Effect of oral administration of robenacoxib on experimentally-induced anterior uveitis in normal cats

by

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Abstract

Objectives- To determine the effect of oral robenacoxib on experimentally-induced anterior uveitis, and to evaluate the ability of robenacoxib to cross an intact blood-aqueous barrier.

Animals- Twelve healthy adult domestic shorthair cats.

Procedures- Cats in the treatment group (n=6) received oral robenacoxib (1.51 ± 0.36 mg/kg) once daily beginning 1 day before experimental induction of uveitis by anterior chamber paracentesis (ACP) and continuing 1 day after paracentesis. Anterior chamber paracentesis was performed using a 30 g needle attached to a 1 mL syringe, and 100 µL of aqueous humor were aspirated over 3-5 seconds. Anterior chamber fluorophotometry was performed in both eyes of each cat immediately before ACP (time 0), and at 6, 24, and 48 hours after ACP. An independent t-test was used to compare percent fluorescein increase in treatment versus control cats at each time point. Values of $p<0.05$ were considered significant. Concentrations of robenacoxib in aqueous humor were measured using liquid chromatography and mass spectrometry.

Results- There was no statistically significant difference between the ACP and control eye at time 0 ($p=0.322$). When comparing the percent fluorescein increase between treatment and control groups, there was no statistically significant difference at any time point ($p>0.05$). Robenacoxib was present in small but detectable levels in 5/6 cats in the treatment group.

Conclusions and clinical relevance- Administration of oral robenacoxib did not significantly lessen experimentally-induced anterior uveitis in normal cats, as assessed by fluorophotometry. Low concentrations of aqueous humor robenacoxib were detectable in the majority of cats receiving the drug.

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List of Abbreviations

Anterior chamber associated immune deviation	ACAID
Arachidonic acid	AA
Anterior chamber paracentesis	ACP
Blood-aqueous barrier	BAB
Cyclooxygenase	COX
Feline diffuse iris melanoma	FDIM
Feline immunodeficiency virus	FIV
Feline leukemia virus	FeLV
Feline infectious peritonitis	FIP
Immunoglobulin M	IgM
Interleukin	IL
Non-steroidal anti-inflammatory drug	NSAID
Phospholipase A_2	PLA_2
Prostaglandin	PG
Tumor necrosis factor-α	TNF-α

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Chapter 1 – Literature Review

Anterior Uveitis

Background

The term uveitis is used to describe inflammation of the uveal tract, which is composed of the iris, ciliary body, and choroid. Inflammation of the uveal tract can occur as a result of primary ocular disease or as a manifestation of systemic disease. Intraocular inflammation can be further categorized by its location within the eye. Anterior uveitis, also known as iridocyclitis, is defined as inflammation of the iris and ciliary body. Posterior uveitis, which may also be referred to as chorioretinitis due to the close association of the choroid and retina, is inflammation of the choroid and overlying retina. Panuveitis is defined as inflammation of all three components of the uveal tract. [1] Uveitis is an important disease in veterinary medicine, as intraocular inflammation can lead to vision threatening consequences, and may be indicative of potentially life-threatening disorders.[2,3]

Inflammatory Cascade

As in other tissues in the body, intraocular inflammation, or uveitis, is initiated by tissue injury. The inflammatory cascade begins with the free arachidonic acid (AA), which can be released from the cell membrane due to a variety of mechanical or chemical stimuli, and subsequent enzymatic action of secretory phospholipase A_2 ($sPLA_2$) or cytoplasmic phospholipase A_2 ($cPLA_2$).[4,5] Free AA is then metabolized by two main enzyme pathways, the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. The COX pathway is responsible for converting AA

to prostaglandin G_2 (PGG_2), which is then converted by peroxidase to prostaglandin H2 (PGH_2). There are various cell specific synthases and isomerases that are responsible for the production of five biologically active PG's, which include prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostacyclin (PGI_2), and thromboxane A_2 (TxA_2).[4] The LOX pathway converts AA to hydroxyl-eicosatetraenoic acids, which can then be metabolized to leukotrienes (Figure 1.1).[4,6]

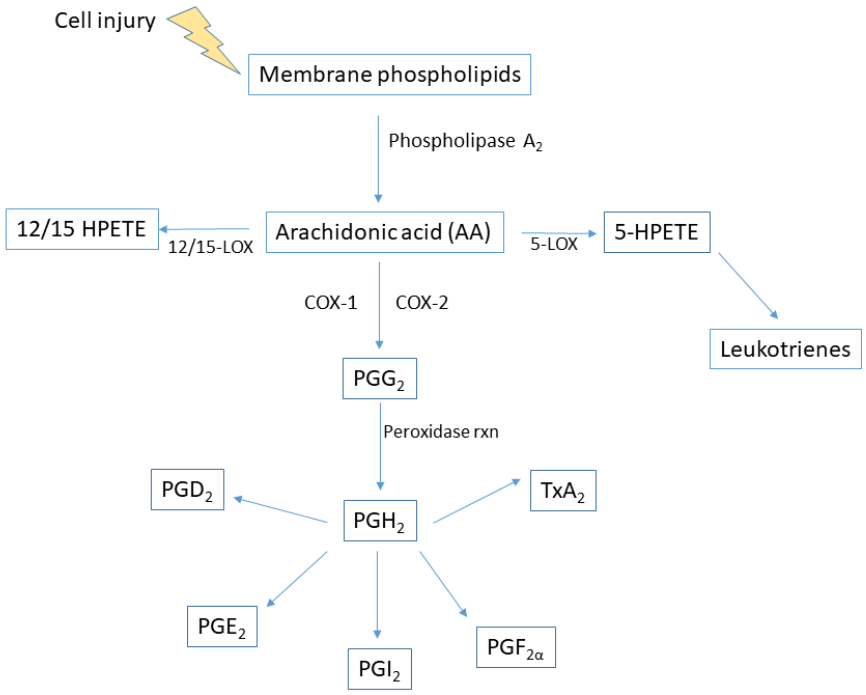

Figure 1.1. Diagram to represent the pathway of prostaglandin synthesis. An initial injury to the cell leads to release of membrane phospholipids, which are converted by phospholipase to arachidonic acid. The LOX pathway produces hydroxyl-eicosatetraenoic acids and leukotrienes while the COX pathway produces biologically active PGs and thromboxane A_2.

Prostaglandins are the best studied mediators of inflammation in the eye. Interestingly, the first report to investigate the production of prostaglandins outside of the reproductive tract identified $PGF_{2\alpha}$ in sheep irides.[7] A few years later, it was shown that injection of PGE into the anterior chamber of rabbits and cats caused increased intraocular pressure and miosis in rabbits, and in cats only caused miosis.[8] A subsequent study demonstrated that PG injected intracamerally in rabbits resulted in significant increase in protein content in the aqueous humor, which is a sign of breakdown of the blood-aqueous barrier (BAB).[9] Prostaglandins can disrupt tight junctions between the non-pigmented epithelial cells of the ciliary body and the endothelial cells of iridal vessels. This leads to protein leakage into the aqueous humor.[10,11] Increased protein concentration causes the clinical appearance of aqueous flare within the anterior chamber, which is a hallmark of anterior uveitis. Other mediators of intraocular inflammation include cytokines, specifically interleukin-1 (IL-1), tumor necrosis factor- α (TNF-α), and IL-6.[12] Studies performed in rabbits and mice have shown that injection IL-1 and IL-6 into the vitreous leads to severe anterior uveitis.[13,14] Tumor necrosis factor-α also causes uveitis when injected into the vitreous of rabbits,[15] but the timing of the inflammation and types of inflammatory cells present suggest that TNF-α may play a more important role in the cell mediated immune response rather than acute inflammation.

Unique Characteristics of Ocular Inflammation

The major events of inflammation in the eye are increased blood supply, increased vessel permeability, and migration of leukocytes to the site of injury.[16] While the events of inflammation are similar to elsewhere in the body, there are features of the eye that make it unique in its susceptibility and response to uveal inflammation. First, there are anterior and

posterior blood ocular barriers to prevent protein movement and limit lymphocyte circulation.[12,17] Next, the aqueous humor concentration of ascorbic acid and other anti-oxidants may help to lessen inflammation secondary to oxidative stress.[18] The ocular immune system demonstrates a unique phenomenon known as anterior chamber associated immune deviation (ACAID). The eye was first described as an immune privileged site in the 19th century when Dutch ophthalmologist van Dooremaal observed extended survival of mouse skin grafts placed in the dog's anterior chamber.[19] Compared to other tissues, the intraocular immunologic response to antigens is characterized by an improved immune tolerance and reduced effector mechanisms. Streilein and colleagues coined the term ACAID in the 1970's after noting that antigens injected into the eye gain access to the systemic lymphatic circulation and induce a generalized down-regulation of the body's cell-mediated immune response to that specific antigen.[20] Finally, the ocular immune response is unique in that the eye does not have an intrinsic lymphatic system. The eye is particularly sensitive to the effects of inflammation. Similar to central nervous system tissue, the eye has limited regenerative capabilities and post-inflammatory changes can have devastating consequences for vision and ocular comfort. These immunologic and anatomic adaptations are therefore crucial to minimize and prevent uveitis, in order to maintain optimal function of the eye (i.e., vision).

