AQUEOUS HUMOR FLOW RATE IN NORMAL CATS AND THE EFFECT OF TOPICAL 2% DORZOLAMIDE ON AQUEOUS HUMOR FLOW AND INTRAOCULAR PRESSURE

by

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Abstract

Objective - To establish the aqueous humor flow rate in normal cats via fluorophotometry, utilizing a noninvasive method previously established in other species and to evaluate the effect of topical 2% dorzolamide on aqueous humor flow rate and intraocular pressure (IOP) in normal cats.

Animals - 20 clinically normal domestic shorthair cats.

Procedures – Topical administration of 10% sodium fluorescein was performed using a 3-drop protocol to establish its use in this species. Aqueous flow was measured using fluorophotometry in the right and left eyes. The subjects were then divided into 2 groups: the first received topical 2% dorzolamide (Trusopt®) and the second received topical artificial tear solution (control). The study was divided into two phases: a 3 day acclimation phase (no treatments given) and a 5 day treatment phase (treatments given three times daily). IOP measurements were taken at 7am, 10am, 1pm, 5pm, and 9pm throughout all phases of the study. Fluorophotometry was performed to measure the aqueous flow rate just prior to and at the end of the treatment phase (days 3 and 9 respectively).

Results - The calculated aqueous humor flow rate for normal cats in the right, left, and both eyes was 5.94 ± 2.30 µl/min, 5.05 ± 2.06 µl/min, and 5.51 ± 2.21 µl/min, respectively. No significant differences were noted between the right and left eyes. In the dorzolamide group, the average flow rate during treatment (3.47 $\pm$ 1.50 µl/min) was significantly lower than prior to treatment (5.9 $\pm$ 2.20 µl/min)($P < 0.001$). The mean IOP during treatment (11 $\pm$ 3 mmHg) was significantly lower than the mean IOP prior to treatment (15 $\pm$ 3 mmHg)($P < 0.001$). In the control group, there were no significant differences in aqueous humor flow or IOP values before or during treatment.

Conclusions - The technique utilized for this study met the standard for accurate fluorophotometric calculation of aqueous humor flow. The average aqueous humor flow rate for normal cats calculated in this study was 5.51 ± 2.21 µl/min. Topical 2% dorzolamide significantly lowers aqueous humor flow (a 41% reduction) and IOP (a 26% reduction) in normal cats.

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Chapter 1 - Literature Review

Aqueous Humor Dynamics

Aqueous Humor

The aqueous humor is a transparent fluid produced within the eye that serves several important roles vital in maintaining proper ocular function. First and foremost it is a pellucid medium filling the anterior and posterior chambers and has a refractive index of 1.33332, allowing for a clear pathway for light entering the eye.[1] It is a relatively acellular, low protein fluid, composed of organic and inorganic compounds necessary to nourish avascular structures within the eye, such as the cornea and lens, for which it also serves as a conduit for waste removal.[2,3] The specific levels of these compounds are species specific, and rely on a stout barrier between the aqueous humor and the blood to prevent equilibration with the systemic circulation.[4] This blood-aqueous barrier is created by tight junctions in the ciliary body epithelium, the iridal vascular endothelium, and the posterior pigmented epithelium of the iris.[5] The aqueous humor also preserves the appropriate alignment of ocular structures necessary for vision by maintaining the intraocular pressure (IOP) within the eye.[1]

Aqueous Humor Production

Aqueous humor is produced by the nonpigmented ciliary body epithelium on the interior surface of the ciliary processes located in the pars plicata region of the ciliary body.[6] Three mechanisms combine to form the aqueous humor found in the eye; they are: diffusion, ultrafiltration, and active secretion. *Diffusion* occurs when solutes move down a concentration gradient from a region of high concentration to low concentration. Carbohydrates and lipid-soluble compounds enter the aqueous humor via this process.[4] *Ultrafiltration* allows for the movement of plasma from an area of high hydrostatic pressure to an area of lower pressure. It is by this method that blood plasma is deposited into the ciliary process stroma from the ciliary capillaries, passing thorough fenestrations in the capillary endothelium.[2] These first two processes create the pool of materials within the ciliary stroma that is then ready to cross the blood-aqueous barrier at the nonpigmented ciliary body epithelium and enter the posterior

chamber.[1] *Active secretion* represents the most important process in the formation of the aqueous humor.[4] It allows for the accumulation of important ionic compounds at higher levels within the aqueous humor, creating a gradient for the diffusion of water and other compounds. Active secretion occurs across the functional syncytium of the pigmented and nonpigmented ciliary body epithelium.[2] Requiring ATP dependent pumps, the secretion that occurs is driven primarily by sodium-potassium-ATPase and carbonic anhydrase, though other active transport mechanisms exist.[1] Sodium, bicarbonate, chloride, and ascorbate are examples of compounds that are actively secreted into the posterior chamber.[4]

Aqueous Humor Drainage

The aqueous builds in the posterior chamber and then flows anteriorly, through the pupil, and into the anterior chamber. In the anterior chamber, convection currents (driven by warmer temperatures at the iris face and cooler temperatures of the cornea) circulate the aqueous until it drains via passive flow following one of two routes: 1) via *conventional outflow*, through the iridocorneal angle, past the trabecular meshwork and into species-specific vascular channels that direct aqueous to the systemic circulation, or 2) via *uveoscleral outflow*, diffusion across the iris and ciliary body and into the suprachoroidal space.[1] The specific amount that is drained by each route is dictated by individual species. In humans, the outflow of aqueous is shared more or less equally between the two routes.[1] In dogs, studies indicate approximately 15% of the aqueous humor is drained via the uveoscleral route, whereas in cats uveoscleral flow represents only 3% of total drainage.[4,7]

Aqueous Humor Flow

In the normal eye, the flow of aqueous is dictated by the rate of formation and the rate of drainage of the aqueous humor.[8] This balance between production and drainage determines the pressure found within the eye itself, or IOP (10-20 mmHg in most species).[4] The rate of formation of the aqueous is relatively constant as the bulk of its production is dictated by active secretion and resultant diffusion. Ultrafiltration, a smaller component process of aqueous production, does not have a large enough contribution to have a great effect on the overall production rate.[9] The rate of drainage of the aqueous humor is dependent upon the resistance to flow present at the outflow channels. Along the conventional outflow path, this resistance is both pressure dependent, by the venous pressure of the collecting vessels, and pressure

independent, by the membranes of vascular collecting channels and extracellular matrix.[4] Interestingly, drainage along the uveoscleral outflow path is independent of IOP; with flow resistance created by the tissues that line the outflow pathways, similar to lymphatic drainage.[1,10] The resultant flow rate has been determined in a variety of species using various methods, with aqueous flow rate averages of 2.5 μl/min in humans,[11-16] 3 μl/min in rabbits,[17-21] 5 μl/min in dogs,[22-25] and 6 μl/min in cats.[26-29]

