treatment as it is in humans, though future studies are needed to examine the contribution of corneal mechano-nociceptors. Future studies are also needed where the effects of diclofenac on corneal sensitivity are evaluated in conscious cats.

Despite its frequent use in veterinary ophthalmology research, the Cochet-Bonnet esthesiometer may not be the ideal instrument for measurement of corneal sensitivity, particularly in healthy young cats. Acclimation to the procedure was performed in this experiment but was limited due to the risk of mechanical trauma to the cornea with repeated use. Although none of our cats required more than gentle manual restraint for this procedure, squinting, head movement, and prolapse of the third eyelid often occurred during testing, making the test difficult to perform and interpret. Even in humans, a strong aversion reaction is observed with approach of the nylon filament to the cornea, making a peripheral approach essential. In humans, training is considered necessary, and the technique requires repeated measurements for accuracy. In addition, the Cochet-Bonnet esthesiometer is limited in its ability to measure subtle differences in sensitivity because the filament is shortened in 0.5 cm increments. The stiffness of the nylon filament also varies with ambient humidity. In human medicine, gas esthesiometers have been developed, where airflow is used to produce a mechanical stimulus. This allows for measurement of a response without corneal contact and increased sensitivity of measurement. As temperature, flow, and chemical composition of the gas can be controlled, they also allow for specific targeting of different types of corneal sensory
fibres.\textsuperscript{76-78} Though validation is needed, gas esthesiometers may be a valuable tool in future investigations of feline corneal sensitivity.

Throughout the experiment, all corneas in both groups remained normal, as assessed by slit lamp biomicroscopy, and all corneas remained fluorescein negative. These findings are consistent with a review of the literature, where reports of diclofenac-associated corneal pathology are rare.\textsuperscript{45-47} The prevalence of diclofenac-associated keratitis is approximately 1\% and lesions reported in human patients include persistent epithelial defects, superficial punctate keratitis, and subepithelial infiltrates.\textsuperscript{45-47} In humans, punctate keratitis is suspected to be associated with decreased corneal sensation, which was not observed in this study.\textsuperscript{45,79}

In human and veterinary medicine, NSAIDs are used to prevent intraoperative or experimentally-induced inflammation and miosis.\textsuperscript{23,38,80-84} Experimentally, there is profound contraction of the feline iris sphincter when PGF\textsubscript{2\alpha} is applied in vitro to muscle strip preparations,\textsuperscript{85} or in vivo with assessment of PD.\textsuperscript{86-88} Though there are few published reports of NSAID use in healthy eyes of any species,\textsuperscript{89} it is likely that NSAIDs do not have an effect on PD unless intraocular inflammation is present and excessive PGs are present. In healthy canine eyes, topical 0.03\% flurbiprofen did not have any effect on pupil size,\textsuperscript{89} and in human cataract patients, there was no significant difference in pupillary diameter prior to corneal incision in patients treated with 0.03\% flurbiprofen pre-operatively as compared to patients who did not receive a topical NSAID prior to surgery.\textsuperscript{90} In our study, a significant treatment effect on PD was not noted when all time points were considered together. However, a treatment effect (mydriasis) was found at
7pm on Day 2, 7am on Day 3, 3pm on Day 4, and 7pm on Day 6. It is unlikely that the differences in pupillary diameter noted are of any clinical significance, though it is possible that NSAIDs, by decreasing endogenous levels of PGs in the eye, resulted in a relative mydriasis. The presence of a treatment effect at a limited number of time points may reflect the difficulty in accurately measuring PD in cats. Despite acclimatization, experimental animals were often excited, and would react to sounds or other stimuli in their environment. To the author’s knowledge, a circadian rhythm for PD has not been established in the cat. A circadian rhythm for PD has been reported in the laboratory rabbit but not in humans.

In our study, no difference in IOP was found between placebo and treated cats at any time point throughout the experiment. To the author’s knowledge, there has been only one feline study to date that has examined the effects of topical NSAIDs on IOP. In this study, increases in IOP in 0.03% flurbiprofen and 0.1% diclofenac –treated eyes were observed between 4 and 26 hours following induction of uveitis by anterior chamber paracentesis. Cats in this study received topical NSAID treatment immediately following paracentesis, and then at 6, 10, and 24 hours following paracentesis. Increases in IOP in NSAID-treated eyes were mild; IOP in diclofenac-treated eyes was 1.7 – 2.1mmHg higher than in control eyes. In the dog, topical 0.03% flurbiprofen and intravenous flunixin pre-treatment have been shown to exacerbate the increase in IOP associated with BAB breakdown following Nd:YAG laser capsulotomy. Flurbiprofen 0.03% has also shown to decrease aqueous outflow in canine eyes, with the decrease in outflow being more marked in eyes undergoing Nd:YAG laser capsulotomy. An increase in IOP has, however, not been documented in all canine studies that have been
performed. There was no significant difference between treated and control eyes in studies examining the effects of topical flurbiprofen,\textsuperscript{94} intravenous flunixin,\textsuperscript{82} or oral aspirin.\textsuperscript{95}

A key difference between our study and previous studies is that in our experiment, topical diclofenac was applied to healthy feline eyes. In other studies, the effects of topical NSAIDs have been examined in the context of experimentally-induced uveitis and the OIR. As the OIR is characterized by an initial elevation in IOP, it has been suggested that topical NSAIDs likely exacerbate the rise in IOP seen during the acute phases of this response.\textsuperscript{39} The initial rise in IOP in the OIR is attributed to uveal vasodilation, and increased ultrafiltration and extravasation of fluid. Blockage of the iridocorneal angle by protein and inflammatory cells likely also contributes to the rise in IOP.\textsuperscript{96} The Nd:YAG laser capsulotomy model of canine uveitis can be used to illustrate the effects of increasing BAB breakdown on IOP, as IOP elevations were more likely when higher levels of energy are used to induce inflammation in the eye.\textsuperscript{39,94}

Although the exact mechanism is unknown, NSAIDs are thought to exacerbate the IOP elevation associated with the initial stages of the OIR through their reduction of PGs. Prostaglandin receptors have been found in both the conventional and uveoscleral aqueous outflow pathways in the human eye.\textsuperscript{28} Prostaglandins are thought to help counteract the rise in IOP associated with the acute phases of the OIR through induction of matrix metalloproteinases and extracellular matrix remodeling in the uveoscleral pathway, enabling increased aqueous outflow.\textsuperscript{97} Continued increase in uveoscleral outflow likely contributes to the ocular hypotony observed with uveitis,\textsuperscript{98} an effect that has been experimentally demonstrated in monkeys.\textsuperscript{99} Thus, decreased levels of PGs may
lead to increased aqueous outflow resistance, decreased aqueous outflow, and increased IOP.\textsuperscript{98}

Although elevations in IOP were not detected with treated animals in our study, changes to aqueous humor dynamics and an effect on IOP cannot be ruled out. In cannulated canine eyes, 0.03\% flurbiprofen caused a decrease in aqueous outflow in eyes with and without induction of uveitis via laser capsulotomy, though the decrease in outflow was more pronounced in uveitic eyes.\textsuperscript{93} It is possible that a mild decrease in aqueous outflow occurred in our study, but the increase in IOP that occurred was too small to be detected via rebound tonometry. In a canine study, 0.03\% flurbiprofen caused a mild but significant mean IOP elevation of 1.1mmHg in treated eyes, with a maximal IOP elevation of 1.8mmHg five days into the treatment period.\textsuperscript{89} Differences of less than 1-2 mmHg are unlikely to be detected, given that the tonometer is calibrated in increments of 1mmHg and factors such as restraint and animal temperament will influence IOP readings. The effects of NSAIDs on aqueous humor dynamics and uveoscleral outflow could potentially be investigated using less invasive techniques such as fluorophotometry,\textsuperscript{100,101} as well as invasive techniques such as the dextran tracer method.\textsuperscript{101,102}

In our experiment, IOP was measured in the hour prior to administration of topical diclofenac. Thus, for those measurements taken at 11am, 3pm, and 7pm, 3.5 to 4 hours had elapsed since administration of the last drop. For the measurement taken at 7am, there was an 11.5-12 hour interval since the last drop the evening before. In the study by Rankin \textit{et. al}, increases in IOP were detected for topical diclofenac administered immediately following paracentesis at 8 and 26 hours following anterior chamber
paracentesis in cats; IOP measurements were taken between 2 and 4 hours following administration of topical medications. In this study, topical diclofenac was administered immediately following paracentesis, and at 6, 10, and 24 hours following paracentesis. In a canine study by Pirie et. al, IOP elevations were detected with topical flurbiprofen pre-treatment between 30 and 120 minutes following Nd:YAG laser capsulotomy. In the Pirie et. al study, between 1 and 3 hours were allowed to elapse between flurbiprofen administration and IOP measurement. Although direct comparisons cannot be drawn because our study was performed on healthy feline eyes, it is possible that IOP elevations occurred in the 3 hours following drop administration but were not detected because of the timing of our IOP measurements. A study on healthy canine eyes utilized a similar study design, with 3 hours between flurbiprofen administration and IOP measurement. In this study, there was a mean IOP elevation of 1.1 mmHg in eyes treated with 0.03% flurbiprofen. The mild IOP elevations in this study and the lack of significant difference between treatment groups in ours suggest that additional time points closer to the time of NSAID administration might have been beneficial. Further studies are needed to determine if NSAIDs are associated with rapidly occurring, transient elevations in IOP in healthy feline eyes as well as eyes with concurrent uveitis.

Although IOP appeared to vary by time of day suggesting a circadian rhythm, comparison to a previous study examining the circadian rhythm of IOP in cats was not possible because IOP measurements were not performed through the night.