Clinical Signs of Uveitis

Patients with uveitis can present with a wide variety of clinical signs. There are some ophthalmic exam findings that are considered to be unique to uveitis, such as aqueous flare and hypotony. Other signs are more general signs of ocular disease, such as blepharospasm, episcleral injection, and conjunctival hyperemia. Epiphora, blepharospasm, enophthalmos, and elevation of the

nictitating membrane are indicative of ocular discomfort. Ciliary body muscle spasm is thought to be the major contributor to pain associated with uveitis, which in humans has been described as a dull ache or throbbing sensation localized to the eye or periorbital region. Photophobia is also thought to be due to ciliary body muscle spasm, and increased lacrimation is thought to occur secondary to photophobia.[21]

Anterior uveitis is associated with increased permeability of corneal endothelial cells, as well as decreased activity of Na^+/K^+ ATPase pumps located along the lateral aspects of the endothelial cells.[22] The normal cornea is composed of 75-85% water, and is relatively dehydrated compared to other tissues in the body. This state of dehydration in the corneal stroma is termed deturgescence, and it helps to maintain corneal transparency and clarity.[23] Reduced activity of Na^+/K^+ ATPase pumps and increased endothelial cell permeability leads to reduced ability to maintain corneal stromal dehydration, and fluid accumulation within the corneal stroma clinically presents as corneal edema.

Aqueous flare is one of the hallmark signs of anterior uveitis, and indicates a breakdown of the BAB. Aqueous flare is visualized on exam as cloudiness in the anterior chamber due to increased protein and cellular components within the aqueous humor. This continuous beam of light reflection throughout the anterior chamber is known as the Tyndall phenomenon. Aqueous flare is graded on a scale from 1+ to 4+, with 1+ being barely detectable, 2+ being moderate flare with details of the iris and lens still visible, 3+ as more severe than 2+ with hazy detail of the iris and lens, and 4+ being intense flare with loss of iris and lens detail.[21] The aqueous humor should normally be a clear ultra-filtrate of plasma. In the cat, the protein concentration in normal

aqueous humor is 0.15-0.55 mg/100 mL, compared with 7.8 mg/100 mL in plasma.[17] Breakdown of the BAB and increased permeability of ocular vessels can also lead to accumulation of inflammatory cells (i.e., hypopyon), red blood cells (i.e., hyphema), or fibrin in the anterior chamber. Aggregates of inflammatory cells and debris that adhere to the endothelial surface of the cornea are called keratic precipitates, and are typically deposited in the ventronasal quadrant due to the convection currents in the anterior chamber.[24]

Hypotony, or low intraocular pressure, can be an early indicator of anterior uveitis. It has been noted that in a variety of species, including cats, that ocular irritation induced by nitrogen mustard solution application is characterized by an initial increase in intraocular pressure, followed by a sustained decrease in intraocular pressure.[25] An initial rise in intraocular pressure, followed by low intraocular pressure, has also been documented in the cat following injection of a high dose of PGE_2 into the anterior chamber.[26] The initial rise in intraocular pressure may be partially due to increased plasma protein in the aqueous humor causing blockage of the trabecular meshwork, or vasodilation that results in a sudden increase in fluid in the ciliary processes, leading to subsequent extravasation of fluid into the eye. The subsequent persistent decrease in intraocular pressure may be due to a combination of both a decrease in production of aqueous humor by the non-pigmented ciliary body epithelial cells and prostaglandin mediated increased outflow via the uveoscleral pathway.[10,27]

Miosis, or constriction of the pupil, is another common clinical sign of anterior uveitis. Miosis occurs due the action of endogenous prostaglandins present in the aqueous humor, specifically $PGF_{2\alpha}$,[27-29] on the iris sphincter muscle. Mild or subclinical uveitis may present as a pupil that

exhibits a slow or incomplete mydriasis following the application of a mydriatic ophthalmic medication. A miotic pupil also leads to a closer proximity between the iris and the lens, which can increase the risk of posterior synechiae. Posterior synechiae occurs when inflammatory cells, fibrin, or fibroblasts cause the iris to adhere to the anterior lens capsule.

Sequelae of Uveitis

Sequelae of anterior uveitis can be painful, vision threatening, and potentially lead to loss of the globe. Potential complications of chronic anterior uveitis include secondary glaucoma, posterior synechiae, peripheral anterior synechiae, cataract formation, lens luxation, phthisis bulbi, and blindness.[1,24,30] In cats, the most common cause of glaucoma is lymphocytic-plasmacytic uveitis, followed by feline diffuse iris melanoma (FDIM).[30] Histopathologic changes associated with chronic uveitis in these globes included collapsed ciliary clefts and peripheral anterior synechiae.[30] Anterior uveitis may lead to glaucoma by pre-iridal fibrovascular membrane formation or through peripheral anterior synechiae, which may lead to physical obstruction of aqueous humor outflow through the iridocorneal angle.[31] Secondary glaucoma can also be due to 360º posterior synechiae, which obstructs aqueous humor flow through the pupil from the posterior chamber and leads to iris bombé. Cataract can result from chronic anterior uveitis, presumably due to the inflammatory mediators in the aqueous humor interfering with normal lens metabolism. Histopathologic changes are nonspecific, and include lens epithelial metaplasia, posterior migration, degeneration and necrosis of lens fibers.[32] Lens luxation in the cat is commonly found in association with uveitis and glaucoma, and may occur secondary to enzymatic degradation of ciliary zonules or stretching of the zonules secondary to buphthalmos.[33] Chronic uveitis can ultimately lead to advanced fibrosis and degeneration of the

ciliary body, where aqueous humor is produced. Once aqueous humor formation has ceased, phthisis bulbi, or shrinking of the globe, may occur. Blindness can occur as a result of glaucoma, phthisis bulbi, or cataract formation, and often develops due to a combination of these sequelae. In cases of glaucoma, the eye is also painful, so removal of the globe (enucleation) may be necessary.

Etiology

Anterior uveitis in the cat can be associated with multiple etiologies. However, in many cases of feline uveitis, a specific underlying etiology is not found, and "idiopathic" is assigned as a diagnosis of exclusion. Exogenous causes of anterior uveitis can typically be readily identified by performing a complete ophthalmic exam, and include trauma, corneal ulcers, or penetrating wounds.[2] Endogenous causes of anterior uveitis require additional diagnostic testing to identify, and include infectious diseases, parasitic diseases, lens-induced inflammation, neoplasia, or sepsis.[34] In a retrospective study evaluating 158 enucleated feline globes with uveitis, the most common histopathologic finding was a diffuse or nodular lymphocytic-plasmacytic anterior uveal infiltrate where a specific cause could not be identified.[35] Other causes identified in this study included feline infectious peritonitis, feline leukemia-virus associated lymphosarcoma, trauma, and lens-induced uveitis. Similarly, a review of 53 cats with anterior uveitis identified that the most common category of uveitis was cats with no other identifiable concurrent ocular or systemic disease (37 out of 53 cases).[36] A recent retrospective study found that in 40.8% of cats with endogenous uveitis, no underlying etiology was found.[37]