Evaluation of Aqueous Humor Flow

Invasive Techniques

The aqueous humor flow rate has been studied extensively over the last 70 years. A variety of techniques have been utilized to assess both path of aqueous drainage and the rate of aqueous humor flow (assessed via drainage and production). Initial attempts at determination of the aqueous humor flow path and rate involved direct cannulization and perfusion, the injection of various tracer compounds, or a combination thereof. These experiments were highly invasive, at times requiring the ligation of renal arteries,[30] and utilized nonhuman test subjects for completion, primarily rabbits, primates, and cats.

Cannulization

Direct cannulization of the eye allowed investigators to perfuse the anterior chamber in an attempt to recreate various normal and abnormal pressure scenarios. Studies utilized either *constant pressure perfusion*: a constant IOP is maintained via perfusate injected at a constant rate or predetermined intervals; or *constant rate perfusion*: the perfusate is injected at a determined rate, allowing for fluctuations of IOP.[4] Many of these studies were designed to evaluate the facility of outflow, or the maximum ability for aqueous to exit via the anterior chamber; however, manipulation of the various experimental setups also allowed for estimations of aqueous production.[7,10,31-33] In 1954, Bárány and Scotchbrook developed the *two-step constant pressure perfusion technique*: an eye is perfused for a few minutes at two different levels (2-3 mmHg and 8-10 mmHg above baseline) and the resultant flow rates are analyzed.[34] This is the most widely used procedure in perfusion studies today.[4,32]

Tracer Compounds

Many perfusion studies were combined with the application of radioactively labeled tracers. A host of various compounds were utilized by a multitude of researchers, to both evaluate aqueous humor flow and also address other important aspects of aqueous humor dynamics. These compounds were introduced into the eye either through direct injection or after being injected into the systemic circulation and allowed to enter the aqueous through natural transport systems. Tracer compounds were also used without direct perfusion in order to prevent the disruption of natural aqueous flow, but were still considered invasive due to the unknown effect many of the compounds would have on the patient. The compounds used varied widely and were designed to meet the specifications for the study goals of each researcher. In their studies O'Rourke and Macri, Macri *et al.*, and Bill used radioactively labeled particles, such as albumin and inulin, as they are large, easy to measure, and readily available.[35-37] Langham used cysteine and ascorbic acid, already found in high levels in the aqueous humor, to concurrently investigate both the aqueous flow rate and properties of aqueous formation.[30] A series of compounds (sodium, p-aminohippurate, sucrose, ethyl alcohol, and propyl thiourea, p-amino hippuric acid, rayopake, diodrast) have been chosen for their different physiologic properties to investigate the different characteristics of aqueous formation and drainage.[38,39] Through these studies, not only have aqueous flow rates been calculated, but they all appeared to decrease at this surprisingly similar rate, indicating aqueous humor was removed in a bulk, nonspecific manner.[38]

While some researchers were establishing aqueous flow rates, other researchers were concurrently evaluating the location of these aqueous outflow routes using similar methods. In 1942, Ascher first documented the flow of aqueous into ocular vasculature, thus allowing a first glimpse into the conventional route of aqueous outflow.[40] That same year, Troncoso's investigations into rabbits revealed these vessels were separated from the anterior chamber, only by the trabecular meshwork.[41] Building upon these discoveries, investigators utilized injected tracers (^{131}I-labeled albumin and ^{14}C-labeled inulin) and the direct cannulization of intrascleral vessels to describe the basic conventional pathway for bulk aqueous flow.[35,37] In 1965, Bill's continued investigations of outflow pathways using labeled albumin led to the discovery of the uveoscleral route for aqueous drainage.[7,10]

Non-Invasive Techniques

As continued progress was made in the evaluation of the aqueous humor flow, researchers' desire to apply this knowledge to human subjects demonstrated the need for a noninvasive method of flow evaluation. Initially, a technique was developed that allowed for tonography to be used to calculate the facility of aqueous outflow.[42,43] The calculated outflow facility was then applied to the Goldmann equation to determine aqueous flow:

$F = (P_i - P_e) \times C,$

where F = rate of aqueous humor formation, P_i = the IOP, P_e = the episcleral venous pressure, and C is the facility of outflow measured tonographically.[43] After Bill's discovery of the uveoscleral outflow pathway, the equation was modified to:

$F = (P_i - P_e) \times C + U,$

where U = uveoscleral outflow.[43] Due to the development of more accurate methods of determining aqueous humor flow, tonography has largely been replaced as a measurement of aqueous humor production; however, it remains a commonly used flow measurement in determining conventional outflow facility and uveoscleral outflow.[18,20,27,44-52]

Starting as early as 1950, the concentration of fluorescein in the anterior chamber was used to investigate aqueous humor flow.[16] Initial attempts utilized systemic administration of fluorescein that, when combined with serial slit lamp evaluations and the collection of blood samples, could be applied to an exceedingly complicated mathematical equation to obtain flow estimation. A few years later, the concept of topical administration of fluorescein was introduced, finally yielding a noninvasive method of flow investigation.[53,54] This early technique, however, was hampered by the lack of an objective, quantitative measurement device for fluorescein concentration calculation. In 1963, Maurice unveiled a new fluorophotometer, which, along with a practical method of measurement introduced by Maurice and Jones three years later, has become the staple of aqueous humor flow measurement today.[11,55]

Fluorophotometry

Concept

Fluorophotometry is the quantified measurement of light emitted by sodium fluorescein after stimulation by blue light. It can be used to evaluate the concentration of fluorescein in

various transparent media and is most commonly used to evaluate intraocular fluorescein concentrations in either the aqueous or vitreous humors. Fluorescein can be introduced systemically or topically and can be utilized to investigate the blood aqueous barrier,[56-60] the blood retinal barrier,[61] corneal endothelial permeability,[14,62,63] tear flow dynamics,[64,65] and aqueous humor flow calculations.[16]