Despite its apparent safety in healthy feline eyes, patients receiving topical diclofenac should be carefully monitored, particularly those with concurrent corneal disease, such as corneal sequestra and feline herpes-associated keratitis, as diclofenac has
been shown to be associated with delayed epithelial healing and altered epithelial cell morphology.\textsuperscript{103,26} Suprofen, another topical NSAID, has also been shown to cause morphological changes at high concentrations in canine corneal epithelial cells propagated in cell culture.\textsuperscript{104} Though very rare and likely multifactorial, topical diclofenac use has been associated with the development of deep, melting, or perforating ulcers in human patients. The higher incidence of keratomalacia associated with the generic diclofenac product, diclofenac sodium ophthalmic solution (DSOS), ultimately lead to this product being removed from the market.\textsuperscript{26} While the exact pathogenesis of NSAID-associated keratomalacia is unknown, due caution should also be used in patients who have had recent intraocular surgery, are concurrently being treated with topical corticosteroids, or who have risk factors for delayed healing such as advanced age or diabetes.\textsuperscript{105-107}

In conclusion, the results of this study demonstrate that topical 0.1\% diclofenac can be safely used in healthy feline eyes up to 4 times a day for 7 days. In general, the medication was well tolerated as mild signs of ocular irritation were the only adverse effect documented with 0.1\% diclofenac treatment. There were no significant effects of 0.1\% diclofenac on aqueous tear production, tear film quality, corneal health, corneal sensitivity, PD or IOP. Limitations of our study design and suggested improvements, including use of a gas esthesiometer and additional IOP measurements, have been discussed. Future studies are needed to evaluate possible adverse effects when 0.1\% topical diclofenac is used for longer periods of time in cases of naturally-occurring or experimentally-induced uveitis.
3.6 Footnotes

a. Personal Communication: Ontario Veterinary College Health Sciences Centre Medical Records Department
b. Liberty Research, Waverly, NY
c. Animal Health Laboratories, Ontario Veterinary College
d. Kowa SL-15, Kowa, Tokyo, Japan
e. Schirmer Tear Test strips, Alcon Canada, Mississauga, Ontario, Canada
f. Timex Ironman Triathlon Women’s Watch, Timex Canada, Markham, Ontario, Canada
g. Fluorets, Chauvin Pharmaceuticals Ltd, Aubenas, France
h. TonoVet, Tiolat Ltd, Helsinki, Finland
i. Heine Omega 2c, Heine Optotechnik, Herrsching, Germany
j. Mydriacyl 1%, Alcon Canada, Mississauga, Ontario, Canada
k. Voltaren Ophtha, Novartis Pharmaceuticals Canada Inc, Dorval, Quebec, Canada
l. Tears Naturel II, Alcon Canada, Mississauga, Ontario, Canada
m. Cochet-Bonnet Esthesiometer, Luneau Ophthalmologie, Chartres, France
n. SAS Institute Inc. 2007, SAS OnlineDoc® 9.2., Cary, North Carolina, USA
### 3.7 Tables

Table 3.1: Ocular tests listed according to time and order performed

<table>
<thead>
<tr>
<th>Time</th>
<th>Ocular Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>7am</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>STT</td>
</tr>
<tr>
<td></td>
<td>IOP</td>
</tr>
<tr>
<td></td>
<td>Slit lamp biomicroscopy</td>
</tr>
<tr>
<td>11am</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>IOP</td>
</tr>
<tr>
<td></td>
<td>TFBUT</td>
</tr>
<tr>
<td></td>
<td>Fluorescein staining</td>
</tr>
<tr>
<td></td>
<td>Slit lamp biomicroscopy</td>
</tr>
<tr>
<td>3pm</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>IOP</td>
</tr>
<tr>
<td></td>
<td>Slit lamp biomicroscopy</td>
</tr>
<tr>
<td>7pm</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>IOP</td>
</tr>
<tr>
<td></td>
<td>Slit lamp biomicroscopy</td>
</tr>
</tbody>
</table>
Table 3.2: Probability of displaying signs of ocular irritation during treatment with topical 0.1% diclofenac and control treatment

<table>
<thead>
<tr>
<th>Sign of ocular irritation</th>
<th>Probability in treated cats (%) (mean ± SE)</th>
<th>Probability in control cats (%) (mean ± SE)</th>
<th>Odds Ratio (95% CI)‡</th>
<th>p-value*</th>
<th># of treated cats with sign</th>
<th># of placebo cats with sign</th>
<th># cats with sign with both placebo and treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival hyperemia</td>
<td>31.08 ± 16.93</td>
<td>5.33 ± 4.11</td>
<td>8 (1.72–37.03)</td>
<td>0.0161</td>
<td>7</td>
<td>5</td>
<td>3‡</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td>2.22 ± 1.50</td>
<td>0.37 ± 0.29</td>
<td>0.0696</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2‡</td>
</tr>
<tr>
<td>Chemosis</td>
<td>0.52 ± 0.11</td>
<td>0.42 ± 0.09</td>
<td>0.4839</td>
<td>2</td>
<td>0</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>0.6798</td>
<td>2</td>
<td>0</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>0.8181</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2‡</td>
</tr>
</tbody>
</table>

* p-value reflects the comparison between probability for treated vs. control animals
† Odds ratio is presented if p <0.05
‡ The same two animals showed blepharospasm, conjunctival hyperemia and ocular discharge in both phases of the study
Table 3.3: Number of cats and number of exams where one or multiple signs of ocular irritation were observed

<table>
<thead>
<tr>
<th># of signs</th>
<th>Prior to drop administration</th>
<th>Following drop administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of placebo cats</td>
<td># of exams on placebo cats (226 exams)</td>
</tr>
<tr>
<td>One</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Two</td>
<td>2*</td>
<td>6</td>
</tr>
<tr>
<td>Three</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Two of the same animals are represented in each of these instances
Table 3.4: Probability of displaying non-specific signs of irritation following application of topical 0.1% diclofenac and placebo treatment

<table>
<thead>
<tr>
<th>Sign of ocular irritation</th>
<th>Probability in treated cats (mean ± SE)</th>
<th>Probability in control cats (mean ± SE)</th>
<th>Odds Ratio (95% CI)†</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Licking</td>
<td>62.60 ± 7.90</td>
<td>19.48 ± 7.90</td>
<td>6.90 (1.31-37.03)</td>
<td><strong>0.0294</strong></td>
</tr>
<tr>
<td>Face rubbing</td>
<td>0.35 ± 0.41</td>
<td>0.41 ± 0.48</td>
<td>0.9192</td>
<td></td>
</tr>
<tr>
<td>Sneezing</td>
<td>0.61 ± 0.37</td>
<td>0.48 ± 0.29</td>
<td>0.7719</td>
<td></td>
</tr>
<tr>
<td>Head shaking</td>
<td>74.03 ± 11.37</td>
<td>52.64 ± 12.43</td>
<td>0.1968</td>
<td></td>
</tr>
</tbody>
</table>

* p-value reflects the comparison between probability for treated vs. control animals
† Odds ratio is presented if p <0.05
Table 3.5: Mean ± SE or mean (95% confidence interval)* Selected ocular variables in cats at baseline, following topical 0.1% diclofenac treatment, or following topical placebo treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Previously published values</th>
<th>Baseline</th>
<th>Placebo</th>
<th>Diclofenac</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>STT (mm/min)</td>
<td>10.8 ± 0.8\textsuperscript{52} 14.3 ± 4.7\textsuperscript{49} 16.9 ± 3.7\textsuperscript{53}</td>
<td>13.5 ± 1.5</td>
<td>14.2 ± 0.7</td>
<td>12.6 ± 0.7</td>
<td>0.0939</td>
</tr>
<tr>
<td>IOP (mmHg)</td>
<td>20.74 ± 0.48\textsuperscript{57}</td>
<td>17.8 ± 0.86</td>
<td>18.2 ± 0.6</td>
<td>18.2 ± 0.6</td>
<td>0.9707</td>
</tr>
<tr>
<td>CTT (g/mm\textsuperscript{2})</td>
<td>1.79 ± 2.33 OD, 1.74 ± 1.65 OS\textsuperscript{56} 4.64 OD, 5.16 OS\textsuperscript{55}</td>
<td>1.08 ± 0.11</td>
<td>1.07 ± 0.11</td>
<td>1.15 ± 0.10</td>
<td>0.5751</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>n/a</td>
<td>8.4, (7.6-9.6)</td>
<td>7.0, (5.9-8.4)</td>
<td>7.5, (6.3-9.0)</td>
<td>0.1653</td>
</tr>
<tr>
<td>TFBUT (s)</td>
<td>16.7 ± 4.5, 9.62 ± 2.06, 22.49 ±3.89\textsuperscript{50}</td>
<td>13.0, (12.12-13.87)</td>
<td>11.2, (10.5-12.0)</td>
<td>11.1, (10.4-11.9)</td>
<td>0.6685</td>
</tr>
</tbody>
</table>

*Values for PD and TFBUT are presented as 95% confidence intervals due to logarithmic transformation of data and asymmetry of the confidence limits
†p-values reflect the comparison between diclofenac and placebo groups, as baseline data is included as a covariable in the model
Table 3.6: Mean (95% confidence interval) Comparison of PDs at select times

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Placebo</th>
<th>Diclofenac</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7pm</td>
<td>6.4, 5.4 – 7.5</td>
<td>7.6, 6.4 – 9.0</td>
<td>0.00039</td>
</tr>
<tr>
<td>3</td>
<td>7am</td>
<td>6.9, 5.8 – 8.2</td>
<td>7.8, 6.6 – 9.2</td>
<td>0.0296</td>
</tr>
<tr>
<td>4</td>
<td>3pm</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>7pm</td>
<td>6.5, 5.5 – 7.7</td>
<td>7.1, 6.0 – 8.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Values for PD are presented as 95% confidence intervals due to logarithmic transformation of data
3.8 Figures

Figure 3.1: Mean (± 95% confidence intervals) Variation in PD in for baseline, placebo and treated animals throughout the day
Figure 3.2: Mean ± SE Baseline and Experimental IOP variation throughout the day
3.9 References


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CHAPTER 4: The Systemic Absorption and Adverse Systemic Effects of Topical 0.1% Diclofenac in Healthy Cats

4.1 Abstract:

**Objective:** To quantify plasma levels of diclofenac following application of topical 0.1% diclofenac and to determine if there are any adverse hepatic or renal effects associated with an aggressive dosing regime in healthy animals.