Infectious causes of feline uveitis include viral, protozoal, bacterial, fungal, and parasitic etiologies. Viral agents that can cause anterior uveitis include feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), and feline coronavirus, which mutates to become the causative pathogen for feline infectious peritonitis (FIP).[38] Feline immunodeficiency virus can cause uveitis through direct viral damage, or chronic immunosuppression may allow opportunistic infections. Similarly, FeLV can cause uveitis primarily, or indirectly when associated with lymphosarcoma. Feline infectious peritonitis causes a widespread vasculitis, and pyogranulomatous anterior uveitis is a common finding. Common ophthalmic signs include fibrin in the anterior chamber, iritis, and keratic precipitates.[3] Protozoal causes of uveitis include *Toxoplasma gondii* and *Leishmania infantum*. *Toxoplasma gondii* can cause both anterior uveitis and chorioretinitis. Diagnosis is based on suggestive clinical signs and serologic testing, specifically immunoglobulin M (IgM) antibodies and calculation of the Witmer-Goldmann coefficient or C-value, which compares aqueous humor-specific antibodies to serum antibodies. It has been suggested that a positive C-value for IgM is a stronger indicator of ocular toxoplasmosis than a positive C-value or IgG.[39] Leishmaniasis in the cat more commonly affects the skin or mucocutaneous tissue, but ocular manifestations of granulomatous conjunctivitis and panophthalmitis with *Leishmania* amastigotes identified has been reported.[40] While bacterial sepsis can lead to uveitis in the cat,[41] primary bacterial uveitis is not well recognized. *Bartonella* species have been the most extensively studied bacterial organism in relation to feline uveitis. The role bartonellosis plays in feline uveitis is unclear. Cats are most commonly infected with *B. henselae* or *B. clarridgeiae*, and once infected, can experience recurrent episodes of bacteremia. Often, cats will exhibit no clinical signs when infected.[42] In regards to anterior uveitis, *Bartonella* specific antibodies have been identified in serum from cats with uveitis (55%), cats

without uveitis that are ill (63%), and healthy cats (70%).[43] Disseminated fungal infections can commonly cause pyogranulomatous panuveitis. Commonly encountered fungal infections include cryptococcosis, histoplasmosis, blastomycosis, and coccidioidomycosis.[44-48] Ophthalmomyiasis, or infestation of the ocular or orbital tissues by fly larvae, is an uncommon disease in cats. *Cuterebra* larvae are the most frequently reported cause of ophthalmomyiasis interna in dogs and cats.[49-52] Clinical signs include fibrin in the anterior chamber, fibrinohemorrhagic clots, aqueous flare, and visualization of the organism within the anterior chamber. Surgical removal of the larva resulted in a visual globe in one case,[49] though complications encountered in other cases of surgical removal included persistent corneal edema[52] and retinal degeneration.[50]

Lens-induced uveitis is an inflammatory response of the ocular immune system to lens proteins, and may be further categorized as phacolytic or phacoclastic.[53] Phacolytic uveitis occurs due to the release of lens protein through an intact lens capsule. Histopathologic changes associated with phacolytic uveitis are lymphocytic-plasmacytic anterior uveitis associated with cataractous changes of the lens, but no evidence of a capsular tear or inflammatory cells within the lens.[53,54] Phacoclastic uveitis occurs as a result of spontaneous or traumatic rupture of the lens capsule. In contrast to phacolytic uveitis, phacoclastic uveitis is characterized by suppurative, lymphocytic, and granulomatous inflammation, intralenticular neutrophils, scrolling of the lens capsule, and prominent fibroplasia associated with chronicity.[53-55] While phacolytic uveitis is only associated with the release of crystallized lens proteins, phacoclastic uveitis results in the release of membrane-associated antigens as well. In either case, there is deviation from the low level, T-cell mediated tolerance of lens proteins, and a cell-mediated hypersensitivity reaction may

occur.[55] Although lens-induced uveitis may occur secondary to cataracts, in the cat cataracts occur most frequently as a sequela of intraocular inflammation. Septic implantation syndrome is a unique form of septic endophthalmitis that occurs as a result of previous penetrating trauma (most commonly a cat claw) that ruptures the lens capsule. Suppurative endophthalmitis is found to be centered around the lens, and bacterial organisms are identified within the lens in the majority of cases.[56] Recently, *Encephalitozoon cuniculi* has been identified in feline cataractous lenses. Cats included in the study presented for evaluation of cataracts and uveitis that were refractory to medical therapy. On histopathologic examination, spores were identified in 15/19 lens samples, and *E. cuniculi* DNA was identified by polymerase chain reaction (PCR) in 18/19 lenses.[57]

Lens instability has also been associated with uveitis. Although the most likely sequence of events in cats is that uveitis leads to lens luxation due to enzymatic degradation of the anchoring zonules, lens mobility within the eye further contributes to intraocular inflammation.[33]

Any intraocular neoplasm can induce uveitis. The most common primary intraocular neoplasm in the cat is feline diffuse iris melanoma. Feline diffuse iris melanoma can cause uveitis, but this seems to be relatively uncommon. Lymphosarcoma is the most common metastatic neoplasm to the eye in cats and dogs.[3] The most common ocular manifestation of metastatic lymphosarcoma in cats is panuveitis.[58] Metastatic adenocarcinomas (i.e., pulmonary, mammary, sweat gland) have also been reported to cause uveitis in cats.[59-61]

Periarteritis is an uncommon disease in cats characterized by fibrinoid necrosis of small and medium arteries. Eventually, these vessels become obstructed and the tissues supplied by these arteries become ischemic.[3] Associated ophthalmic signs include fibrinous exudate in the anterior chamber, lymphocytic-plasmacytic inflammation of the ciliary body with multifocal areas of necrosis, cyclitic membranes, and retinal detachment. The underlying cause of this disease is not well understood, but is suspected to be a hypersensitivity to collagen.[62]

Anatomic considerations for anterior uveitis

Blood Ocular Barriers

The blood ocular barriers are composed of the BAB anteriorly and the blood-retinal barrier posteriorly. The BAB is composed of tight junctions between the nonpigmented ciliary epithelial cells, posterior pigmented iridal epithelium, and endothelial cells of the iridal blood vessels. The blood-retinal barrier is composed of an outer layer at the level of the retinal pigmented epithelium and an inner barrier at the level of the retinal blood vessel endothelium.[17,63] Capillaries within the ciliary body are highly fenestrated, so the tight junctions of the ciliary epithelium prevent plasma proteins from entering the posterior chamber. The BAB is considered to be a barrier between the blood and the ocular tissues and fluids posterior to the iris, including the lens, vitreous, and retina.[64] The BAB is considered a less effective barrier than the blood-retinal barrier, as proteins can pass into the aqueous humor by pinocytosis and through openings from the iris root and anterior iris face.[17] Stability of the BAB can vary significantly by species. The relative stability of the BAB in vertebrate species has been described by Bito[25] as follows:

rabbits < guinea pigs < cats < owl monkeys < rhesus monkeys < ducks < chickens. Age may impact permeability of BAB, as one study found that iris capillaries in adult cats were less permeable to larger molecules than iris capillaries of kittens.[65] However, a similarly designed study using sodium fluorescein as a marker rather than fluorescein-labeled dextrans of various sizes found no significant difference between the adult and neonatal cats in regards to BAB permeability.[66] A recent study using anterior chamber fluorophotometry to evaluate the effect of age on BAB permeability in dogs found that dogs less than one year of age and greater than 11 years of age had increased BAB permeability compared to other age groups, and showed a positive correlation between aging and BAB permeability when dogs less than one year of ago were excluded from analysis.[67]

Experimental Induction of BAB Breakdown

There are various experimental techniques that have been shown to induce breakdown of the BAB. Nitrogen mustard applied topically or administered subconjunctivally in rabbits produces an initial rise in intraocular pressure, increased protein in the anterior chamber evidenced clinically as aqueous flare, and severe miosis.[68] Mechanical injury to the iris was also shown to produce a similar response.[69] Both topical application[11] and intracameral injection[8] of prostaglandins induce signs of anterior uveitis in rabbits. Injections of calcitonin gene-related peptide into the anterior chamber of rabbits and cats produced clinical signs consistent with BAB breakdown in both species, but rabbits were much more severely affected than cats at each dosing group.[70] This is consistent with previous findings that rabbits have a significantly more labile BAB than cats.[25] Intracameral injection of leukotriene C4 and leukotriene D4 in cats

produced equipotent dose dependent iris constriction, but there was no effect on aqueous humor protein concentration..[71]

Anterior chamber paracentesis (ACP) has been used in a variety of species to induce BAB breakdown.[72-74] Studies have shown that the main site of BAB disruption is ciliary processes.[75-78] A study in rhesus monkeys used scanning electron microscopy and evaluation of fluorescein within the anterior chamber to evaluate the site of BAB breakdown following ACP. The authors noted that within 3 to 4 minutes after intravenous fluorescein administration, there were multifocal areas of fluorescence of the ciliary processes, and when evaluated using scanning electron microscopy, the ciliary processes were swollen and a substance suspected to be fibrin was present within the posterior chamber and adhered to the ciliary processes.[78] These findings were consistent with a study performed in Cynomolgus monkeys, which also found the anterior pars plicata to be the primary site of BAB disruption following paracentesis.[75] Within 15 minutes of ACP, the ciliary processes in Cynomolgus monkeys exhibited disruption of the pigmented epithelium basement membrane, enlargement of intracellular space between pigmented and nonpigmented ciliary body epithelium, and fibrin aggregates between dilated capillaries and the pigmented ciliary body epithelium.[75] These findings contrast with a study that used horseradish peroxidase as a tracer to evaluate the permeability of the BAB in rhesus monkeys, where the authors did not find any difference in permeability in the ciliary epithelium or iridal vascular endothelium after paracentesis.[79] The duration of BAB breakdown likely varies depending on the severity of intraocular inflammation, duration of BAB breakdown, and the species being evaluated. In rabbits, PGE_2 and protein concentration in the aqueous humor increased significantly within 10 and 30 minutes, respectively, following ACP, and returned to baseline

levels 48 hours after ACP, indicating return of BAB stability.[80] A study in Cynomolgus monkeys used light and scanning electron microscopy to assess changes to the BAB following ACP, and found that 7 days post-ACP there were still morphologic abnormalities noted within the anterior pars plicata.[75] A study in dogs found that following ACP, there was reestablishment of the BAB (as assessed by anterior chamber fluorophotometry) by day 5 post-ACP.[81] In the previously mentioned study[81] the authors evaluated the use of different needle size (25 gauge, 27 gauge, and 30 gauge) on the degree of BAB breakdown and the effect on intraocular pressure, and found that the use of a 25 gauge needle resulted in a significant increase in anterior chamber fluorescence, as well as a significant increase in intraocular pressure, 20 minutes after ACP. No significant difference in anterior chamber fluorescence was noted between the use of a 27 gauge or 30-gauge needle.