The computerized scanning electronic fluorophotometer was introduced in 1963 by Maurice as a new, quantitative method for the evaluation of sodium fluorescein concentrations in the eye.[55] Though more advanced models have been created, the basic principle remains the same.[12] The machine is essentially a modified slit lamp with a fiber optic probe that emits a focused beam of blue (480nm) light. A barrier filter allows only the green (520nm) light that indicates peak fluorescence to be evaluated, thereby reducing the effect of scatter. The digital radiometer then compares the emitted fluorescein to known fluorescein curves to determine the concentration seen at each measured point.[66] These concentrations are then monitored over a set period of time. This decline of fluorescein concentration is then attributed to the drainage of the fluorescein-aqueous mix and further dilution as it is replaced by new aqueous humor that is being produced. The aqueous humor flow calculation given by fluorophotometry is an estimation of aqueous outflow of both the conventional and uveoscleral routes.[52]

Equation Derivation

Maurice and Jones established the basic calculations utilized for fluorophotometric evaluation of aqueous flow shortly after the introduction of the new fluorophotometer.[11] Their evaluation is based upon three assumptions: a) the topically applied sodium fluorescein is homogenously distributed throughout the cornea and aqueous humor during the measured time points; b) after the initial sodium fluorescein application, no further sodium fluorescein is applied to the cornea; and c) the corneal and aqueous humor sodium fluorescein concentrations decrease at the same rate over the course of the measured time points. Utilizing these assumptions, the basis for fluorophotometric calculation relies on 3 equations:[11]

$$(1)\quad dC_c/dt = k_{c.ca}(C_a - C_c),$$

where $k_{c.ca}$ is the transfer coefficient from the cornea to the aqueous, C_a is the effective aqueous fluorescein concentration, and C_c is the effective corneal concentration of fluorescein;

$$(2)\quad dC_a/dt = K_oC_a + k_{a.ca}(C_c - C_a),$$

where K_o is the anterior chamber loss coefficient and $k_{a.ca}$ is the transfer coefficient from the aqueous to the cornea; and

$$(3)\quad dm_t/dt = - C_aV_aK_o,$$

where m_t is the total mass of fluorescein and V_a is the volume of aqueous humor.

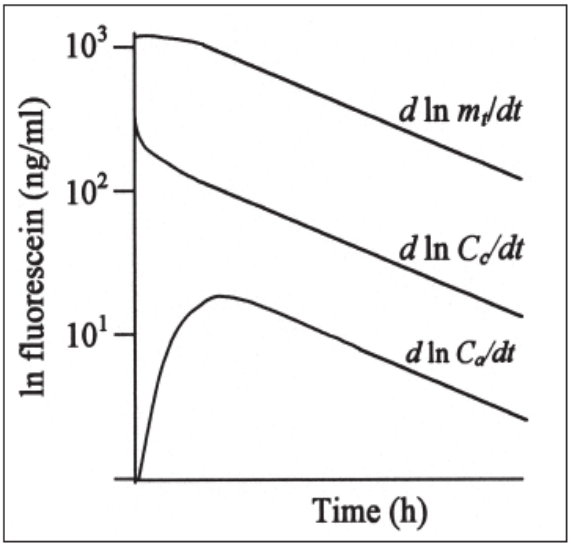
Before continuing with further mathematical derivation, it is important to point out that several assumptions have been made to make these equations possible. For the first equation, it is assumed that all fluorescein present within the cornea is exchanged with the aqueous humor and that no exchange occurs between the cornea and limbal blood vessels. For the second equation, it is assumed that the concentration of fluorescein in the blood is negligible. And for the third, that fluorescein is lost by the cornea to the aqueous humor alone.

Because fluorophotometric evaluation occurs at steady state, equation (2) can be further reduced to:

$$(2)\quad dC_a/dt = K_oC_a$$

Since we assume that the concentrations of the cornea and aqueous will be decreasing at the same rate at steady state, and thus the rate of fluorescein egress from the cornea to the aqueous and the aqueous to the blood stream are the same, we can plot the three equations and their slopes are represented by the differential expressions: $d\ln m_t/dt$, $d\ln C_c/dt$, and $d\ln C_a/dt$. (Figure 1.1)

Figure 1.1 Plotted Equations of Fluorescein Egress



The terminal slope of these curves are all the same and is represented by the equation:

(4) $A = -V_a C_a K_o / m_t$

In 1978, Yablonski *et al.* introduced a modification to the original calculations to avoid direct measurement of m_t.[13] Because the total mass of fluorescein is equal to the mass of fluorescein in the anterior chamber and the cornea, then $m_t = V_a C_a . + V_c C_c$, which means that:

(4) $A = -V_a C_a K_o / (V_a C_a . + V_c C_c)$

By solving for $K_o$this equation can be further modified to:

(4) $K_o = -A \times (1 + [V_c C_c / V_a C_a])$

Before completing the final aqueous humor flow equation, two correction factors have been introduced to assure more exact aqueous humor flow rates, both of which are inherent to the fluorophotometric method of flow calculation. Maurice and Jones noted, at equilibrium, the fluorescence of the cornea and aqueous humor differed by a factor of 1.2.[11] In 1993, van Best *et al.* added a second correction factor to account for the fact that the diamond-shaped intersection

of the fluorophotometer's excitation and emission beams was larger than the corneal thickness, thus underestimating corneal fluorescence.[14] Called the spatial resolution correction factor ($k_{spat.res}$), this value was represented by the equation:

$$(5)\ k_{spat.res} = 1 / (1 - Q \times e^{Bd}),$$

where Q =0.9622, B = -1.848, and d is the thickness of the cornea in millimeters. These corrections leave a final equation of:

$$(4)\ K_o = -A \times (1 + [k_{spat.res} V_c C_c / 1.2 V_a C_a])$$

After obtaining fluorophotometric values (C_c and C_a) over at least two time points during the steady state fluorescein egress, the slope of that decline can be plotted and representative C_c and C_a values used to obtain K_o. Also required are the previously obtained corneal and aqueous humor volume values and corneal thickness (which allows for $k_{spat.res}$ calculation). K_o can then be used to calculate aqueous humor flow using the equations:

$$(6)\ \text{Flow} = K_o V_a$$

Using their new technique, Maurice and Jones calculated the human aqueous humor flow rate to average 2.5 µl/min with an average aqueous humor turnover rate (K_o) of 0.015 min, or 1.5%/min.[11] This number was similar to earlier studies that had been performed with invasive techniques on cadaver eyes,[67] using less advanced fluorophotometry equipment,[16,68] and with more complicated methods developed at a similar period.[53,54] At the present time, fluorophotometry using this method is considered highly accurate for determining aqueous humor flow and, as an entirely noninvasive procedure, has allowed for the inclusion of human data to an already large pool of animal data.[69]