**Animals:** 9 healthy male cats.

**Procedures:** A randomized, placebo-controlled cross-over study design was used. Either topical 0.1% diclofenac (n = 4 cats) or an artificial tear solution (n = 4 cats) was administered to both eyes 4 times daily (8am, 12pm, 4pm, 8pm) for 7 days. Data for a trial animal (n = 1) is also included. There was a 12-day wash-out period before cats were crossed-over. Serial plasma samples (0-240 min) were collected following the last dose on Days 1 and 7 of each phase for pharmacokinetic analysis. Plasma samples were analyzed using HPLC-MS, and C\text{max}, t\text{max} and AUC\text{0-240} were determined. CBC, serum biochemistry panel, urinalysis, UPCR and GFR were performed before the start of the experiment and at the conclusion of each 7-day phase.

**Results:** C\text{max} (87.6±23.3 ng/ml) and AUC\text{0-240} (16,502±4,568 min*ng/ml) on Day 7 were significantly higher than C\text{max} (46.8±24.4 ng/ml) and AUC\text{0-240} (10,953±3,502 min*ng/ml) on Day 1 (p<0.05). There were no clinically relevant abnormalities detected for any animal on CBC, serum biochemistry panel, urinalysis or UPCR, and no significant treatment effect was detected on statistical analysis of these tests. Treated animals (2.75 ± 0.16 ml/kg/min) were found to have a significantly lower GFR than control animals (2.31
± 0.16 ml/kg/min) in the second phase of the study (p=0.0013) but not in the first phase of the study (p = 0.8320).

**Conclusions and Clinical Relevance:** Detectable systemic levels of diclofenac are achieved with topical application of 0.1% diclofenac, with accumulation over 7 days. Systemic absorption of diclofenac may be associated with reduced GFR, particularly in animals that are in a volume- contracted state. Topical 0.1% diclofenac should be used with caution in at risk cats such as those that are dehydrated, hypovolemic or have concurrent systemic diseases.

**4.2 Introduction:**

Anterior uveitis is a common ocular condition in the domestic cat. Causes of feline uveitis include corneal injury or trauma, idiopathic, neoplastic, lens-induced, and infectious.\textsuperscript{1-18} Regardless of cause, uveitis is initiated by tissue injury, and is associated with breakdown of the BAB. Though many mediators are involved, PGs are considered one of the main mediators of ocular inflammation.\textsuperscript{19,20} Thus, anti-inflammatory drug, including corticosteroids and NSAIDs, are the mainstay of uveitis treatment.\textsuperscript{21-23} Topical corticosteroids are commonly administered as the primary therapy in the treatment of feline anterior uveitis, as they are potent anti-inflammatory agents and are generally well tolerated by cats.\textsuperscript{22-24} However, topical NSAIDs may be used with caution in cases where topical corticosteroids are contraindicated, such as in cases of corneal ulceration or infection.\textsuperscript{22,25} Topical NSAIDs can also be used to treat mild inflammation or can be used in combination with topical corticosteroids in cases of severe inflammation.\textsuperscript{22,23} NSAIDs exert their therapeutic effect through inhibition of COX, which converts AA to PGs.\textsuperscript{24,26}
It has long been recognized that NSAIDs need be used with caution in cats due to their limited capacity for hepatic glucuronidation.\textsuperscript{27,28} Due to decreased metabolism of NSAIDs, accumulation and subsequent toxicity are considered more likely in cats. Many NSAIDs, including carprofen and acetylsalicylic acid, have a much longer elimination half-life ($t_{\text{1/2}}$) in the cat as compared to the dog.\textsuperscript{29,30} The risk of adverse effects associated with systemic NSAID use has led to a lack of licensed systemic NSAIDs available for cats.\textsuperscript{1} Compared to dogs, there are very few licensed systemic NSAIDs in cats, and most licensed products can only be administered perioperatively or for a period of only a few days.\textsuperscript{1} All topical ophthalmic NSAIDs are currently used off-label in cats.

Studies in other species have shown that topical application of NSAIDs allow for high concentrations of medication to be achieved in the aqueous humor and anterior uvea,\textsuperscript{31-33} while minimizing systemic exposure to NSAIDs as compared to systemic routes of administration.\textsuperscript{33-35} It is assumed that similar absorption kinetics occur in the cat. Topical NSAIDs have been shown to be effective in reducing BAB breakdown in both canine and feline models of uveitis,\textsuperscript{35-39} and may thus be a valuable therapeutic option in the treatment of naturally-occurring uveitis. Alternatives to systemic NSAIDs are especially important in the cat, given its limited ability to metabolize NSAIDs and the lack of available licensed systemic products. Therapy of anterior uveitis, particularly in cases of idiopathic lymphocytic-plasmacytic uveitis, often requires a slow tapering of the administered dose. Repeat courses of anti-inflammatory therapy are not uncommon as relapse of uveitis can occur. Topical NSAID therapy may be initiated as frequently as once every 2 hours in severe cases,\textsuperscript{22,23} but are typically applied 2 to 4 times per day.\textsuperscript{22,23}
Although one goal of topical therapy is to limit systemic absorption and adverse systemic effects, a significant 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{40-42} Topical medications may also be swallowed with absorption via the gastrointestinal system.\textsuperscript{40-42} To the author’s knowledge, there are no published studies in cats that have characterized the amount of systemic absorption that occurs with topical NSAID use, and little is known about adverse systemic effects associated with long-term use of topical NSAIDs. Cats accounted for 14 of 110 cases and 20 of 77 cases of adverse drug experiences (ADEs) associated with systemic NSAID use reported to the UK’s Veterinary Medicines Directorate in 2009 and 2011.\textsuperscript{43} In these reports, renal insufficiency was reported in cats, particularly following off-label use of systemic NSAIDs, though exact numbers are not given.\textsuperscript{44} Unfortunately, at present, the exact incidence and distribution of ADEs associated with both systemic and topical NSAID use in cats is not available in the published literature. In dogs, 64\% of NSAID-related adverse drug experiences (ADEs) reported to the US Federal Drug Administration are related to the gastrointestinal tract, 21\% to the renal system, and 14\% to the hepatic system.\textsuperscript{45}

The goal of this experiment was to determine if a topically applied 0.1\% diclofenac dosed according to a clinically aggressive regime would reach significant plasma levels to cause adverse systemic effects. Another goal was to determine whether or not accumulation occurs with repeated doses. Though GI adverse effects are of concern, the emphasis of this research project was on hepatic and renal function. It was
hypothesized that 0.1% diclofenac would not be associated with any significant accumulation or adverse renal or hepatic effects.

4.3 Materials and Methods:

Nine male intact, barrier-raised, purpose-bred domestic shorthair cats of approximately one year of age were included in the study. As systemic adverse effects were not anticipated, sample size was based on the power analysis performed for the ocular outcomes (intraocular pressure) being concurrently evaluated in this experiment. One cat was used as a trial animal to verify sample design, and its data was included because none of the systemic parameters required any subjective evaluation. Cats were purchased from a commercial supplier. Cats weighed between 5.02 and 6.63kg, with a mean starting weight of 5.60 ± 0.20 (mean ± SE). Prior to inclusion, cats received a complete physical and ocular examination, which were within normal limits. Complete blood count, serum biochemistry, UA (including sediment exam) and UPCR were also performed. Ocular examination including neuro-ophthalmic exam (palpebral reflex, menace response, pupillary light reflexes), slit lamp biomicroscopy, Schirmer Tear Test, fluorescein staining, rebound tonometry, and indirect ophthalmoscopy were performed prior to the study. In three animals, superficial focal white corneal opacities were present, and were consistent with corneal scarring from previous injury. As there were no signs of active corneal inflammation or any other ocular abnormalities, these animals were included in the study.

Cats were exposed to an automated 12-hour light-dark cycle (light phase from 7am to 7pm, dark phase from 7pm to 7am). Cats were acclimatized to handling and
ocular procedures for one month prior to the start of the experiment. This study was approved by the University of Guelph Animal Care and Use Committee.

Physical examinations, including measurement of vital parameters, were performed on a daily basis during the experiment. Inspection of each animal’s kennel and litter tray was also monitored throughout the experiment for signs of vomiting or diarrhea.

A CBC, serum biochemistry panel, UA and UPCR were performed on each cat approximately 2 weeks prior to the start of the experiment to ensure that all cats were healthy, and to collect baseline values. Baseline urine samples were collected via cystocentesis.

Two days prior to the experiment, long-stay jugular catheters were placed to facilitate serial blood sampling. Catheters were placed under anesthesia using ketamine (5mg/kg, IM), buprenorphine (0.02 mg/kg, IM), and acepromazine. (0.05mg/kg, IM). Propofol was given to effect.

One day prior to the beginning of treatment, all cats underwent a GFR study via plasma clearance of 99mTc-diethylenetriaminepentaacetic acid (99mTc-DTPA). Glomerular filtration rate was determined using an established protocol. In order to minimize the quantity of blood collected, a modified protocol was used. Two milliliters of blood was collected via a jugular catheter at 15 min, 30 min, 120 min, and 240 min for gamma counting. 2ml of heparinized 0.9% sodium chloride solution was used to flush the jugular catheter and replace the blood volume withdrawn following each sample collection.
Prior to the beginning of the experiment, the cats were randomly assigned to control (n=4) and treatment (n=4) groups by drawing names out of a hat. The study consisted of two 7-day treatment periods (Phases 1 and 2), with a 12-day washout period in between periods. During the 7-day treatment phase (Days 1-7), cats in the treatment group received one drop (50ul) of 0.1% diclofenac ophthalmic solution and control cats received one drop (50ul) of an artificial tear in both eyes at 8am, 12pm, 4pm, and 8pm. This timing of drug administration was chosen to simulate a regime that might be used clinically.