Pilocarpine has also been used to initiate experimental uveitis in dogs, but its effects on the BAB are inhibited by application of topical proparacaine.[82] A study in cats comparing topical 2% pilocarpine application and ACP found that paracentesis resulted in more severe breakdown of the BAB than pilocarpine.[83] Intravitreal injection of lipopolysaccharide in cats produces significant hypotony and increases aqueous humor protein concentration for 45 days post-injection. This model may be more comparable with naturally occurring uveitis, and could be considered for future studies evaluating anti-inflammatory therapy.[84]

Quantification of BAB Breakdown

In a clinical setting, the severity of anterior segment inflammation is evaluated subjectively using slit-lamp biomicroscopy. Aqueous flare is graded from 1+ to 4+, based on the ability to clearly visualize iris detail. Cells present in the anterior chamber can be graded with a similar scale, with number of cells counted in a "wide beam with a narrow slit" corresponding to grade 1+ to 4+.[21,85] Measurement of aqueous humor protein concentration has been utilized as a means to subjectively quantify degree of BAB breakdown, but this method cannot be used serially because ACP to collect aqueous humor induces BAB breakdown.[86] Assays measuring prostaglandin concentration in the aqueous humor have also been evaluated in veterinary species as an objective measurement of uveitis. Aqueous humor levels of PGE_2 have been evaluated in dogs,[87,88] and prostacyclin (PGI_2) has been evaluated in horses.[72] However, as with aqueous humor protein concentrations, the process of obtaining aqueous humor for analysis of PG concentrations induces BAB breakdown, therefore only one measurement can be taken from each subject. Laser flare-cell photometry is another method to objectively quantify severity of aqueous flare and cell count. Light that is scattered by aqueous flare or cells in the anterior chamber is detected by photomultipliers in the machine. Flare is expressed in photon units/msec and cells are expressed as cells/mm^3.[85] Sedation or anesthesia may be necessary for laser flare-cell photometry to be performed in veterinary patients.[83,89] Fluorophotometry also quantitatively assesses breakdown of the BAB. In order to assess permeability of the BAB, fluorescein is administered parenterally (most commonly intravenously), and the fluorescence in the anterior chamber is quantified in ng/mL.[74,90] Anterior chamber fluorophotometry may utilize diffusion or permeability coefficients, peak concentration of anterior chamber fluorescence, comparison to fluorescence in the fellow eye, or comparison of fluorescence between sequential readings.[90,91] Risks of fluorophotometry include the need to administer intravenous fluorescein, which can

lead to allergic reactions,[92,93] and in veterinary patients heavy sedation may be necessary in order to properly position the subject in front of the fluorophotometer.

Treatment of Anterior Uveitis

Categories of Anti-inflammatory Medications

Treatment of anterior uveitis must be targeted at addressing the underlying disease process when identified. Concurrent anti-inflammatory therapy is also necessary, and is most often accomplished through the use of corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs). Goals of anti-inflammatory therapy are to reduce production of prostaglandins and other metabolites of the arachidonic acid pathway (Figure 1.2)

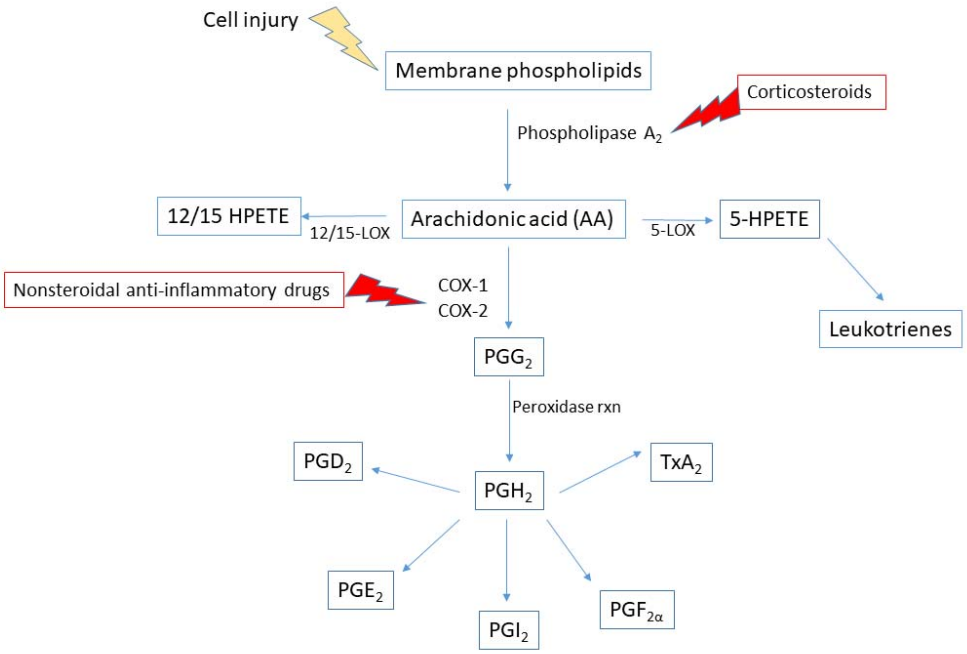

Figure 1.2. Diagram to represent the site of action of corticosteroids and NSAIDs. Corticosteroids block the action of phospholipase A_2 to inhibit release of arachidonic acid from membrane phospholipids. Nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase-1 and cyclooxygenase-2 to prevent conversion of arachidonic acid to PGG_2.

Corticosteroids decrease inflammation predominantly by inhibiting synthesis of arachidonic acid, thereby inhibiting formation of inflammatory metabolites such as prostaglandins and leukotrienes.[94] Corticosteroids may also decrease prostaglandin production by directly contributing to COX inhibition and blocking PGE isomerase to prevent conversion PGH_2 to PGE_2.[95] Additionally, corticosteroids help to maintain cell membrane integrity, decrease vasodilation and capillary permeability, and reduce the recruitment of leukocytes to the site of inflammation.[96] Nonsteroidal anti-inflammatory drugs inhibit COX, whose isoforms catalyze two sequential reactions following the release of arachidonic acid. As illustrated in Figure 1.2, NSAIDs prevent the conversion of arachidonic acid to PGG_2.[4] In contrast to corticosteroids, NSAIDs have no effect on the lipoxygenase pathway. The initial COX reaction converts arachidonic acid to prostaglandin G_2. The subsequent peroxidase reaction reduces prostaglandin G_2 to prostaglandin H_2, which is then converted by various cell specific isomerases and synthases to produce five biologically active prostaglandins that include prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, prostacyclin, and thromboxane A_2.[4] Prostaglandins are considered to be key mediators of ocular inflammation, and have been shown to induce breakdown of the BAB.[8,9,11] In general, the COX-1 isoenzyme is considered constitutive, and the COX-2 isoenzyme is classified at inducible. COX-2 is considered pathological since it is known to be induced by cytokines associated with inflammation, such as interleukin-1, tumor necrosis factor-α, and bacterial lipopolysaccharide.[5,97] Cycloxygenase-1 produces prostaglandins

important for many normal physiologic functions, such as gastrointestinal mucosal protection, renal blood flow, and normal platelet function.[98] However, this is an overly simplistic description of the physiologic behavior of these isoenzymes as COX-2 is constitutively expressed in the gastrointestinal tract[99] and kidneys[100] of dogs and maintains homeostatic functions. Additionally, a recent study[101] found that inhibition of either COX-1 or COX-2 could lead to duodenal ulceration in cats, suggesting that both isoforms play a role in protection of duodenal mucosa.