Fluorophotometric Results

Since the development of their technique, the fluorophotometric method established by Maurice and Jones has been utilized in hundreds of investigations that have tested various aspects of aqueous humor flow. These studies have confirmed the average aqueous humor flow rate in the normal human eye is around 2.5 µl/min.[16,43] Taking the next step, investigators have also evaluated changes that occur in aqueous flow rate on both a daily and lifelong basis. Investigations have revealed normal circadian rhythms diminish aqueous flow by nearly half during sleep, although it changes very little over the course of the waking hours.[15,52,70] It has also been found that aqueous humor flow slowly decreases with age (approximately 2-4% per decade of life), although the aqueous humor turnover rate actually increases due to a concurrent decrease in anterior chamber size.[16,40]

Though initially developed for human use, fluorophotometry has often been utilized in animal models before subjecting humans to the possibly dangerous effects of various pharmacologic compounds being tested. These studies, combined with the volumes of historic data from more invasive research, have provided a large base of knowledge for the animal species utilized: rabbits,[17,18,20,21,38,71-73] primates,[18,32,44-49,73-76] and cats.[26-30,35,36,38,47,48,67,72,73,76] Recently, the continued expansion of veterinary-specific research has also started compiling further data on these animals, as well as dogs, one of the more predominant species of interest in veterinary medicine.[22,24,77]

The aqueous humor flow rate in the normal eye has been determined for a handful of species, with some species having numerous values available for comparison. The following species have been evaluated via fluorophotometry and the average flow rates are: cynomolgus monkey (1.7 µl/min),[18,32,45-49,74,76,78] owl monkey (2.15 µl/min),[75] rabbit (3 µl/min),[17-21] dog (5 µl/min),[22,24,77] and the cat (6 µl/min).[26-29] With a few exceptions, fluorophotometrically determined aqueous humor flow values tend to be fairly similar across studies performed.[25,73]Unlike in human studies, fluorophotometric comparisons of aqueous flow have differed greatly from invasive studies performed in the past, especially in the feline species where historic values range from 10-20 µl/min, far above 6 µl/min.[30,31,35-38] This difference has yet to be explained.

Intraocular Pressure

Tonometry

Intraocular pressure is created by the balance between the formation of aqueous humor and the resistance to aqueous outflow. Tonometry is the measure of tension or pressure and is performed noninvasively to determine the pressure within the eye created by this balance in aqueous humor. The first ocular tonometer was introduced by von Graefe in 1862.[79] His device was a form of indentation tonometry, where direct pressure is placed on the eye, and resistance measured. In 1905, Schiøtz refined the indentation tonometer and his device became the most widely used tonometer and is still used today.[79]

In 1885, Maklakov introduced the first applanation tonometer.[43] This device utilized a suspended block of known weight that was covered in dye; the block was allowed to rest on the cornea, which would remove the dye in the area the cornea was flattened to allow contact. This area could then be used to calculate the pressure required to flatten the cornea to the degree noted.[80] Maklakov's design was improved with the development of the Goldmann tonometer in 1954. Attached to a slit lamp, Goldmann's technique utilized topical fluorescein which, when aligned with a corneal contact prism, allows calculation of the force required to applanate a known area with great accuracy.[79] Due to the fixed nature of the tonometer, the requirement for the patient to remain still for several seconds, and the inability for the Goldmann tonometer to take pressures in diseased and edematous corneas, more practical tonometers have been developed.[81]

With the development of the Mackay-Marg tonometer in 1959, the electronic applanation tonometer was born.[81] This tonometer utilized a ceramic or quartz-actuating rod that is housed within a steel probe.[79,81] As the probe is pressed against the cornea, the rod depressed, activating a position transducer that enables the device to calculate pressure. Though also quite accurate, a handheld device similar to the Mackay-Marg device, the TonoPen, was introduced in the late 1980's and has largely replaced the Mackay-Marg device due to its portability, position independency, and ease of use.[82] This has been especially true in veterinary medicine, where patients are highly mobile and often resistant to IOP measurement. With this ease of use, does come some loss of accuracy, as the TonoPen is known to overestimate IOP at low levels and will

underestimate IOP at high levels; however, these inaccuracies have been deemed clinically unimportant.[83]

Though the concept for a dynamic or rebound tonometer is decades old,[84] a method for clinical use has only recently been introduced.[85,86] Rebound tonometry relies on a small (1mm) round, plastic tipped probe that is expelled from the tonometer electromagnetically. The probe will then bounce off the surface of the cornea and return to its original position. The machine notes the deceleration of the probe and this is translated into a pressure measurement (the faster the probe decelerates, the higher the pressure in the eye).[86] Used in both human and veterinary medicine, rebound tonometers are highly portable and do not require topical anesthesia.[79] Investigations into many different species have demonstrated they are accurate, comparable to other accepted methods of tonometry or invasive manometry, easy to use, and accepted by patients of all species.[87-90]

Various tonometric studies utilizing the techniques described above have established normal ranges for IOP for various species: humans (average 15 mmHg, range 10-21 mmHg),[1,91] cats (average 19-22, range 10-30 mmHg),[83,87,92] and dogs (average 12-21, range 10-25 mmHg).[4,88,90] The value of this measurement cannot be underestimated, as it is often our only insight into the aqueous humor dynamics of the clinical patient. An important part of the clinical evaluation of ocular disease, it can be used to document abnormal IOPs that occur during various disease processes. Elevated pressures are most often attributed to a disruption of normal aqueous outflow, which can occur transiently in the case of post-operative ocular hypertension (a common sequela to recent intraocular surgery), or can represent a more permanent and devastating condition such as glaucoma. In the intact globe, low IOPs are caused by decreased aqueous humor production. This can occur as a sequela to old age, active uveitis causing active inflammation within the ciliary processes, or chronic uveitis that permanently damaged the ciliary body.