Blood samples were collected prior to the first dose in both phases (0) to ensure that no circulating levels of diclofenac could be detected. Blood samples were collected for pharmacokinetic analysis following the 8 pm dosing on Days 1 and 7 of each phase of the study. Timing of the dose on Day 1 was chosen to maximize the likelihood of being able to detect diclofenac. Following administration of the eye drops, blood samples (2 mL) were collected at 5, 15, 30, 60, 120 and 240 minutes post-treatment. To control for volume of blood collected, the same number of blood samples were collected for both control and treated cats. Due to the large volume of blood collection, following each sample, 2 ml of 0.9% saline was replaced (1:1 replacement) to minimize volume depletion. Samples were collected into tubes containing sodium heparin and kept on ice, before being centrifuged (3,000g for 10 minutes). Plasma was collected and stored at -80°C until the conclusion of the study, then shipped on dry ice for diclofenac level determination via HPLC-MS. For treated animals, all samples ((0), 5, 15, 30, 60, 120 and 240 minutes) were submitted for analysis. However, for control animals only the (0) and
240-minute sample was analyzed to ensure that accidental dosing with diclofenac had not occurred.

Blood samples for CBC and serum biochemistry were also collected following the 8pm dose on Day 7 of each phase at the same time as the 5 minute pharmacokinetic sample. Free catch urine samples for urinalysis and UPCR were collected on Day 7 using plastic litter beads. All samples of urine collected during the day were taken immediately upon collection to the laboratory for analysis to decrease the chances of cast dissolution for sediment exam. If a free urine sample could not be obtained, cystocentesis was performed under butorphanol sedation (0.2-0.4 mg/kg SQ) upon completion of the pharmacokinetic study. These samples were refrigerated overnight and delivered to the laboratory the morning following collection. GFR was performed the day following Day 7 of each phase. To minimize the volume of blood collected, CBC and Chemistry panels were not repeated prior to the second phase of the experiment if results following the first phase were deemed to be within normal limits. If at the end of Phase I there were any significant abnormalities on CBC, Chemistry, urinalysis, UPCR or GFR, these tests were repeated prior to the start of Phase II to ensure that values returned to within normal range. A schedule of blood sampling is presented (Table 4.1).

Prior to the GFR and pharmacokinetic studies, PCV was measured to ensure that no animal was anemic. Animals were not allowed to proceed in the study unless they had a minimum PCV of 25%. A maximum of 22 ml of blood was collected within a 24 hour period when combining the volume collected for the CBC and Chemistry panels (2 ml), pharmacokinetic study (12 ml) and GFR study (8 ml). Oscillometric blood pressure,
heart rate, and pulse quality was monitored throughout and following the GFR and pharmacokinetic studies.

Throughout the study, jugular catheters were monitored and flushed twice daily with heparinized saline (2 ml), and replaced as needed. To prevent self-trauma, all animals wore Elizabethan collars for the duration of the study.

**Pharmacokinetic Analysis:**

Plasma concentrations of diclofenac were determined with high-pressure liquid chromatography with triple quadruple mass spectrometry. Reference standards and test samples were processed in an identical manner. Plasma, 0.2 mL, was added to 0.8 mL acetonitrile containing the internal standard, meclofenamic acid (62.5 ng/mL) to precipitate the plasma proteins in a microcentrifuge tube. The microcentrifuge tubes were vortexed for 5 seconds, centrifuged for 5 minutes at 15,000 g, and the supernatant transferred to a clean culture tube. The supernatant was evaporated to dryness at 40° C under an airstream for 25 minutes. Test samples or reference standards were reconstituted with 0.2 mL 10% methanol in deionized water, vortexed, transferred to a microcentrifuge tube, and centrifuged for 5 minutes at 15,000 g, to sediment any particulates. The supernatant was transferred to an injection vial and 0.05 mL was the injection volume. The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid in deionized water. The mobile phase started at 85% from 0 to 0.5 minutes, followed by a linear gradient to 25% B at 4 minutes followed by a linear gradient to 85% B at 5 minutes with a total run time of 6.5 minutes. Separation was achieved with a phenyl column maintained at 40° C. The qualifying and quantifying ions for diclofenac were m/z 296.12
and 215.00, respectively. The qualifying and quantifying ions for meclofenamic acid were \(m/z\) 296.07 and 243.00, respectively. Reference standard curves were constructed in blank feline plasma spiked with diclofenac providing concentrations of 0, 10, 50, 100, 500, and 1000 ng/mL, and were accepted if the correlation coefficient was at least 0.99 and predicted concentrations were within 15% of the actual concentration. The accuracy and coefficient of variation were determined on replicates of 5 at each the following concentrations: 10, 100, and 1000 ng/mL. The accuracy of the assay was 99.8, 100.1, and 101.0% of the actual concentration at 10, 100, and 1000 ng/mL, respectively. The coefficient of variation of the assay was 3.3, 3.8, and 3.6% at 10, 100, and 1000 ng/mL, respectively.

Pharmacokinetic analysis of plasma diclofenac drug levels were determined with PK functions for Microsoft Excel\textsuperscript{\textregistered} using non-compartmental methods. The following parameters were generated or calculated: the area under the curve (AUC) from 0 to 240 minutes, which was determined with the linear trapezoidal rule; and the maximum plasma concentration (\(C_{\text{max}}\)) and time to \(C_{\text{max}}\) (\(T_{\text{max}}\)), which were both determined directly from the data.

**Statistical Analysis**

Parameters of interest were analyzed with a general linear mixed model that included baseline values as a covariable (ANCOVA).\textsuperscript{w} Baseline values were included as a covariable because preliminary statistical analysis showed that baseline values had a significant effect (\(p<0.05\)) on experimental values obtained during the experiment. For example, cats that had an ALT low within the normal range tended to continue having
relatively low ALT values. Parameters were fitted to the best covariance structure to allow for making repeated measures over time. Effects in the model included cat, period, and treatment. All interactions of effects in the model were accounted for and removed if not significant. Data was assessed for normality using a Shapiro-Wilk test and examination of the residuals. Data was logarithmically transformed if it improved normality. Post hoc tests used included a Tukey or Dunnett’s adjustment depending on the comparison. Standard errors presented are based on pooled standard error.

Pharmacokinetic parameters were assessed for differences on day 1 and day 7 with a significance level of p<0.05. The AUC$_{0-240}$ and C$_{\text{max}}$ were assessed statistically\textsuperscript{x} (SigmaPlot 12, Systat Software Inc., San Jose, CA USA) for differences using a paired t-test as the data were normally distributed with uniform variance. The t$_{\text{max}}$ was assessed statistically for differences with a Wilcoxon signed rank test as the data were not distributed normally.

4.4 Results:

No detectable levels of diclofenac (<10 ng/ml) were found in the samples collected prior to the start of the experiment. Similarly, no detectable levels of diclofenac were present prior to the start of Phase 2, confirming that the 12-day wash-out period was adequate. No detectable levels of diclofenac were found in any placebo animals except for one, where a single dose of diclofenac had been accidentally administered at 4pm on Day 1 of Phase 1. In this animal, the plasma concentration of diclofenac at 240 min was 24.3 ng/ml. Diclofenac levels in this animal were below the limit of detection by the Day 7 pharmacokinetic study.
Plasma levels of diclofenac were detected out to the last sample time point at day 1 and day 7 of the study (Figure 4.1). \(C_{\text{max}}, T_{\text{max}},\) and \(AUC_{0-240}\) values were determined for each animal and summarized (Table 4.2). Values for \(C_{\text{max}}\) and \(AUC_{0-240}\) on Day 7 were significantly greater than on Day 1 (\(P<0.05\)).

All animals completed the study without any adverse effects detectable on physical examination. General physical examinationss were unremarkable throughout the experiment, with no signs of dehydration, weakness, lethargy or abdominal pain. Vomit was found on a single occasion in the kennel of one diclofenac treated cat the morning following the end of the first phase. All cats maintained and increased their body weight throughout the experiment, with a mean starting weight of 5.60 ± 0.20 and mean end weight of 6.12 ± 0.19. Mean blood pressure in all animals exceeded the minimum cut-off value of 100mmHg. In many cases, blood pressure measurements were difficult to obtain due to excitement of the animals and resistance to restraint. In these cases, individual assessments were made based on general physical examination findings such as demeanor, heart rate and pulse quality. All cats met the requirement of a minimum PCV of 25% prior blood sampling in both phases of the study. However, a decline in PCV and TP were noted. (Table 4.3)

Immediately prior to the GFR study at the end of Phase II, one cat was found dead. This cat had been receiving the placebo treatment. The cat had undergone anesthesia for placement of a jugular catheter following the pharmacokinetic study the night before, and had recovered uneventfully. There were no significant findings on post-mortem examination or on the CBC and serum biochemistry panels collected the day
prior. The cause of death is unknown but is suspected to be associated with a thromboembolic accident following placement of the jugular catheter.

Baseline CBC and serum biochemistry panels were within normal limits with some exceptions that were not deemed clinically significant. On serum biochemistry, three cats had mild elevations in phosphorus (2.45-2.71 mmol/L, reference range 0.80-2.29 mmol/L), four cats had mildly decreased glucose (3.9-4.2 mmol/L, reference range 4.4-7.7 mmol/L), one cat had mildly decreased TP (64, 66-84 g/L), and two cats had mildly decreased globulin concentrations (22-25 g/L, reference range 27-48 g/L). Elevated phosphorus levels were consistent with growth and bony remodeling seen in young animals, and the abnormalities in glucose, TP, and globulin were considered incidental. Urinalysis was unremarkable for all cats at baseline. Urine protein to creatinine ratio, determined from urine samples collected via cystocentesis, was borderline in four cats at 0.4, and elevated in three cats (0.5-0.7). Normal values for UPCR are typically less than 0.2, while 0.2-0.4 is considered borderline, and greater than 0.4 is usually considered abnormal. The cause for UPCR elevation prior to the start of the experiment is unknown and was not investigated as UA and serum biochemistry were unremarkable. (Table 4.4).