Routes of Anti-inflammatory Drug Administration

Treatment of uveitis can vary, and is based on the portion of the uveal tract involved, considerations for underlying etiology, and severity of inflammation. Potential routes of administration include topically, subconjunctivally, intraocularly (intracameral or intravitreal), and systemically. In general, topical anti-inflammatory drugs are used to treat anterior uveitis and systemic anti-inflammatory drugs are used to treat posterior uveitis. Potential etiology and species being treated must be taken into consideration when deciding whether to administer a systemic corticosteroid or NSAID. When an infectious etiology is suspected or confirmed, a systemically administered NSAID is preferred over a systemically administered corticosteroid.[102] However, due to cats' reduced ability for hepatic glucuronidation,[103,104] which is the major mechanism for metabolism and excretion of NSAIDs, there are concerns for an increased risk of toxicity in this species.

Topical Anti-inflammatory Drugs in Cats

There have been several studies evaluating topical anti-inflammatory drugs in dogs,[82,105-108] but only one study evaluating effects of topical anti-inflammatory drugs in cats.[109] In this study, topical prednisolone 1%, flurbiprofen 0.03%, dexamethasone 0.1%, or diclofenac 0.1% was administered immediately after ACP was performed to induce breakdown of the BAB, and at 6, 10, and 24 hours following paracentesis. Aqueous humor protein concentration was estimated by using a laser flare-cell meter. Topical prednisolone significantly decreased aqueous flare compared to the control eye at 4, 8, and 26 hours following ACP, and topical diclofenac significantly decreased flare in the treated eye compared to the control eye at 8 and 26 hours following ACP. Flurbiprofen and dexamethasone applied topically did not significantly reduce aqueous flare. It is also noted in this study that topical diclofenac and flurbiprofen caused a significant increase in intraocular pressure. Therefore, it is recommended that topical NSAIDs be used cautiously or avoided in cases of feline uveitis and concurrent ocular hypertension or glaucoma.

Systemic effects and absorption of topical NSAIDs have been evaluated in cats. In one study, diclofenac 0.1% ophthalmic solution was administered topically four times per day for one week. At the beginning and end of this treatment period, plasma concentrations of diclofenac were measured, along with complete blood count, serum biochemistry, urinalysis, urine protein-to-creatinine ratio, and glomerular filtration rate. The investigators found that glomerular filtration rate was significantly lower in the treatment group, but only in the second phase of the crossover study. The authors hypothesize that this may be secondary to iatrogenic hypovolemia from repeated blood samplings rather than a true effect of the drug.[110] A similar study evaluated both diclofenac 0.1% ophthalmic solution and flurbiprofen 0.03% ophthalmic solution applied

topically in healthy cats to determine systemic absorption and effects on complete blood count, serum biochemistry, and urinalysis. Each drug was administered 4 times per day for 14 days, and plasma concentrations of diclofenac and flurbiprofen were evaluated before, during at multiple time points, and after discontinuation of the drugs. Neither diclofenac nor flurbiprofen caused any significant alterations in serum blood urea nitrogen, creatinine, or urine specific gravity. Flurbiprofen achieved higher plasma concentrations than diclofenac, and flurbiprofen was detected in plasma up to 48 hours after discontinuing treatment, whereas diclofenac was detected for 24 hours following the final treatment.[111]

Systemic Anti-inflammatory Drugs in Cats

It has long been recognized that NSAIDs should be used with caution in cats due to the species' reduced capacity for hepatic glucuronidation[103,104], which is the primary mechanism of metabolism of many NSAIDs. A diminished ability to metabolize NSAIDs could lead to prolonged drug action or increased risk of adverse effects, explaining why there are limited options for NSAIDs in cats. At this time, commercial availability of approved NSAIDs in cats in the United States is limited to two options: 1) meloxicam as a single subcutaneous injection, and 2) robenacoxib (Onsior®), a new COX-2 selective NSAID.

Robenacoxib is approved for once daily oral dosing in cats for three consecutive days. The recommended minimum dosage for robenacoxib in cats is 1 mg/kg once daily, and safety studies have shown no adverse effects with doses up to 10 mg/kg twice daily for 42 days.[112] The safety index for robenacoxib is thought to be due in part to its high selectivity for COX-2 inhibition, where it is has been shown that at a dose of 2 mg/kg, mean robenacoxib blood concentrations

corresponded with a 5% inhibition of COX-1 and a 90% inhibition of COX-2.[113] Clinically, robenacoxib has proven effective as a peri-operative analgesic, with similar analgesic properties when compared to meloxicam and buprenorphine.[114,115]

A study by Rankin et al[73] evaluated the inhibitory effects of oral administration of acetylsalicyclic acid, meloxicam, prednisone, and prednisolone on breakdown of the BAB in normal cats. Experimental uveitis, or breakdown of the BAB, was induced by performing ACP. Oral anti-inflammatory drugs were administered two days before paracentesis, the day of paracentesis, and two days after paracentesis. Breakdown of the BAB was quantified using anterior chamber fluorophotometry. Cats that received oral prednisolone had significantly lower anterior chamber fluorescein concentration than control cats at 24 and 48 hours post-ACP. Cats that received meloxicam had significantly lower anterior chamber fluorescein concentration than control cats at 48 hours post-ACP. Orally administered prednisone and acetylsalicylic did not have any significant inhibitory effect on BAB breakdown.

Chapter 2 – Effect of Oral Administration of Robenacoxib on Inhibition of Paracentesis-induced Breakdown of the Blood-aqueous Barrier in Normal Cats

Introduction

Uveitis is a common and important disease in feline ophthalmology, and causes can be categorized as exogenous and endogenous. Exogenous causes include trauma, corneal ulceration, or lens luxation, and are usually identified during a routine ophthalmic examination. Endogenous causes of uveitis include immune-mediated, infectious, neoplastic, or idiopathic diseases.[2] A recent study evaluating endogenous causes of anterior uveitis in 120 cats found no underlying etiology in 40.8% of cases.[37] Treatment for uveitis includes topical and systemic anti-inflammatory medications, as well as antimicrobial agents when an infectious etiology is identified. The goals of treatment are to reduce ocular discomfort, decrease inflammatory mediators, and prevent sequelae associated with intraocular inflammation. Complications of uveitis include glaucoma, cataract formation, synechiae, phthisis bulbi, and blindness.[3,73]

Anterior uveitis can be experimentally induced by disrupting the BAB using several previously described methods, including ACP.[74,81,116] Methods used to quantify degree of breakdown of the BAB include laser flaremetry,[83,117] measurement of PGE_2,[87,88,118] and anterior chamber fluorophotometry.[73,74,81] Topical anti-inflammatory drugs have been evaluated in the treatment of experimentally induced uveitis in cats, and both 1% prednisolone acetate and 0.1% diclofenac sodium significantly reduced ACP-induced uveitis.[109] A separate study evaluating orally

administered anti-inflammatory drugs in cats found that prednisolone and meloxicam significantly reduced ACP-induced uveitis.[73]

The purpose of this study was to evaluate whether oral administration of a new COX-2 selective NSAID, robenacoxib, lessens the degree of ACP-induced uveitis in normal cats, as assessed by anterior chamber fluorophotometry. A secondary objective was to determine if detectable levels of robenacoxib are present in the aqueous humor of cats with an intact BAB. We hypothesized that cats receiving oral robenacoxib would have less ACP-induced uveitis compared to cats in the control group and that detectable levels of robenacoxib would be present in the aqueous humor of treated cats.

Materials and Methods

Twelve domestic shorthair cats that were part of an established research colony at Kansas State University were included in the study. Each cat received a complete ophthalmic examination and physical examination prior to inclusion. The ophthalmic examination was performed by a board certified veterinary ophthalmologist (JMM), and included slit lamp biomicroscopy,[a] rebound tonometry,[b] and indirect ophthalmoscopy.[c] Cats were excluded if there was evidence of previous or active intraocular inflammation, or any signs of systemic disease on physical examination. The study was approved by the Institutional Animal Care and Use Committee at Kansas State University.

The cats were housed in a temperature controlled environment and exposed to 12 hours of light and 12 hours of darkness. Cats were fed their full ration once daily in the evening. The cats were

given a two-day acclimation period. On day 3, each cat was pre-medicated with maropitant citrate[d] (1 mg/kg SC) to reduce likelihood of emesis following sedation. Cats were then sedated with IM dexmedetomidine[e] (0.015 mg/kg) and ketamine hydrochloride[f] (5 mg/kg). While sedated, jugular venipuncture and cystocentesis were performed to obtain samples for complete blood count, serum biochemistry profile, and urinalysis. Intravenous catheters were placed in the cephalic or medial saphenous vein. Sedation was reversed with IM atipamezole[g] (0.06 mg/kg).