Physiologic Effects on Intraocular Pressure

In the assessment of IOP, it is important to consider the various normal physiologic processes that can impact IOP measurement. First and foremost, proper handling and positioning of the patient is necessary to ensure accurate readings. In human medicine, IOPs should be taken in a sitting or standing position, whereas felines and canines should be restrained

in a sitting, laying, or standing position.[93-95] Various reports have documented differences in IOPs, depending on the position of the head in relation to the body in several species.[93-95] If manual restraint is required, it is also important to avoid the inadvertent increase of IOP by placing direct pressure on the globe (digital or by pulling the eyelids tight) or by indirectly increasing IOP via jugular vein occlusion. The stress of the patient should also be considered, as the fight or flight response in some animals adds increased pressure on the globe from eyelid or extraocular muscle pressure.[4] Indeed, a recent study showed that IOP slowly decreased over the 5 minute time period when dogs were held in the same position.[93] It is plausible to assume that the patients involved had become more accustomed to their restraint over that time period. In a research setting, it is exceedingly important that IOP be measured the same way in each patient over the course of the study to ensure that all of these factors are constant and do not constitute a variable that could bias results.[93]

Another physiologic effect on IOP to consider is the natural circadian rhythms that have been shown to effect pressure levels. In humans, circadian rhythms have been variably recorded and contradicting data has been presented.[71] The majority of studies have demonstrated a basic rhythm exists for IOP, with the peak pressure found during the night.[96-99] These daily variations have been found to influence IOP differently based on the position of the subject, the disease status of the eye studied, and even the effects of pharmacologic compounds that alter IOP.[52] Similar rhythmic fluctuations in IOP have been noted in other species, which have revealed that the peaks and valleys of the rhythmic pressure fluctuations often depend on the time of day the animal is most active, with nocturnal animals, such as cats and rabbits, experiencing peak IOPs different times of the day than animals who are active during the day, such as cynomolgus monkeys.[71,92,100-107]

Relation to Aqueous Humor Flow

As discussed earlier, circadian rhythms have also been documented affecting aqueous humor flow.[15,52] With the continued development of aqueous humor flow studies and the seemingly obvious link between flow and IOP, researchers have often concurrently studied them together in order to better understand the interaction between the two. What has so far been elucidated is surprising. In the majority of studies utilizing human subjects, both normal and diseased eyes, changes in aqueous humor flow are not necessarily similarly manifested by

changes in IOP. In a study specifically designed to assess the interaction between the two, Carlson *et al*. altered subject body position to increase IOP and simultaneously measure aqueous flow using fluorophotometry.[95] They found significant alteration of IOP, with only mild changes in aqueous flow with the highest pressures. Sit *et al*. demonstrated in normal eyes that although aqueous humor flow decreased by half from day to night (2.26 µl/min to 1.12 µl/min), IOP was minimally reduced (18.9 mmHg to 17.8 mmHg).[52] Similar findings have also been seen in research performed on abnormal or diseased eyes. An evaluation by Yablonski *et al*. demonstrated that no change in fluorophotometric flow was noted, despite significant decreases in IOP in patients after trabeculectomy surgery.[108] A recent review by McLaren summarized the data of 20 separate studies investigating eyes of people suffering from systemic or ocular disease. In the study, the aqueous flow was compared to IOP. The findings indicate that IOP and aqueous humor flow are independent of each other, revealing no significant compensatory relationship. Included within this summary were 5 studies investigating the effects of ocular hypertension and open-angle glaucoma in humans.[16]

At first glance, the data reported would seem to contradict our basic conception of how IOP is determined. As stated earlier, IOP is created by the production of the aqueous humor and the resistance to its outflow. If we manipulate the Goldmann equation presented earlier to reflect IOP, we see that:

$$P_i = (F - US)/C + P_e,$$

where F = rate of aqueous humor formation, P_i = the IOP, P_e = the episcleral venous pressure, U = uveoscleral outflow and C is the facility of outflow measured tonographically. Aqueous humor flow, as determined by fluorophotometry, accounts only for F and U within the Goldmann equation. As discussed earlier, active secretion and resultant diffusion (the main components of aqueous humor production, F) and uveoscleral outflow (U) are both considered independent of IOP, thus flow measurements would be expected to be independent of pressure. It would therefore stand to reason, that changes in IOP seen in the studies listed above were due to changes in the facility of outflow and episcleral venous pressure. It is important to note that no in vivo fluorophotometric studies have been performed at excessive IOP or on subjects suffering from closed angle glaucoma, the most common type affecting veterinary species.

Pharmacologic Alteration of Aqueous Humor Dynamics

The study of aqueous humor dynamics has helped researchers to understand the intricacies of the aqueous flow pathway in the many species utilized for their investigations. A large part of that body of research focused on various pharmacological compounds designed to alter normal dynamics in order to reveal the exact mechanisms involved. As a side product of the use of these compounds, and ultimately the true goal of the research itself, various compounds were found that could be used therapeutically in humans and animals that suffered from alterations in normal dynamics.

The compounds investigated vary widely in their effect on the eye, as well as their efficacy for altering IOP and aqueous flow. This has demonstrated the complexity of the regulation of aqueous humor dynamics within the eye and the importance in understanding the actions of these medications. A brief review of the compounds found to alter IOP and aqueous humor flow (conventional and uveoscleral) can be found below.

β-Adrenergic Antagonists

Propranolol was discovered to reduce IOP when administered systemically in 1967 and topically in 1972.[16] Since that time, several β-blockers have been introduced including betaxolol, levobunolol, and timolol. As this class of medications is one of the most widely used in the management of glaucoma in humans,[109] numerous reports exist, which have revealed these compounds reduce IOP and the flow of aqueous humor, suggesting a decrease in aqueous humor production was the source of β-blockers' hypotonic effect.[75,92,106,110-114] The exact mechanism is, as yet undiscovered, but the traditional view purports β-blockade at the level of the ciliary processes alters neuronal control of aqueous humor formation.[113]

α_2 Adrenergic Antagonists

Makabe initially discovered the ability for clonidine to lower IOP in 1966.[16] Para-amino-clonidine (apraclonidine) was subsequently developed as a topical preparation and was also found to reduce IOP. Later studies, revealed aqueous humor flow was similarly decreased and so the presumed method of action was a reduction in aqueous humor production.[115] It is currently postulated that these medications exact their effects by inhibiting norepinephrine release at the sympathetic nerve-ciliary body junction.[109]