Throughout the experiment, means for all parameters were within normal limits, except for TP, where both treated (63.9 ± 0.9 g/L, reference range 66-84 g/L) and placebo animals (63.9 ± 0.9 g/L, reference range 66-84 g/L) were mildly hypoproteinemic in both phases of the study. There were no significant difference in TP between phases of the study (p = 0.7641). There were no significant difference between treated animals and placebo animals for albumin (p = 0.0758), globulin (p = 0.2643), TP (p = 0.2149), ALP
(p = 0.1546), ALT (p = 0.5108), GGT (p = 0.6517), urea (p = 0.3283), creatinine (p = 0.4967), phosphorus (p = 0.7473), potassium (p = 0.7712), USG (p = 0.7837) and UPCR (p = 0.4389). (Table 4.4).

Mild abnormalities were noted in individual animals in both groups. Specifically, a mild hyperphosphatemia was observed in 2/9 placebo animals and 1/9 treated animals (2.47-2.49 mmol/L, reference range 0.80-2.29 mmol/L). All cases of hyperphosphatemia were represented by the same two cats, both of whom were hyperphosphatemic at baseline. A mild hypoproteinemia was observed in 8/9 diclofenac-treated animals and 5/9 placebo-treated animals (59-65 g/L, reference range 66-84 g/L). A mild hypoglycemia was found in 1/9 diclofenac-treated animals and 2/9 placebo-treated animals (4.0-4.2 mmol/L, reference range). Mild elevations in ALT were noted in 2/9 diclofenac-treated animals and 3/9 placebo-treated animals (127-171 U/L, reference range 31-105 U/L). Elevations in ALT were seen in the same two cats in each of the phases of the experiment. ALT levels dropped to within normal range at the end of the washout period in both of these animals, but were once again increased at the end of the second phase. UPCR was abnormal (>0.5) in 3/9 diclofenac-treated animals and 4/9 placebo-treated animals. Urinalysis was unremarkable for all cats throughout the experiment.

Baseline GFR was within normal limits for all cats (3.07 ± 0.22 ml/min/kg), where normal is considered to be greater than 2.0 ml/min/kg. When GFR data from both Phase 1 and 2 were combined, no adverse treatment effect was found (p = 0.2879). However, GFR in treated animals was significantly lower than in placebo animals following the cross-over in the second phase of the experiment (p=0.0013). The GFR for all cats treated with diclofenac in the first phase of the study was maintained or increased
in the second phase of the study where these cats received placebo. In contrast, all cats receiving placebo in the first phase of the study had a decrease in GFR following the second phase of the study, where they received diclofenac. (Table 4.5)

4.5 Discussion:

In this study, topical 0.1% diclofenac was applied bilaterally 4 times daily for 7 days in clinically normal eyes to simulate an aggressive dosing regimen administered on an outpatient basis. In hospitalized patients with severe uveitis, topical anti-inflammatory can be used as often as every 2 hours with doses continued through the night. In contrast, when owners are administering medications on an outpatient basis, the frequency of application typically does not exceed 4 times daily and most doses are given during daylight hours. Thus, in our experiment, 0.1% diclofenac was administered 4 times between 8 and 8pm. Diclofenac 0.1%, (Voltaren)\textsuperscript{m}, was chosen because it is commonly used by veterinary ophthalmologists and is commercially available. Although topical NSAIDs have been shown to be effective in canine and feline models of uveitis,\textsuperscript{35-39} little is currently known regarding systemic absorption and possible systemic adverse effects, particularly after repeated long-term administration. By performing this study, our goal was to characterize the systemic absorption of topical 0.1% diclofenac, as well as establish its renal and hepatic safety in healthy cats. Based on the results of this experiment, systemic levels of diclofenac are achieved, with systemic accumulation after multiple doses. No adverse hepatic effects were detected. Renal effects were not present in the first phase of the cross-over experiment, but became apparent in the second phase,
suggesting a potential risk of renal toxicity with topical diclofenac 0.1% use in volume-contracted animals.

**Drug disposition of diclofenac following topical ocular administration in cats**

Plasma levels of diclofenac were determined following topical application in this study using HPLC-MS. To date, there are only two published studies that have evaluated plasma levels of diclofenac following topical application. In both experiments, single dose pharmacokinetic studies of topical 0.1% diclofenac were performed in rabbits (Table 4.6). Despite species differences and the multiple dosing regimen used in our study, the $C_{\text{max}}$ and $T_{\text{max}}$ determined in our study were similar to those obtained in the study performed by Gonzalez-Penas et al.$^{34}$ In contrast, the $C_{\text{max}}$ determined in our study is considerably higher than the $C_{\text{max}}$ determined in the Palmero et al study.$^{33}$ As sampling did not begin until 30 minutes following administration of eye drops in the Palmero et al. study,$^{33}$ it is suspected that the true peak concentration may have been missed. In addition to $C_{\text{max}}$, $T_{\text{max}}$ in the Palmero et al. study was significantly later than in the present study. In the Palmero et al study, it is possible that as plasma diclofenac levels did not fit a biexponential curve, curve fitting could not be accurately performed, leading to overestimation of $T_{\text{max}}$. Despite differences in $C_{\text{max}}$ and $T_{\text{max}}$, the AUC determined by our experiment, particularly after Day 1, is similar to that determined by Palmero et al. suggesting that total exposure was similar between studies.

Mild hypoproteinemia was observed in both placebo and treated groups in this study, likely due to repeated blood sampling. Given that diclofenac is highly protein bound,$^{48}$ it might be anticipated that decreased protein levels could lead to increased
concentrations of unbound active drug, thus increasing the potential for toxic effects, such as the reduction in GFR detected in the second phase of the study. It is, however, unlikely that hypoproteinemia contributed to the suspected renal toxicity. Recent literature in humans suggests protein binding is less clinically relevant than previously thought. Drugs such as diclofenac, which are classified as high extraction drugs i.e. blood-flow limited, are thought to distribute with a relatively large volume of distribution, and without saturation of hepatic extraction and metabolism.49 As hepatic metabolism is dependent on hepatic blood flow and not protein binding, metabolism and elimination of the drug increase to compensate for the increased concentrations of free drug.

Further research on the effects of hypoproteinemia on NSAID metabolism in the cat is, however, required. Although hypoproteinemia in humans is of little consequence clinically with high extraction drugs, hypoproteinemia may play a larger role in the cat given the limited ability for hepatic glucuronidation in this species. In the cat, should hepatic metabolism become saturated, hepatic extraction could become rate-limited. Metabolism of diclofenac occurs via glucuronidation in the dog and the rat,50 and remains to be determined in the cat. In addition, it is possible that in this study, due to withdrawal of large amounts of blood, that both hypovolemia and hypoproteinemia were present. Hypovolemia yielding subsequent redistribution of blood away from the organs of elimination could presumably lead to decreased hepatic blood flow and decreased hepatic extraction, and therefore decreased clearance of the unbound drug fraction.

In this study, due to a limited number of sample points, only $C_{\text{max}}$, $T_{\text{max}}$, and $AUC_{0-240}$ were determined. However, for the purposes of developing a better understanding of the pharmacokinetics of diclofenac in the cat, $t_{1/2}$ and accumulation ratio
were also calculated. As these estimations are crude and do not adhere to standard guidelines, the following sections should be interpreted with caution in light of the limitations presented by our data.

Due to an insufficient number of sample points, it was not possible to accurately determine elimination half-life ($t_{1/2}$). However, by interpolation, an estimate of $t_{1/2}$ in our experiment was 355 ($\pm$ 142) minutes or 5.9 ($\pm$ 2.4) hours. (mean $\pm$ SE), with a range for $t_{1/2}$ between 166 and 707 minutes or between 2.8 and 11.8 hours. Though not a direct comparison, the $t_{1/2}$ of diclofenac following topical application to the cat does not appear to be significantly longer than with other NSAIDs dosed by other routes of administration to cats. For example, $t_{1/2}$ for 0.3 mg/kg of meloxicam administered subcutaneously in cats is 15 hours, $t_{1/2}$ for 0.3 mg/kg of piroxicam administered intravenously or orally is 12 hours, and $t_{1/2}$ for 4 mg/kg of carprofen administered intravenously or orally is 19 hours. Similar to studies on carprofen and acetylsalicylic acid, a large amount of individual variation in the pharmacokinetics of diclofenac was found in our study.

Systemic drug accumulation occurs when a drug is incompletely eliminated from the body before the next dose is administered. Accumulation of drug continues with successive doses until steady state is reached, where the rate of drug input equals the rate of elimination. Plasma accumulation of diclofenac was observed in our experiment, with plasma concentrations of diclofenac on Day 7 being significantly greater than on Day 1. As steady state is typically achieved at approximately 5 $t_{1/2}$s, assuming that $t_{1/2}$ in this experiment was between 2.8 and 11.8 hours, we hypothesize that by Day 7 of drug administration, steady state had have been achieved. Steady state is also defined as the dosing interval in which AUC for that interval is equal to the single dose AUC$_{0-\infty}$. As
samples were only collected to the 240 minute point, we would expect that with additional time points (approaching infinity on Day 1), the Day 1 AUC would be higher and more similar to that determined on Day 7. The accumulation ratio, R, is the ratio of drug concentrations at steady state to those achieved following the first dose. C<sub>max</sub> and AUC at steady state, and following the first dose, can be used to calculate R.\textsuperscript{55} In our experiment, sample collection was performed following the fourth dose rather than following the first dose of drug administration to maximize the likelihood of detecting drug concentrations. Thus, estimations of accumulation ratio based on C<sub>max</sub> and AUC are inaccurate and underestimate the true value of R. However, using average Day 1 C<sub>max</sub> and AUC values, an accumulation ratio of 1.55 (using C<sub>max</sub>) and 1.87 (using AUC) can be calculated, respectively. This suggests that steady state plasma diclofenac concentrations are approximately 1.5x to 2x those observed after the fourth dose. The accumulation ratio can be easily calculated for one-compartment intravenous models using ratios between the dosing interval, τ, and t<sub>1/2</sub>.\textsuperscript{54,55} However, as plasma levels of diclofenac in this study are dependent upon absorption across cellular membranes and redistribution to the central plasma compartment, additional pharmacokinetic parameters, namely the absorption rate constant (k<sub>a</sub>) as well as the elimination rate constant (k<sub>e</sub>), are needed to calculate R by this method.\textsuperscript{56} Additional sample points are needed for calculation of these parameters and more accurate determination of R.