Cats were randomly assigned to a treatment or control group. The treatment group received a 6 mg robenacoxib tablet[h] within a Pill Pocket®[i] by mouth once every 24 hours for 3 days. The control group received an empty Pill Pocket® at the same dosing interval. The investigators performing fluorophotometry (JMM and EKS) were masked to the group assignment of each cat. The cats received robenacoxib or an empty Pill Pocket the day before, the day of, and the day after ACP (Figure 2.1).

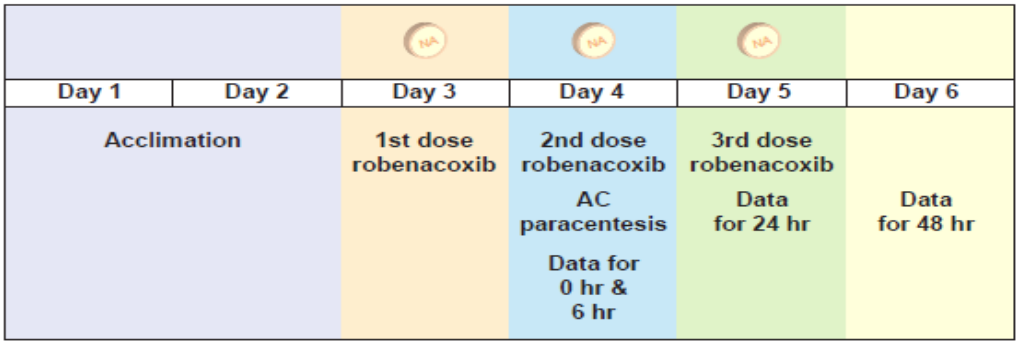

Figure 2.1. Study timeline. Administration of oral robenacoxib in relation to ACP and fluorophotometry scans. The first dose of robenacoxib was administered on day 3, prior to ACP. The second and third doses were administered on days 4 and 5, respectively. Fluorophotometry scans were performed at times 0 (immediately prior to ACP) and 6 hours on day 4, at time 24 hours on day 5, and at time 48 hours on day 6 of the study.

Anterior chamber paracentesis was performed in 1 randomly selected eye of each cat, as described in similar studies,[74,81] to induce breakdown of the BAB. Briefly, each cat was pre-medicated with maropitant citrate (1 mg/kg IV) to reduce likelihood of emesis. Each cat was then sedated with IV dexmedetomidine (0.0075 mg/kg) and ketamine hydrochloride (5 mg/kg). One drop of proparacaine[j] was applied to the eye selected to undergo ACP. A 30-gauge needle was attached to a 1 mL syringe. The needle was inserted 1 mm anterior to the limbus, and advanced into the center of the anterior chamber. Aqueous humor (100 µL) was slowly aspirated over 3-5 seconds. The cats were monitored after ACP for any signs of discomfort (epiphora, blepharospasm), conjunctival hyperemia, or severe intraocular inflammation. The aqueous humor was stored at -80°C until liquid chromatography and mass spectrometry were performed.

Sodium fluorescein solution[k] (20 mg/kg IV) was administered to each cat one hour prior to each fluorophotometry reading. One hour was deemed to be sufficient time for fluorescein concentrations in the anterior chamber to plateau based on a previous study that showed that fluorescein is stable in the anterior chamber from 30 minutes to 90 minutes after intravenous administration.[74] Cats were sedated immediately prior to each fluorophotometry reading using the described protocol of IV dexmedetomidine (0.0075 mg/kg) and ketamine hydrochloride (5 mg/kg). Anterior chamber fluorophotometry was performed using a computerized scanning fluorophotometer with an anterior chamber adapter.[l] Fluorophotometry was performed in both eyes of each cat with less than 2 minutes elapsing between each eye. Scans were performed immediately prior to ACP (time 0), and at 6, 24, and 48 hours after paracentesis. Disruption of the BAB was quantified by measurement of fluorescein concentration in the central portion of the anterior chamber.

Aqueous humor from recently euthanized cats with no prior history of robenacoxib administration was obtained for calibration standards and controls. Aqueous humor samples were stored at -80° C until analyzed for robenacoxib concentrations using liquid chromatography and mass spectrometry.[m] The mass to charge ratio (*m/z*) for the qualifying ion to quantifying ions for robenacoxib was 328.44→247.02, 262.06, 282.14 and for the internal standard celecoxib were 382.15→303.26, 362.13. The lower limit of quantification was 0.5 ng/mL with standard curves linear from 0.5-500 ng/mL using celecoxib as the internal standard. The accuracy of the assay on replicates of 2 at 0.5, 10, 100 ng/mL were 119%, 102% and 99%, respectively. Aqueous humor was directly injected. Ketamine (*m/z* 238.06→124.95, 179.0), medetomidine (*m/z* 201.12→68.02, 94.99) and maropitant (*m/z* 469.38→147.14, 177.15) were also monitored, but not quantified in the aqueous humor.

Data analysis

Statistical analysis was performed using a commercially available software program.[n] Fluorescein concentration in the anterior chamber was compared between eyes that underwent ACP and eyes that did not undergo ACP at each time period by two-tailed independent t-test. A repeated measures ANOVA was used to compare values in the eye that underwent ACP over time and in the opposite eye over time. Percent fluorescein increase was compared between the eye that underwent ACP and the opposite eye at each time period by two-tailed independent t-test. Disruption of the BAB was expressed as the percentage increase in fluorescein concentration between the eye that had paracentesis of the anterior chamber and the eye that did

not have paracentesis of the anterior chamber performed. The following equation was used based on a previous similarly designed study[73]:

$$\% \text{ fluorescein increase} = ([F]_{\text{paracentesis eye}} - [F]_{\text{control eye}})/([F]_{\text{control eye}}) \times 100$$

P values <0.05 were considered significant.

Results

The twelve cats included one spayed female, 4 intact females, and 7 castrated males. The weight of the cats ranged from 2.9 – 6.23 kg (mean ± SD, 4.18 ± 0.98). The age of the cats ranged from 1.3 – 3.6 years (mean ± SD, 1.7 ± 0.71). The mean dose of robenacoxib in the treatment group was 1.51 ± 0.36 mg/kg. Complete blood count, serum biochemistry profile, and urinalysis showed no clinically relevant abnormalities. One of the cats in the control group experienced fibrin in the anterior chamber noted at 6 hours following ACP, which resolved by 24 hours post-ACP. One of the cats in the control group experienced an adverse reaction after administration of intravenous sodium fluorescein at the 6-hour time point. Within 30 seconds after completing fluorescein administration, the cat began to hypersalivate, and experienced a syncopal episode. The cat recovered over the course of approximately 5 minutes, but was weak and ataxic during that period. Physical examination, including Doppler evaluation of systolic blood pressure, were normal when examined during recovery from the episode. Fluorophotometry readings were obtained for the 6-hour time point for this cat, and the cat was excluded from the remainder of the study.

There was no significant difference in the fluorescein concentration between the ACP and the non-ACP eyes at time 0 (P=0.322), which represents the baseline fluorescein concentration prior

to ACP. There was a significant difference in the percent fluorescein increase in the ACP eye compared to the non-ACP eye (P=0.002) from time 0 (baseline) to 6 hours after ACP was performed, indicating that experimental breakdown of the BAB was successfully achieved. Mean ± SD of fluorescein concentrations for the ACP eye and non-ACP eyes for the treatment and control groups are summarized in Table 1. When comparing the percent fluorescein increase between the treatment and control groups, the 6-hour data trended toward significance (P=0.077), but there was no statistically significant difference at any time point (Table 2).

Detectable concentrations of robenacoxib were identified in 5 of 6 cats in the treatment group. Robenacoxib was not detected in any cats in the control group. The lower limit of quantification was 0.5 ng/mL. Concentrations of robenacoxib in the aqueous humor ranged from 0.9 – 16 ng/mL (mean 5.32 ng/mL). Ketamine and maropitant were also detected in the aqueous humor, but not dexmedetomidine.

Discussion

This study identified no significant difference in the degree of experimentally induced uveitis in cats receiving a single pre-treatment dose of oral robenacoxib followed by two subsequent daily doses, when compared to a control group of cats. We also determined that low concentrations of robenacoxib were detectable in the aqueous humor of cats in the treatment group, suggesting that robenacoxib crosses an intact BAB.