Adrenergic Agonists

The study of adrenergic agonists (primarily epinephrine) effects has provided conflicting results, but they are generally considered to reduce both aqueous humor production and increase aqueous outflow.[29,40,116] The ability for this medication class to exert its effects is enhanced during the night, when it is believed low levels of endogenous adrenergic hormones are in circulation.[40] Dipivefrin is the lipophilic prodrug of epinephrine that is commercially available for clinical use. Aqueous production is believed to be reduced through decreased blood flow to the ciliary body via vasoconstriction. It is unknown how these medications exert their effect on aqueous outflow.[117]

Cholinergic Agonists

Direct-acting compounds such as pilocarpine and carbachol, as well as indirect-acting compounds such as demecarium bromide have been shown to decrease IOP and increase trabecular outflow facility.[118-121] Fluorophotometric studies have shown less aqueous humor flow effects,[16,40] and in some cases, the addition of pilocarpine appeared to decrease the efficacy of other medications in reducing aqueous humor flow.[122] The contraction of the ciliary muscle in response to stimulation from these compounds is believed be the source of increased outflow. This contraction is more effective in humans and other animals with a scleral spur.[109]

Prostaglandins and Prostaglandin Analogues

The prostaglandin and prostaglandin–associated compounds have been the focus of extensive research over the last two decades. This is due in large part to the significant reductions in IOP demonstrated through their use. This research has documented these compounds exert their effects through receptor-mediated channels that are highly species-specific, and derivative compounds that stimulate several receptors are the most effective in altering aqueous humor dynamics.[26,32,123-131] Though a handful of studies have shown mild increases in aqueous humor flow, the majority of studies have shown no fluorophotometric increase in aqueous flow, instead finding significant increases in the calculated facility of uveoscleral outflow, as summarized by Toris in 2008.[124] This would indicate these compounds reduce IOP by increasing uveoscleral outflow and, despite the miosis created, do not significantly influence conventional outflow (though increases in trabecular outflow facility have

been demonstrated inconsistently).[124] Several prostaglandin analogue compounds are now available for clinical use, including latanoprost ($PGF_{2\alpha}$), bimatoprost ($PGF_{2\alpha}$), travoprost ($PGF_{2\alpha}$), and unoprostone ($PGF_{2\alpha}$).[124] As all of these compounds specifically target the FP prostaglandin receptor (designed to receive $PGF_{2\alpha}$, they have been demonstrated to be ineffective at lowering IOP in cats (who lack FP receptors along the uveoscleral outflow pathway).[123] Cats do have FP receptors associated with their irises and these compounds do lead to miosis in this species.[123] Other compounds currently in or awaiting clinical trials include tafluprost ($PGF_{2\alpha}$) and butaprost (EP_2).[32,124]

Carbonic Anhydrase Inhibitors

Carbonic anhydrase catalyzes the reaction that changes carbon dioxide (CO_2) and water (H_2O) into carbonic acid (H_2CO_3), also helping to dissociate an H^+ atom, thus creating bicarbonate (HCO_3^-).[1] Carbonic anhydrase has been subcategorized into seven isoenzymes (CA I-VII), with various isoenzymes creating bicarbonate ions in many places in the body, including the red blood cells, renal tubules, and the ciliary body epithelium. Bicarbonate is vital in maintaining normal homeostatic functions in all three areas, including buffering the blood, maintaining normal proximal tubular ion exchange, and contributing to the production of aqueous humor.[4]

Acetazolamide was the first carbonic anhydrase inhibitor (CAI) developed for clinical use, and was the initial step toward pharmacologic manipulation of aqueous humor dynamics. In 1955, Becker first documented that acetazolamide reduced IOP.[67] Subsequent studies documented reductions in aqueous humor flow, indicating CAIs exerted their effect on IOP at the level of aqueous humor production.[132] Researchers have since discovered carbonic anhydrase creates bicarbonate within the epithelial layers of the ciliary processes. This bicarbonate is then excreted into the posterior chamber of the eye along with actively secreted Na^+ ions, which creates an osmotic diffusion gradient that pulls water into the posterior chamber, forming a substantial percentage of the aqueous humor volume.[4] It is also hypothesized that the presence of bicarbonate is necessary for normal function of active Na^+ secretion.[1] The proposed mechanisms by which CAIs reduce the secretion of Na^+ include: decreasing the available HCO_3^- necessary for direct transport with Na^+, reducing HCO_3^- concentration leading to a reduction in intracellular pH preventing proper function of Na^+-K^+-ATPase, or by decreasing availability of

H^+ used for Na^+ transport.[1] CAIs suppress the production of bicarbonate by attaching to the carbonic anhydrase enzyme at the site of the carbonic acid receptor using a sulfonamide group, thereby preventing carbonic acid attachment.[133] In the ciliary body epithelium, CA II, III, and IV have been demonstrated to be present, and CA II is considered the most important target for CAIs.[134] To be effective 99% of the carbonic anhydrase II enzymes present in the ciliary body must be inhibited.[135]

Systemic Carbonic Anhydrase Inhibitors

Acetazolamide has been found to be 40-60% successful in reducing IOP in various human studies.[1] In dogs and cats, it has been proven to be similarly effective.[136] Unfortunately, as a systemically administered medication, important side effects have been noted with the use of acetazolamide. Those side effects include metabolic acidosis, hypokalemia, diuresis, and gastrointestinal disturbances.[109] Not surprisingly, these side effects can be traced back to the other regions of carbonic anhydrase activity. Cats are especially susceptible to these side effects, to the point that acetazolamide is no longer recommended for use in this species.[109] Methazolamide, another systemic CAI, is more potent than acetazolamide, has been shown to exert its effects over a longer time period, and has been demonstrated to be effective in lowering IOP.[24,137-139] For these reasons, it can be administered at lower overall levels allowing for patients to experience less severe systemic side effects.