The effect of reduced GFR on the elimination of diclofenac must also be considered. At present, the route of elimination of diclofenac in the cat is unknown. Excretion of diclofenac may be predominantly biliary, as in humans and rhesus monkeys, or urinary, as in rats.\textsuperscript{57} If diclofenac is renally eliminated in the cat, a reduction in GFR
may cause elimination to be delayed with subsequent accumulation and development of a cycle of increased diclofenac levels and reduced GFR. In human patients with renal insufficiency, initial accumulation of diclofenac metabolites occurs due to decreased GFR, but is of little clinical concern as diclofenac and its metabolites are likely eliminated via alternate compensatory mechanisms, such as biliary excretion, with no net decrease in elimination or accumulation over time.\textsuperscript{58} Thus, human patients with renal insufficiency do not currently require adjustment of their diclofenac dose.\textsuperscript{58} Other NSAIDs, such as ketorolac, do however show accumulation in human patients with renal insufficiency.\textsuperscript{59} In addition to its role in the elimination of drugs, the kidney may also play a limited role in metabolism.\textsuperscript{60} Some drugs, such as acetaminophen, require metabolism in the renal parenchyma, which in humans accounts for approximately 15\% the metabolic capacity of the liver.\textsuperscript{60} Thus renal compromise may also lead to accumulation of drugs due to reduced metabolism. At present, the mechanisms underlying the metabolism and elimination of diclofenac in the cat are unknown, and the effects of reduced GFR on the pharmacokinetics of diclofenac in this species also remain to be determined.

\textit{Evaluation of Possible Adverse Effects on Serum Biochemistry Parameters}

The major finding in the serum biochemistry panel was hypoproteinemia. As hypoproteinemia was observed in both control and treatment groups (p = 0.2149), it was likely due to repeated blood sampling and replacement of blood with crystalloid fluids. Decreased protein synthesis due to hepatic dysfunction is unlikely, as elevation of hepatic enzymes was rare, and other compounds synthesized by the liver, such as urea and glucose, were not concurrently deceased. Development of a protein-losing nephropathy is
also unlikely as increased loss of protein in the urine was not detected via UPCR. Gastrointestinal loss is also unlikely in the absence of gastrointestinal signs such as vomiting and diarrhea or other signs such as weight loss.

**Evaluation of Possible Adverse Hepatic Effects**

Based on serum biochemistry panels, there was no evidence for hepatic toxicity associated with topical 0.1% diclofenac use. At present, there are no known literature reports of hepatic toxicity following use of NSAIDs in cats. There was no evidence of hepatic toxicity in cats receiving long-term meloxicam, piroxicam or robenacoxib therapy.\(^4\)\(^{,}61\)\(^-\)\(^63\) AST elevations were noted with perioperative meloxicam and carprofen administration in cats undergoing ovariohysterectomy. However, no other hepatic parameters were abnormal and the significance of this elevation is not clear as AST is not specific to the liver.\(^64\) Further research is needed to confirm that the use of topical diclofenac has no adverse hepatic effects in the cat. In particular, larger sample numbers are needed as the incidence of hepatic toxicity is expected to be low. In humans, the incidence of NSAID-associated hepatotoxicity is low; it is estimated that there are between 1-8 cases of hepatotoxicity for every 100,000 NSAID prescriptions.\(^65\)\(^,\)\(^66\) Interestingly, diclofenac and sulindac are considered to pose a higher risk than other NSAIDs.\(^65\)\(^,\)\(^67\) NSAID-associated hepatotoxicity is incompletely understood, but is attributed to two main mechanisms in human patients: hypersensitivity reaction and alteration of hepatic metabolism.\(^68\) Rat hepatocytes exposed to diclofenac exhibit mitochondrial swelling, and toxicity appears to be more pronounced in drug-metabolizing cell lines than non-metabolizing lines.\(^69\)
Evaluation of Possible Adverse Renal Effects

The risk of NSAID-associated renal toxicity was assessed through serum biochemistry panels, UA, UPCR, and GFR studies. No abnormalities were detected in either placebo or treatment group using serum biochemistry panels or urinalyses. There were also no significant changes in UPCR throughout the experiment in either group. The cause of the borderline and abnormal UPCR values at baseline and during the experiment is unknown. Although no changes were detected using more traditional tests of renal function, a difference in treated animals was detected using GFR, which allows for early detection of renal dysfunction prior to development of azotemia. Thus, it is likely that a sufficient amount of systemic diclofenac absorption occurred to cause inhibition of renal PG synthesis or cytotoxicity, and mild compromise to renal function. In particular, treated animals had a lower GFR than placebo animals in the second phase of the study. No difference between groups was detected during the first phase of the study, prior to the crossover. Reduction of GFR in treated animals during the second phase of the study is likely due to induction of a volume contracted state with repeated blood sampling with cumulative effects becoming apparent only later in the study. A range of GFR values using different techniques have been published in cats. Most published values range between 2.5 ml/min/kg and 3.5 ml/min/kg but values as low as 2 ml/min/kg have also been reported in normal cats.

Through inhibition of PG synthesis, NSAIDs blunt the kidney’s ability to counteract decreased renal perfusion in volume-contracted states. In the euvolemic state, PGs do not contribute significantly to renal perfusion. As renal blood flow does not depend on PGs, NSAIDs do not have a significant effect on renal function. In cats,
there were no changes in GFR following a 5-day course of meloxicam in healthy, euvoilemic animals. Similarly, though studies in cats are lacking, in young and elderly euvoilemic human subjects, diclofenac administration has no effect on GFR administered perioperatively or over several weeks. ⁸⁰,⁸¹

In contrast, with volume contraction, there is stimulation of the renin-angiotensin-aldosterone system and increase in sympathetic outflow, both of which promote renal vasoconstriction. ⁷⁷,⁷⁹,⁸² In the face of increased systemic levels of vasopressors, PGs play an important role in maintaining renal perfusion by stimulating compensatory renal vasodilation at the level of renal medullary and cortical arterioles. ⁸³-⁸⁵ In the cat, PGE₁ and PGE₂ have been shown to have potent dilatory activity in the renal vascular bed. ⁸⁶ Also in the cat, prostaglandins E₁, E₂ and A₂ have been shown to counteract vasoconstriction secondary to sympathetic stimulus and increased angiotensin levels. ⁸⁶ Though a volume-contracted state is suspected based on GFR results in our study, there were few detectable clinical signs of hypovolemia during the experiment. All animals remained bright and responsive throughout the study, and maintained a mean blood pressure of greater than 100mmHg following blood sampling. More accurate and consistent determination of blood pressure could potentially have been achieved through acclimatization of the animals to the procedure prior to the study. The effects of repeated blood sampling were evident in the reduction of hematocrit and TP recorded during the experiment. However, as all animals met the minimum cut-off for PCV of 25% and a no cut-off for TP was established, we did not suspect during the experiment that repeated blood sampling would be of clinical relevance. In addition, while decreases in PCV and TP reflect loss of circulating blood volume, they do not allow for an accurate assessment
of the actual circulating volume. Accurate determination of plasma volume could theoretically be attained through techniques such as the T-1824 and iodinated albumin methods.\textsuperscript{87,88} These methods lack feasibility due to the requirement for further serial blood sampling. Comparison of plasma renin levels at baseline versus during the experiment could potentially be used to quantify the body’s response to hypovolemia, though such an approach would require validation in the cat.\textsuperscript{89}

In susceptible human patients, such as those with pre-existing renal insufficiency or concurrent furosemide therapy, short-acting NSAIDs such as ibuprofen result in sharp decreases in GFR. However, as long as the drug $t_{1/2}$ is short relative to dosing interval, GFR returns to normal within the dosing interval prior to administration of the next dose. Thus, there are no long-term adverse effects on renal function in humans.\textsuperscript{90,91} By contrast, for NSAIDs with a long $t_{1/2}$, renal function is considered to be less likely to return to normal during a dosing interval, leading to sustained and more clinically significant reduction in GFR. Data in humans supports this hypothesis; epidemiological research suggests that renal toxicity is more likely to occur with NSAIDs having a $t_{1/2}$ greater than 12 hours.\textsuperscript{92} In our experiments, $t_{1/2}$ was estimated to be between 3.3 and 11.6 hours, while the dosing interval was 4 hours during the day, and 12 hours between the 8pm and 8am treatments. Thus, in our experiment, a relatively long $t_{1/2}$ and short dosing interval, in addition to likely volume contraction, probably contributed to reductions in GFR. Fortunately, NSAID-associated renal complications are considered to be reversible if NSAID therapy is discontinued promptly upon detection of renal dysfunction.\textsuperscript{77} Additional GFR studies in the days and weeks following the experiment would be needed to confirm the return of GFR to baseline values.
Topical administration of diclofenac according to an aggressive clinical regime for 7 days may be associated with a reduction of GFR in the context of hypovolemia. An additional factor that may have contributed to reduction in GFR is a short dosing interval relative to the elimination half-life of diclofenac. Despite the reduction in GFR, there were no signs of more severe manifestations of NSAID-associated renal toxicity. These include acute renal failure, hyperkalemia, and sodium retention with edema. In severe cases of renal ischemia with NSAID use, acute renal failure may occur. Acute renal failure is typically accompanied by azotemia, potassium elevations, weight gain, and decreased urine production. Sodium retention and hyperkalemia with NSAID use occur due to inhibition of PGs that inhibit sodium transport in the thick ascending limb of the Loop of Henle and PGs that stimulate renin secretion, which indirectly decreases potassium excretion. Interstitial nephritis likely occurs due to shunting of arachidonic acid metabolites away from the COX pathway towards the lipoxygenase pathway, with the production of leukotrienes. Interstitial nephritis is characterized by the development of proteinuria. Diclofenac-associated cytotoxicity was not examined in this study, but has been documented in mice and rats. Proposed causes for cytotoxicity include induction of oxidative stress, uncoupling and inhibition of oxidative phosphorylation, and genomic fragmentation.