There is some evidence that NSAIDs with less COX-2 selectivity may be more effective at decreasing intraocular inflammation. A study evaluating injectable NSAIDs showed that when

compared to carprofen, meloxicam, and saline, dogs receiving flunixin meglumine, a nonselective COX inhibitor, had significantly lower prostaglandin E_2 concentrations in the aqueous humor.[88] Another study evaluating oral NSAIDs found that dogs receiving oral tepoxalin, a preferential COX-1 and lipooxygenase inhibitor, had a significantly lower concentration of prostaglandin E_2 in the aqueous humor compared to dogs receiving carprofen, meloxicam, or no medication.[87] However, another report found that dogs administered oral carprofen had reduced PGE_2 present in the aqueous humor compared to dogs that did not receive carprofen.[118]

There are two studies that evaluate the effect of anti-inflammatory medications on ACP-induced breakdown of the BAB in normal cats. The first study evaluated the effect of topically applied diclofenac, prednisolone acetate, flurbiprofen, and dexamethasone in normal cats with experimentally induced uveitis, and using laser flaremetry to quantify breakdown of BAB, found that topically applied diclofenac and prednisolone acetate significantly reduced ACP-induced uveitis.[109] Rankin et al. evaluated the effect of oral anti-inflammatory medications on breakdown of the BAB in cats, determining that prednisolone at 24 and 48 hours after ACP and meloxicam at 48 hours after ACP, reduced the severity of BAB breakdown as measured by anterior chamber fluorophotometry.[73] It is interesting to note that cats receiving meloxicam had a reduction in breakdown of the BAB, while in our study there was no difference between cats receiving oral robenacoxib and the control group. When comparing the COX selectivity of meloxicam and robenacoxib, a study evaluating *in vitro* COX inhibitor selectivity found that when meloxicam achieves 80% or greater inhibition of COX-2, there is a corresponding inhibition of COX-1 of at least 40%.[119] In contrast, when robenacoxib achieves 90% inhibition of

COX-2, there is a corresponding 5% inhibition of COX-1.[113] While highly selective COX-2 inhibitors may have an improved gastrointestinal safety profile in healthy animals when compared to less selective or non-selective COX inhibitors, whether COX-2 selective inhibitors are less effective at reducing intraocular inflammation warrants further investigation. Another consideration for the difference in anti-inflammatory effects in these similar studies is that in the study from Rankin et al., cats were pre-treated with meloxicam two days before ACP, the day of, and two days after ACP. In our study, cats received robenacoxib one day before ACP, the day of, and one day after ACP. It is possible that additional pre-treatment may have provided more stabilization of the BAB. One study in dogs with experimentally induced uveitis found that pre-treatment with carprofen resulted in a 68% inhibition of flare as measured by laser flaremetry.[89] It would be of interest to evaluate whether administering robenacoxib for additional days prior to ACP would have any impact on BAB stabilization.

A recent study evaluated COX-2 expression in feline globes with and without uveitis.[120] This study found no COX-2 expression in the normal feline globe, while uveal COX-2 immunostaining was found in less than half of the globes with uveitis.[120] Additional studies are necessary to evaluate constitutive expression of COX-1 within the feline globe, and to evaluate how duration and severity of uveitis impacts COX-2 expression.

Detectable concentrations of robenacoxib were present in the aqueous humor of 5 of 6 cats receiving oral robenacoxib, indicating that low concentrations of robenacoxib are able to cross an intact BAB. In our study, the time between the second daily dose of robenacoxib and ACP ranged from 1.5 to 6 hours after oral administration of robenacoxib. The mean robenacoxib

concentration in the aqueous humor was 5.32 ng/mL. In comparison, the reported maximum concentration (C_{max}) of robenacoxib in feline plasma after oral administration ranges from 692 ng/mL (after food) to 1159 ng/mL (fasted).[121] The median time of maximum concentration (T_{max}) in the same study was 30 minutes when food was withheld, and the median T_{max} was 1 hour when cats were fed an entire ration. After oral administration, the terminal half-life of robenacoxib is approximately 2 hours.[121] A study using a tissue cage model of acute inflammation found a mean residence time of 3.3 hours in blood after oral administration of robenacoxib, but a mean residence time in exudate of approximately 24 hours.[122] That study also found that inhibition of COX-2 persisted in exudates for 24 hours, which supports the rationale for once daily dosing.

There are limited studies that report aqueous humor concentrations of NSAIDs after systemic administration. One study found that in cats administered carprofen intravenously (4.4 mg/kg), a serum concentration 40 minutes after administration of 25.8 µg/mL corresponded to cats without intraocular inflammation, and a serum concentration of 24.3 µg/mL corresponded to cats with severe uveitis. The mean aqueous humor concentration of carprofen ranged from a median of 0.07 µg/mL in cats without intraocular inflammation to a median of 2.52 µg/mL in cats with severe uveitis, which suggests increased BAB permeability and drug penetration into the eye.[123] A separate study in horses found that mean serum concentrations of orally administered flunixin meglumine were 20.4 ng/mL on day 3 and 57.5 ng/mL on day 5, which corresponded with aqueous humor concentrations of 0.4 ng/mL and 0.8 ng/mL, respectively. This study also evaluated orally administered firocoxib, and measured serum concentrations of 35.2 ng/mL on day 3 and 55.2 ng/mL on day 5, which corresponded with aqueous humor concentrations of 4.7

ng/mL and 6.4 ng/mL, respectively.[124] The aqueous humor values of flunixin meglumine and firocoxib are comparable to values of robenacoxib obtained in our study. However, the aqueous humor concentrations from cats administered carprofen[123] are much higher than aqueous humor robenacoxib concentrations in the present study. Further investigation into the ocular distribution of parenterally and orally administered NSAIDs in domestic species is warranted.

Study limitations

Limitations of this study include the small number of cats and the variability in anterior chamber fluorescein concentration between cats. The number of animals was chosen based on previous similarly designed studies[4] and availability of cats. It is possible that a significant difference between the treatment and control groups may have been identified with a larger number of cats. The variability in fluorescein concentrations before and after ACP may be due to individual variation in the stability of the BAB between cats. Another limitation of this study is the lack of plasma concentrations to correspond with aqueous humor concentrations of robenacoxib. Future studies should evaluate whether increasing plasma concentrations correspond with increased concentrations of robenacoxib in the aqueous humor, and the effect of pre-existing uveitis on these levels. Although not evaluated in this study, it is possible that there may be improved efficacy of robenacoxib in cats with naturally occurring uveitis due to increased permeability of the BAB when compared to the ACP model. Future studies are needed to investigate the effects of robenacoxib in cats with naturally occurring uveitis, the inducible COX expression in the feline uveal tract, and to determine whether robenacoxib may have detectable ocular anti-inflammatory effects if the duration or severity of inflammation were increased. Additional studies could also evaluate whether timing of administration (only before or only after ACP) or a

different route of administration (IV, SC) would impact the ocular anti-inflammatory effects of robenacoxib.

Conclusions

In summary, administration of oral robenacoxib did not significantly decrease ACP-induced breakdown of the BAB in normal cats, as assessed by fluorophotometry. Low concentrations of aqueous humor robenacoxib were detectable in the majority of cats receiving the drug. Future studies are suggested to evaluate the effects of robenacoxib on intraocular inflammation in cats with naturally occurring uveitis, varying degrees and duration of uveitis, and to further evaluate ocular distribution and serum concentration of orally administered robenacoxib.

Chapter 3 – Ocular Cyclooxygenase Expression

Ocular Cyclooxygenase Expression in Normal and Diseased Globes

Introduction

Overall, there is relatively limited information regarding cyclooxygenase (COX) expression in feline eyes. In other species, the expression of COX has been evaluated in various ocular disease states, and might provide additional insight into pathophysiology, prognosis, and targeted treatment options.