Topical Carbonic Anhydrase Inhibitors

For many years, it was thought that topical formulations of CAIs would not be able to reach the 99% saturation required to effectively reduce IOP.[1] The introduction of dorzolamide disproved that theory. Numerous studies have shown topically applied dorzolamide is effective at lowering IOP in various species, as well as reducing aqueous humor flow.[22,103,104,110,140-144] Dorzolamide is a topical CAI formulation that is most sensitive for CA II, and has been shown to produce blood levels $1/200^{th}$ the level necessary to see systemic side effects in humans.[135] This makes it an ideal compound to manipulate aqueous production in the ciliary body without affecting carbonic anhydrase in other areas of the body, even other CA II present systemically. Concurrent administration of dorzolamide and systemic CAIs have shown a similar response to systemic CAIs alone, but no additive effect on IOP has been documented.[137,143] With a pH of 5.6, ocular irritation upon application is the most common side effect.[109,145]

Chapter 2 - Evaluation of the Aqueous Humor Flow Rate in Normal Cats Using Fluorophotometry

Introduction

The flow of aqueous humor is an important aspect of normal ocular health in all animals. The function of the eye relies on the normal turnover of aqueous humor in order to provide nutrients to and remove waste products from avascular structures within the eye, including the corneal endothelium and lens.[1] Aqueous humor flow is also a vital component in establishing normal IOP, which provides the framework for appropriate visual function by maintaining structural alignment for the cornea, lens, and retina.[4] Alterations in normal aqueous humor flow can result from serious ocular diseases, such as glaucoma and uveitis, which can have damaging effects on the eye and threaten vision.Though the changes in flow may not be the direct cause of these damaging sequelae, a proper understanding of flow during these events may help elucidate the specifics of the disease process or enable objective evaluation of potential therapeutic interventions.

Fluorophotometry is an accurate tool for noninvasive assessment of aqueous humor flow. The first objective fluorophotometer was introduced by Maurice in 1963 and was followed three years later by the introduction of a specific mathematical model by Jones and Maurice.[11,55] This method estimates the rate of aqueous flow based on a reduction in fluorescence of the aqueous humor over time following topical administration of sodium fluorescein. The model is predicated upon the assumption that a steady state is reached as the fluorescein passes from the cornea into the aqueous humor and eventually exits via the aqueous outflow tracts. Decay curves of this steady decline are formed using periodic fluorophotometric measurements taken hours after initial fluorescein application to the cornea. The slopes of these decay curves are the basis for aqueous flow measurement, once applied to the aforementioned equations. This model has been utilized, with slight modification, in several species including humans,[13,16,40,116,141] dogs,[22-25] and cats[26-29] and is the predominant model utilized for flow investigations today.

Fluorophotometric flow studies have been reported in the feline species, though previous investigations utilized this information largely as an adjunct to the evaluation of other aspects of aqueous humor dynamics. The protocols for these evaluations are varied and often require large

numbers of topical drops and long durations between drop application and fluorophotometric evaluation. In these feline studies, the application of the fluorescein, fluorescein concentration, and flow measurement protocol varied significantly. Consequently, reported aqueous humor flow values have fallen across a wide range, from 3.5 to 22.5 µl/min.[26-29] As the focus of flow determination has been on creating a base value to which treatment values can be compared, this variability has largely been ignored and has made it difficult to compare feline values to other species. Recently, a more streamlined fluorescein application protocol was created for the canine species.[23] Subsequent investigations have since confirmed the viability of this technique.[22,24] In the feline species, a more streamlined protocol would reduce the time necessary to perform fluorophotometry as well as eliminate the variables associated with excessive drop application and long durations between fluorescein application and flow measurement. It would then be important to demonstrate this protocol successfully met the assumptions required to trust the flow data. The purpose of this study was to evaluate the aqueous humor flow rate of normal cats using a method that has proven successful and repeatable in the canine species with the intent to compare values between species as well as to previous investigations in the feline species.

Materials and Methods

Animals

This study was performed using 20 cats (8 neutered males and 12 intact females) weighing between 2.5 and 5.2 kg. The average age of the cats used in our study was 11 months.The cats were purchased from various commercial suppliers and provided by the .[a] A full physical examination including complete ophthalmic examination was performed prior to the study. Cats were assessed via slit-lamp biomicroscopy,[b] indirect ophthalmoscopy, corneal fluorescein staining,[c] and rebound tonometry.[d] All cats were determined to be in good health with normal ocular examinations prior to inclusion in the study. The animals were housed individually or in pairs throughout the study. This study adhered to the guidelines of the institutional animal care and use committee at Kansas State University.

Fluorophotometry Protocol

A random number generator was used to randomly assign the cats to 1 of 4 groups with 5 cats in each group. Group assignment dictated the day fluorophotometry would be performed. The initial group of 5 cats was utilized to assess the feasibility of the proposed fluorophotometry protocol. The 3-drop protocol described by Ward *et al.* was used to obtain homogenous fluorescein introduction into the cornea and anterior chamber: one drop of 10% sodium fluorescein[e] was applied via a sterile dropper bottle into both eyes and allowed to remain for 5 minutes; this process was repeated until 3 drops total had been given.[23] Five minutes after the final drop, the eyes, face, and front limbs were thoroughly rinsed to assure no fluorescein stain remained that could be reintroduced during grooming. Cages and bedding were cleaned or removed if any obvious fluorescein drops were present. One hour and fifty-five minutes after the rinse, the cats were sedated using intramuscular ketamine[f] (5 mg/kg) and medetomidine[g] (0.03 mg/kg) to facilitate patient positioning

At 2 hours post-rinse, fluorophotometric readings were taken of both eyes using a computerized, scanning fluorophotometer with anterior chamber adapter.[h] The head and eyes were aligned using a small platform, manual restraint, and a plastic eyepiece adaptor designed to approximate appropriate eye position for the holder. Fluorescein concentrations were measured in the cornea and mid-central anterior aqueous humor using a scanning ocular fluorophotometer fitted with an anterior chamber adapter. Repeat measurements were taken at 4, 5, 6, 7, 8, 9, and 10 hours. Sedation was repeated at reduced doses (usually 0.01-0.015 mg/kg medetomidine only) as necessary to achieve the desired state of compliance for patient positioning. During the periods between measurements, patients' eyes were kept closed with tape to prevent corneal desiccation.

The remaining 3 groups, 15 cats, were evaluated using the same 3-drop protocol. The same technique and sedation protocols were utilized, with the exception that measurements were taken at 5, 6, 7, and 8 hours only.