In our study, one cat developed asthma in the week following completion of the second phase of the study, where he received topical diclofenac. As only one individual developed such signs of asthma, the significance of this finding is unknown. However, isolated cases of asthma attacks following topical NSAID application have been reported with indomethacin and diclofenac have been reported in humans. The proposed
mechanism in NSAID-induced asthma is COX inhibition within the respiratory tract, leading to shunting of arachidonic acid from the cyclo-oxygenase pathway to the lipoxygenase pathway and production of leukotrienes. Leukotriene accumulation causes spasm of non-vascular smooth muscles within the bronchi. Epidemiological studies are needed in cats to determine if topical NSAIDs either trigger or exacerbate feline asthma.

**Limitations**

A limitation of this study is the likely induction of a volume-contracted state, despite an effort to limit the number of blood samples. Future experiments should consider separation of the pharmacokinetic and GFR studies to decrease the volume of blood collected over a given time period. Separation of the studies will also permit a greater number of samples to be collected during the pharmacokinetic study, allowing for a more complete analysis (see below). Alternatively, a longer wash-out period could have been used. In this study, blood volumes collected, and wash-out periods observed, adhered to the guidelines published by the European Federation of Pharmaceutical Industries Associations (EFPIA) and the European Centre for the Validation of Alternative Methods (ECVAM) for multiple sampling. In addition, blood collected was replaced 1:1 with physiologic saline, a regime chosen to minimize the risk of hypovolemia while minimizing dilution of the blood volume, which could cause compromise of the pharmacokinetic and GFR studies. In the future, an appropriate wash-out period could be determined using a small group of pilot animals, or a parallel rather than cross-over study design used. Use of more stringent minimums for PCV and
inclusion of a minimum for TP prior to proceeding with additional phases of the study could also decrease the likelihood of hypovolemia.

Limitations in the pharmacokinetic study include an insufficient number of sample points, timing of sample collection, and the site of sample collection. Due to a limited number of sample points, the information that could be derived from our pharmacokinetic study is limited. Additional sample time points, particularly during the elimination phase, would allow for more accurate determination of pharmacokinetic parameters such as AUC. More accurate determination of AUC would allow for additional parameters such as clearance (CL) to be calculated. Increased sample points are also required for the determination of $t_{1/2}$, absorption constant ($k_a$) and elimination constant ($k_e$). Also, if detection of lower drug levels could be achieved, Day 1 sampling following the first drop (as opposed to the fourth drop) would allow for determination of the accumulation ratio.

In this study, the jugular vein was used as the sampling site due to the ease of blood collection, ease of placement, and ready availability of jugular catheters. However, as the ocular circulation drains indirectly into the jugular vein, sampling for jugular catheters may have led to an overestimation of plasma concentrations achieved in peripheral circulation. Jugular catheters were considered a viable option as it was assumed that ocular blood flow contributes only a small fraction of total jugular blood flow, with rapid dilution of the blood from ocular circulation. Sample collection from a more peripheral site, such as the femoral vein, would result in lower plasma drug concentrations that are more reflective of true peripheral plasma levels.
Conclusion

In conclusion, topical application of 0.1% diclofenac resulted in detectable plasma diclofenac levels. Application of one drop to each eye 4 times a day for 7 days resulted in systemic drug accumulation. Though very low, the levels of diclofenac attained are suspected to have led to reduction in GFR. Deterioration in GFR is thought to have occurred secondary to NSAID use in the face of a volume- contracted or hypovolemic state. Volume contraction is thought to have occurred secondary to serial blood sampling. Thus, the results of this study suggest that topical 0.1% diclofenac should be used with caution in patients in volume- contracted states. Patients who may be at risk for adverse renal effects include those affected by hypovolemia, dehydration, cardiac disease, hepatic disease, renal disease, and dietary sodium restriction.77,79,82,104-106 As feline anterior uveitis can be secondary to systemic diseases such as infection and neoplasia,22,23 careful patient selection may be necessary. There were no detectable hepatic effects associated with topical diclofenac use in this experiment. Future studies are needed for more accurate determination of pharmacokinetic parameters and to determine the effects of topical NSAIDs without the confounding effects of hypovolemia.
4.6 Footnotes

a. Liberty Research, Waverly, NY
b. Kowa SL-15, Kowa, Tokyo, Japan
c. Schirmer Tear Test strips, Alcon Canada, Mississauga, Ontario, Canada
d. Fluorescets, Chauvin Pharmaceuticals Ltd, Aubenas, France
e. TonoVet, Tiolat Ltd, Helsinki, Finland
f. Heine Omega 2c, Heine Optotechnik, Herrsching, Germany
g. NeoMedical V-Cath PICC, NeoMedical Inc., Fremont, California, USA
h. Vetalar, Bioniche Animal Health Canada Inc., Belleville, Ontario, Canada
i. Vetergesic, Reckitt Benckiser Healthcare Ltd., Dansom Lane, Hull, UK
j. Acepromazine 2mg/ml, Ontario Veterinary College Pharmacy, Guelph, Ontario, Canada.
k. Diprivan, Astra Zeneca, Mississauga, Ontario, Canada
l. 99mTc-DTPA, Bristol-Myers Squibb Imaging, Montreal, Quebec Canada
m. Voltaren Ophtha, Novartis Pharmaceuticals Canada Inc, Dorval, Quebec, Canada
n. Tears Naturel II, Alcon Canada, Mississauga, Ontario, Canada
o. Nosorb, Catco Inc., Cape Coral, FL, USA
p. Schuco Refractometer, Schuco International Ltd, London, UK
q. Cardell Veterinary Monitor, Model 9401, Midmark, Tampa, FL, USA
r. Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, MD, USA
s. API 2000, Applied Biosystems, Foster City, CA, USA
t. Dr. Butch Kukanich, DVM, PhD, Diplomate ACVCP, College of Veterinary Medicine, Kansas State University
u. Thermo Hypersil, 150x3 mm, 5uM particle size, Fisher Scientific, Pittsburgh, PA, USA
v. Usansky, Desai, Tang-Liu, Irvine, CA, USA
w. SAS Institute Inc. 2007, SAS OnlineDoc® 9.2., Cary, North Carolina, USA
x. SigmaPlot 12, Systat Software Inc., San Jose, CA USA
y. Metacam (meloxicam) 5 mg/ml injectable product monograph, Boehringer Ingelheim, St. Joseph, MO, USA
z. Personal Communication : OVC Cardiology Service
### 4.7 Tables

Table 4.1: Schedule of blood sample collection and 1:1 fluid replacement

<table>
<thead>
<tr>
<th>Timing</th>
<th>Test*</th>
<th>Volume of blood (ml)</th>
<th>Fluid replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrival (18d prior to Phase I)</td>
<td>CBC/Chem Diclofenac</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td>Pre-Phase I (1d prior to Phase I)</td>
<td>GFR Study</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>Phase I Day 1</td>
<td>PK Study</td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td>Phase I Day 7</td>
<td>CBC/Chem PK Study</td>
<td>14</td>
<td>Yes</td>
</tr>
<tr>
<td>Post-Phase I (1d after Phase I)</td>
<td>GFR Study</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>Pre-Phase II (1d prior to Phase II)</td>
<td>Diclofenac</td>
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<td>Phase II Day 1</td>
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</tr>
<tr>
<td>Post-Phase II (1d after Phase II)</td>
<td>GFR Study</td>
<td>14</td>
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</tr>
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</table>

* Abbreviations: Chem = serum biochemistry panel, PK = pharmacokinetic, Diclofenac = plasma diclofenac level
Table 4.2: Mean ± SD Plasma pharmacokinetic parameters of diclofenac after topical ocular administration to healthy cats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( \text{C}_{\text{MAX}} )</th>
<th>( \text{AUC}_{0-240} )</th>
<th>( \text{T}_{\text{MAX}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units</td>
<td>ng/mL</td>
<td>min*ng/mL</td>
<td>min</td>
</tr>
<tr>
<td>Day</td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>46.8* ±24.4</td>
<td>10,953* ±3,502</td>
<td>23.1±15.5</td>
</tr>
<tr>
<td></td>
<td>87.6* ±23.3</td>
<td>16,502* ±4,568</td>
<td>24.6±22.2</td>
</tr>
<tr>
<td>Minimum</td>
<td>10.6</td>
<td>6702</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>50.5</td>
<td>8664</td>
<td>5.0</td>
</tr>
<tr>
<td>Median</td>
<td>59.7</td>
<td>11578</td>
<td>22.5</td>
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<tr>
<td></td>
<td>106.0</td>
<td>19781</td>
<td>30.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>85.0</td>
<td>15471</td>
<td>60.0</td>
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<tr>
<td></td>
<td>114.0</td>
<td>21553</td>
<td>60.0</td>
</tr>
</tbody>
</table>

* P<0.05 within a pharmacokinetic parameter, comparison of Day 1 vs. Day 7
Table 4.3: Mean ± SE Select PCV and TP testing prior to blood sampling