Humans

In normal human globes, COX-1 and COX-2 were constitutively expressed most prominently in the nonpigmented ciliary body epithelial cells. In cases of primary open angle glaucoma, COX-1 expression was unchanged, and COX-2 expression was significantly reduced in the nonpigmented ciliary body epithelium. The authors discussed that this is in contrast to other types of glaucoma in humans, such as primary closed angle glaucoma or juvenile glaucoma, where the COX-2 expression was unchanged. The authors discussed that since prostaglandins are known to play a role in regulation of aqueous humor outflow, this reduction in COX-2 expression may reduce prostaglandin production, and ultimately contribute to outflow resistance seen in primary open angle glaucoma.[125] When COX-2 was evaluated in the aqueous humor of Mexican patients with glaucoma, there was no significant difference between COX-2 levels in normal patients and patients with glaucoma. The authors suggested that low levels of COX-2 may be necessary for normal ocular functions.[126]

A study evaluating the expression of COX-2 and uveal melanoma found that 90.6% of melanomas expressed COX-2, and that there was a statistically significant correlation between COX-2 expression, both in intensity and in overall score, and risk of metastatic death.[127]

Rabbits

In contrast to normal human eyes, in rabbits, constitutive COX-1 and COX-2 were expressed predominantly in the iris and ciliary body stroma, and were not expressed in the ciliary body epithelium. The authors speculated that this variation in expression could contribute to the differences in blood aqueous barrier stability between rabbits and humans.[128] A study evaluating a COX-1 selective inhibitor (FR122047) and a COX-2 selective inhibitor (FR188582) in rabbits with experimentally induced uveitis found that both drugs reduced BAB breakdown.[129] Expression of COX-1 and COX-2 has also been evaluated during corneal wound healing in New Zealand albino rabbits. COX-1 expression remained unchanged within the corneal epithelium, stroma, and endothelium following corneal wounding, while COX-2 expression increased in the corneal epithelial cells and reached maximum mRNA expression 2 hours after wounding, followed by declining expression at 6 hours after wounding. The authors suggested that COX-2 may play an important role in facilitating the migration of corneal epithelial cells.[130]

Canine

In a study evaluating COX-2 expression in canine glaucomatous eyes, the authors found that in normal eyes, the only area where COX-2 was expressed was the ciliary body epithelium, and only minimal immunoreactivity was noted. In glaucomatous eyes, immunoreactivity for COX-2 was identified in all examined ocular tissues, including the trabecular meshwork, angular

aqueous plexus, and ciliary body epithelial cells. Strongest staining scores, however, were noted in the corneal epithelium, stroma, and endothelium.[131]

When evaluating COX-2 immunohistochemical staining in pre-iridal fibrovascular membranes (PIFM) in dogs, retrocorneal membrane spindle cells, corneal epithelium, keratocytes, corneal endothelium, nonpigmented ciliary body epithelium, spindle-shaped cells lining trabecular meshwork, and the majority of the vascular endothelium stained positive. Additionally, the anterior-most PIFM spindle cells were often prominently positive. Normal canine globes were also evaluated, and COX-2 immunoreactivity was detected in conjunctival goblet cell epithelium, corneal epithelium, corneal endothelium, vascular endothelium of few larger uveal and retinal vessels, and nonpigmented ciliary body epithelium.[132]

In a study evaluating the expression of COX-2 in canine uveal melanocytic neoplasms, globes with neoplasia appeared to express COX-2 in similar sites and with similar intensity as globes without neoplasia. The authors were only able to definitively identify intraneoplastic expression of COX-2 in 3.5% of the melanocytic neoplasms evaluated. However, the authors proposed that this may be an underestimate of the expression of COX-2 in these tumors because ocular tissues that frequently abutted or invaded by the uveal neoplasm (such as the iris, ciliary body, aqueous outflow tract, and anterior sclera) expressed COX-2, but only expression of COX-2 specifically identified in a neoplastic cell was included in analysis.[133]

Feline

A recent study evaluated COX-2 expression in feline globes with and without naturally occurring uveitis.[120] This study found no COX-2 expression in normal feline globes, while uveal COX-2 immunostaining was found in less than half of the globes with uveitis. Of the 16 globes in which COX-2 expression was detected, 11 eyes were diagnosed with lymphoplasmacytic uveitis, four were diagnosed with neutrophilic uveitis, and one with diffuse iris melanoma-induced uveitis. The severity of uveal inflammation was positively correlated with COX-2 expression in the uvea and the corneal endothelium.[120] Additional studies are necessary to evaluate constitutive expression of COX-1 within the feline globe, and to evaluate how duration of uveitis impacts COX-2 expression.

Equine

The research investigating the role of COX in equine ophthalmology is primarily focused on the relationship between ocular or adnexal squamous cell carcinoma and the expression of COX-2. The first study evaluated the expression of COX-1 and COX-2 in squamous cell carcinoma affected tissues from equine cornea, eyelid, and third eyelid, as well as unaffected site-matched tissue from control horses. Cyclooxygenase-1 and COX-2 expression was significantly higher in corneal squamous cell carcinoma than control cornea. The expression of COX-1 and COX-2 was also increased in eyelid squamous cell carcinoma, but this difference was not statistically significant.[134] Similarly, a study that evaluated COX-2 in cases of equine ocular and periocular squamous cell carcinoma found that only cases of corneal squamous cell carcinoma exhibited increased COX-2 expression.[135] The authors also identified that COX-2 immunoreactivity was significantly associated with mitotic index.[135] Smith, et al.[136] found that of squamous cell

carcinoma samples that exhibited positive COX-2 immunoreactivity, 65% were limbal, 30% were from the nictitating membrane, and 5% were from the eyelid. An interesting finding from this study was that while ocular or adnexal squamous cell carcinoma or carcinoma *in situ* had significantly higher expression of COX-2 than control tissue, the percentage of cells that expressed immunoreactivity for COX-2 was very low. Of the samples that were COX-2 positive, 90% showed immunoreactivity in only 1% of neoplastic cells.[136]

Conclusions

A recent study demonstrated increased expression of COX-2 in enucleated feline globes with evidence of uveitis. This finding suggests that COX-2 expression is inducible in the feline eye, since there was no COX-2 expression in normal feline globes. Therefore, there may be benefit in selective COX-2 inhibition in cases of feline uveitis. However, more research is needed to further elucidate the expression of COX-1 and COX-2 in normal and diseased feline eyes, since there is evidence in other species that the expression of the isoenzymes within the eye is more complex than simply COX-1 as constitutive and COX-2 as inducible. Further research investigating specific products of the COX pathway (prostaglandins) may also be useful in developing and evaluating targeted therapeutic options for anterior uveitis in cats.

Footnotes

a. SL-14, Kowa Co Ltd, Tokyo, Japan

b. TonoVet, Tiolat Ltd, Helsinki, Finland

c. Keeler binocular indirect ophthalmoscope; Keeler Instruments Inc., Broomall, PA

d. Cerenia® Zoetis Inc. Kalamazoo, MI

e. Dexdomitor® Zoetis Inc. Kalamazoo, MI

f. Zetamine® VetOne, Boise, ID

g. Antisedan® Zoetis Inc. Kalamazoo, MI

h. Onsior®, Elanco US Inc, Greenfield, IN

i. Greenies Pill Pockets®, The Nutro Company, Franklin, TN

j. Proparacaine hydrochloride 0.5% ophthalmic solution, USP, Akorn, Lake Forest, IL

k. AK-Fluor 25%, Akorn Inc, Lake Forest, IL, USA

l. FM-2 Fluorotron Master, OcuMetrics Inc, Mountain View, CA

m. Acquity Prominence UPLC and Acquity TQD, Waters Corp. Milford, MA USA

n. WINKS SDA Basic 6.0, Texasoft, Cedar Hill, TX, USA

Table legend

Table 1. Mean ± SD fluorescein concentration in ACP and non-ACP eyes at 0, 6, 24, and 48 hours after ACP. Fluorescein concentration (mean ± SD) measured in non-ACP eyes in the robenacoxib group was compared with that of the control group, and no statistically significant difference was detected. Similarly, fluorescein concentration (mean ± SD) measured in ACP eyes in the robenacoxib group was compared with that of the control group, and no statistically significant difference was detected. Data for one cat in the control group was not available for 24 and 48 hours.

Table 2. Mean ± SD percent fluorescein increase in control and robenacoxib groups at 0, 6, 24, and 48 hours after ACP. Data for one cat in the control group was not available for 24 and 48 hours.

Table 1.

	Non-ACP eye (fluorescein concentration ng/mL) **(mean ± SD)**	P-value	ACP eye (fluorescein concentration ng/mL) **(mean ± SD)**	P-value
Time				
0 hours Robenacoxib Control	 1478.9 ± 465.5 1082.9 ± 521.5	0.195	 1499.5 ± 343.2 1088.8 ± 510.9	0.133
6 hours Robenacoxib Control	 1899.8 ± 1078.8 1168.7 ± 418	0.169	 2324.2 ± 750.9 2300.3 ± 940.6	0.962
24 hours Robenacoxib Control	 1354.8 ± 750.6 755.2 ± 329.1	0.134	 2076.3 ± 1069.3 1201.8 ± 503.8	0.129
48 hours Robenacoxib Control	 1284.5 ± 581.9 1021.8 ± 368.9	0.407	 1651.7 ± 684.8 1545.2 ± 560.1	0.787

Table 2.

	% [F] increase (mean ± SD)		
Time	Control	Robenacoxib	P-value
0 hour	0.645 ± 4.18	4.53 ± 21.12	0.676
6 hours	96.34 ± 40.04	41.08 ± 55.79	0.077
24 hours	63.47 ± 30.89	56.84 ± 53.28	0.812
48 hours	54.24 ± 20.55	31.21 ± 26.55	0.149

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