<table>
<thead>
<tr>
<th>Timing</th>
<th>Phase</th>
<th>PCV (%)*</th>
<th>TP (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to Phase I</td>
<td>Pre-Phase I</td>
<td>36.8 ± 1.2</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>(1d prior to Phase I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior to Phase I GFR Study</td>
<td>Post-Phase I</td>
<td>30.1 ± 0.1</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>(1 d after Phase I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior to Phase II</td>
<td>Pre-Phase II</td>
<td>35.4 ± 1.5</td>
<td>6.8 ± 0.3</td>
</tr>
<tr>
<td>(1d prior to Phase II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior to Phase II GFR Study</td>
<td>Post-Phase II</td>
<td>29.7 ± 1.4</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>(1 d after Phase II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of Study</td>
<td>Post-Phase II</td>
<td>29.5 ± 1.2</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>(after last GFR sample)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All cats needed to meet the minimum PCV of 25% to participate in PK and GFR studies. No cat failed to meet this minimum.
Table 4.4: Mean ± SE Selected serum biochemistry and urinalysis variables in cats at baseline, following topical 0.1% diclofenac treatment or following topical placebo treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference Range**</th>
<th>Baseline</th>
<th>Placebo</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>0.0758</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>0.2643</td>
</tr>
<tr>
<td>TP (g/L)</td>
<td>66-84</td>
<td>66 ± 2</td>
<td>63.9 ± 0.9</td>
<td>62.4 ± 0.9</td>
<td>0.2149</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.4-7.7</td>
<td>4.74 ± 0.49</td>
<td>4.76 ± 0.09</td>
<td>4.65 ± 0.09</td>
<td>0.0758</td>
</tr>
<tr>
<td>ALP (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>0.1546</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>0.5108</td>
</tr>
<tr>
<td>GGT (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>0.6517</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>6-12</td>
<td>8.0 ± 0.4</td>
<td>8.93 ± 0.33</td>
<td>8.77 ± 0.33</td>
<td>0.3283</td>
</tr>
<tr>
<td>Creatinine (umol/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>0.4967</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>0.7473</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.6-5.2</td>
<td>4.7 ± 0.1</td>
<td>4.68 ± 0.10</td>
<td>4.73 ± 0.10</td>
<td>0.7712</td>
</tr>
<tr>
<td>USG</td>
<td>&gt;1.035</td>
<td>1.0464 ± 0.0026</td>
<td>1.0474 ± 0.0003</td>
<td>1.0510 ± 0.0003</td>
<td>0.7837</td>
</tr>
<tr>
<td>UPCR</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>0.4389</td>
</tr>
</tbody>
</table>

* p-values are a comparison between placebo and diclofenac animals, where baseline values have been built into the analysis model as a covariate

** AHL, Ontario Veterinary College, Guelph, ON, Canada
Table 4.5: Mean ± SE GFR values in cats at baseline, following topical diclofenac and following topical placebo treatment

<table>
<thead>
<tr>
<th>GFR (ml/kg/min)</th>
<th>Baseline</th>
<th>Placebo</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>0.2879</td>
</tr>
<tr>
<td>Phase I</td>
<td>2.87 ± 0.16</td>
<td>2.75 ± 0.14</td>
<td>0.8320</td>
<td></td>
</tr>
<tr>
<td>Phase II</td>
<td>2.75 ± 0.16</td>
<td>2.31 ± 0.16</td>
<td>0.0013</td>
<td></td>
</tr>
</tbody>
</table>

* p-values are a comparison between placebo and diclofenac animals, where baseline values have been built into the analysis model as a covariate
Table 4.6: Comparison of experimental pharmacokinetic parameters to those obtained in other studies (mean +/- SD unless otherwise indicated)

<table>
<thead>
<tr>
<th>Study</th>
<th>Mass DCF (mg/route)</th>
<th>Subjects</th>
<th>Approximate Dose (mg/kg)</th>
<th>C(_{\text{max}}) (ng/mL)</th>
<th>T(_{\text{max}}) (min)</th>
<th>AUC (min*ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present Study</td>
<td>0.1 (top) QID</td>
<td>Cats 1-2y, 5-6kg</td>
<td>0.01</td>
<td>Day1: 46.8 ± 24.4</td>
<td>Day1: 26.3 ± 15.5</td>
<td>Day1: 10,953 ± 3,502</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day7: 87.6 ± 23.3</td>
<td>Day7: 24.6 ± 15.5</td>
<td>Day7: 16,502 ± 4,568</td>
</tr>
<tr>
<td>Gonzalez – Penas 1998(^{34})</td>
<td>0.06 (top)</td>
<td>Rabbits 3kg</td>
<td>0.02</td>
<td>72.7 ± 14.2 (SE)</td>
<td>15</td>
<td>n/a</td>
</tr>
<tr>
<td>Palmero 1999(^{33})</td>
<td>0.03 (top)</td>
<td>Rabbit 2.5kg</td>
<td>0.008</td>
<td>6.1</td>
<td>82.3</td>
<td>6,540</td>
</tr>
</tbody>
</table>
4.8 Figures

Figure 4.1: Mean ± SD Plasma concentrations of diclofenac after topical ocular administration in healthy cats.
4.9 References


15. Nasisse MP, Van Ee RT, Wright B. Ocular changes in a cat with disseminated


31. Riegel M, Ellis PP. High-performance liquid chromatographic assay for


CHAPTER 5: General Discussion, Conclusions, and Future Directions

5.1 General Discussion, Conclusions, and Future Directions

Topical NSAIDs have been shown to decrease intraocular inflammation in cats,\(^1\) and clinically, may be used in cases where topical corticosteroids are contraindicated or where adjunctive therapy is needed.\(^2-4\) As with any other medication, however, veterinarians must consider potential adverse effects in addition to therapeutic efficacy when prescribing topical NSAIDs. The objectives of this study were thus to determine if there are any adverse ocular, hepatic or renal effects associated with topical NSAID use in healthy cats. Another objective was to quantify systemic diclofenac levels following topical administration for comparison to adverse systemic effects. Through characterization of any changes to the healthy eye, it was our hope that the results of our study could help veterinarians better anticipate and recognize any adverse ocular or systemic effects associated with topical 0.1% diclofenac use. Early recognition of adverse drug effects is especially important in treating feline uveitis, where patients may have concurrent ocular diseases, such as corneal ulceration, and may be at risk for sequelae of uveitis, including glaucoma.\(^2-4\) Despite the small quantities administered through the topical route, characterization of potential systemic effects was also important, due to the cat’s limited ability for hepatic glucuronidation and the potential for systemic accumulation and toxicity.
Based on the results of this experiment, topical 0.1% diclofenac was well tolerated and has good ocular safety when dosed 4 times a day for 7 days in healthy cats. Mild conjunctival hyperemia was the only adverse ocular effect documented during the study. There were no significant effects of 0.1% diclofenac on STT, TFBUT, corneal health, CTT, PD or IOP. Topical administration of 0.1% diclofenac resulted in detectable plasma levels with accumulation over the 7-day dosing period. No hepatic or renal adverse effects were detected via conventional laboratory testing; no abnormalities were detected on serum biochemistry panel, urinalysis or UPCR. However, a significant reduction in GFR was detected in treated cats in the second phase of the study, where animals may have been in a subclinical volume-contracted state. Thus, as is the case with systemic NSAIDs, topical NSAIDs may need to be used with caution in at-risk feline patients, including those that are hypovolemic, dehydrated or have concurrent systemic disease. In at risk patients, topical NSAIDs should be tapered to the lowest effective dose, and monitoring of renal function may be indicated, particularly if prolonged or frequent NSAID use.

Having established that topical 0.1% diclofenac can be safely used in healthy eyes, future experiments need to be performed in cats with either experimentally-induced or naturally-occurring uveitis. Due to increased intraocular concentrations of PGs with uveitis, adverse effects associated with topical NSAID use in uveitis eyes may differ from healthy eyes. As has been previously discussed, elevation in IOP with NSAID use may be associated with uveitic eyes but not healthy eyes. It is also plausible that in eyes that are already irritated or sensitized due to intraocular inflammation, the irritation associated with 0.1% diclofenac may be exacerbated and corneal sensitivity may be altered.
Pharmacokinetic analysis following induction of intraocular inflammation should also be performed, as systemic absorption could hypothetically increase as the permeability of ocular blood vessels increase with breakdown of the BAB.

Experiments should also be performed using longer courses of topical NSAID administration (ie. weeks to months) and at increased frequencies (ie. every 2 hours) as longer courses and increased frequencies of administration may be required in clinical patients. Longer courses of topical diclofenac could be associated with additional ocular and organ effects, particularly those that may develop gradually, such as corneal degeneration. A shortened dosing interval could be associated with additional accumulation of diclofenac. Inclusion of a larger number of study animals would also increase power, thus increasing the chances of detecting differences exist between treated and control animals. Additional topical NSAIDs, in addition to diclofenac, should also be tested.

In addition, given the reduction in GFR detected in treated animals, additional tests can be used in future experiments to better characterize the effects of diclofenac on the kidney. Urinary fractional excretion of electrolytes could have been helpful in determining if repeated topical diclofenac administration affects renal electrolyte excretion. Renal plasma flow could also have been measured, and compared to GFR. In addition, measurement of urinary PG levels could have been performed to confirm the effect of diclofenac on renal PG levels and the pathogenesis of NSAID-induced renal toxicity. To better characterize the effects of topical NSAID use on the liver, additional tests such as bile acid stimulation and hepatic ultrasound, and biopsy may be
added. Future experiments should examine the effects of NSAIDs on other affected systems, namely the gastrointestinal tract and clotting pathways.

The information in this study suggests that topical 0.1% diclofenac dosed 4 times daily for one week in healthy cats is well tolerated and is not associated with any significant adverse ocular effects. Systemic absorption of diclofenac was detected in this study, with accumulation over 7 days. Topical 0.1% diclofenac therapy was associated with a reduction in GFR in the second phase of the cross-over study, suggesting that volume-contracted animals receiving topical NSAID therapy may be at risk for impairment of renal function.
5.2 References


15. Blasingham MC, Nasjletti A. Differential renal effects of cyclooxygenase inhibition