Aqueous Flow Rate Calculation

For each cat, fluorophotometric scans were evaluated at each time point to determine the corneal and aqueous humor fluorescence levels. The landmarks used for this assessment were the apex of the corneal peak and mid-central plateau of the aqueous readings. These data points

were then natural log transformed, plotted, and regression analysis was performed to derive the slopes of the two lines (cornea and aqueous humor). The equations utilized to determine the aqueous flow rate were derived by Jones and Maurice and later modified by Yablonski *et al.* and Van Best *et al.:* Flow = K_oV_a . Where K_o = -A x (1 + [$k_{spat.res.}$ V_cC_c/1.2 V_aC_a]), A= average of the slopes of the decreasing cornea and aqueous humor fluorescein levels, V_c= corneal volume, C_a= anterior chamber volume, C_c = corneal fluorescein concentration, C_a = anterior chamber fluorescein concentration, and $k_{spat.res.}$ = a correction factor necessary to compensate for underestimation of corneal fluorescence inherent to fluorophotometry measurement.[11,13,14] This underestimation results because the focal diamond created by the fluorophotometer for measurement of fluorescein concentration is wider than the thickness of the feline cornea. $k_{spat.res.}$ = 1/(1 – Q x e^{Bd}), where Q =0.9622, B = -1.848, and d is the thickness of the cornea in millimeters. A value of 0.565 mm has been reported for the corneal thickness in cats less than 1 year of age and was utilized for d in this study, resulting in $k_{spat.res.}$ = 1.51.[146] The corneal and anterior chamber fluorescein concentrations ($C_c$and C_a) were obtained from the midpoint of the fluorescein decay curves. Published averages for the feline anterior chamber volume (V_a = 820 µl) and corneal volume (V_c =165 µl) were used.[27] The denominator value of 1.2 represents a second correction factor established by Jones and Maurice designed to account for an inherent difference in fluorescence between the cornea and the aqueous humor that is suggested to be present in all species.[11]

Data Analysis

Regression analysis of the natural log transformed corneal and aqueous fluorescein concentration was utilized to create linear decay curves. Correlation coefficients were calculated to assess the fit of the four time points to the approximated straight line. The slopes of these curves were then compared to ensure they were decreasing in a reasonably parallel fashion. Aqueous humor flow rates were calculated for the right, left, and mean of both eyes as described above. A paired t-test was used to compare the results between the right and left eyes. Eyes were excluded from flow calculation if correlation or slope ratio values represented extreme outliers (defined as greater than 3 times the interquartile range).

Results

Evaluation of corneal and aqueous humor fluorescence revealed the topically applied sodium fluorescein was homogenously distributed within the cornea and aqueous humor at the four-hour time point and beyond in all cats. Fluorescein concentrations were noted to slowly decline after the four-hour time point.

The calculated correlation coefficients for the logarithmically transformed fluorescein concentration data utilized for aqueous humor flow calculation and the slope ratios between the regression lines calculated for the cornea and aqueous humor fluorescein concentration levels are summarized below in Table 2.1.

Table 2.1 Correlation coefficients and slope ratios calculated for the change in fluorescein concentration over time in the cornea and aqueous humor.

EYE	CORNEAL CORRELATION	AQUEOUS HUMOR CORRELATION	SLOPE RATIO
Left	0.78 ± 0.23 (0.19-0.97)	0.92 ± 0.12 (0.59-1.00)	1.61 ± 1.1 (0.23-2.19)
Right	0.85 ± 0.20 (0.15-0.98)	0.90 ± 0.14 (0.56-1.00)	1.13 ± 0.71 (0.28-4.31)
Both	0.82 ± 0.21 (0.15-0.98)	0.91 ± 0.13 (0.56-1.00)	1.37 ± 0.96 (0.23-4.31)

Values reported as mean ± s.d. (range)

The aqueous humor flow rate values obtained for normal feline eyes and the corresponding anterior chamber turnover rates are summarized in Table 2.2. No significant difference was detected between aqueous humor flow values for the right and left eyes ($p = 0.2005$).

Table 2.2 Aqueous humor flow rate and anterior chamber turnover rates determined fluorophotometrically in clinically normal cats.

EYE	AQUEOUS HUMOR FLOW RATE (μL/MIN)	ANTERIOR CHAMBER TURNOVER RATE (RAW/MIN)
Left	5.05 ± 2.06 (1.17-9.89)	0.006 ± 0.003 (0.003-0.012)
Right	5.62 ± 2.40 (2.56-10.67)	0.007 ± 0.003 (0.001-0.013)
Both	5.51 ± 2.21 (1.17-10.67)	0.007 ± 0.003 (0.001-0.013)

Values reported as mean ± s.d. (range)

Discussion

Utilizing the 3-drop protocol for fluorescein application described by Ward *et al.* and established fluorophotometric methods developed by Maurice and Jones, as modified by Yablonski *et al.* and van Best *et al.*, the average baseline feline aqueous humor flow rate is 5.5 μl/min in normal cats. This is the first study to utilize this more streamlined protocol for fluorescein administration in cats and the only investigation to date to focus solely on the determination of normal aqueous flow in this species. For this reason, it was important to fully evaluate the technique to ensure the tenability of the reported results.

When utilizing fluorophotometry to evaluate flow it is important to ensure certain assumptions are met for the mathematical model to be valid. They are as follows: a) the topically applied fluorescein is homogenously distributed throughout the cornea and aqueous humor during the measured time points; b) after the initial fluorescein application, no further fluorescein is applied to the cornea; and c) the corneal and aqueous humor fluorescein concentrations decrease at the same rate over the course of the measured time points. In dogs, Ward *et al.* demonstrated these assumptions could be met using the 3-drop protocol.[23] Utilization of this same protocol in this study proved to be equally effective in cats. Initial fluorophotometric readings using 5 cats demonstrated that homogenous, steady state fluorescein levels in the cornea and aqueous humor were attained by 4 hours (similar to the time point found in the canine species). A steady decline in fluorescein concentration after that time point allowed flow measurements to be taken from 5 hours on, enabling flow calculations to be performed in the same day.

The correlation coefficients of the corneal and aqueous humor slopes showed high correlation between data points and calculated slopes for this study, indicating the slopes obtained were a reliable representation of the data points collected and a reasonable approximation of the true rate of decline (Table 1). Much like previous studies in other species, these correlation values obtained after natural logarithm transformation again demonstrate this decline to be semilogarithmic in nature. The analysis of slope ratios reveals a reasonably parallel rate of decay between the cornea and aqueous humor. This allows for the assumption that they are decreasing at the same rate, fulfilling the necessary requirements to proceed with flow calculation using the described equations.[11,13,14]

The average correlation values and slope ratios determined for cats in this study were not as high as previous reports in dogs. This could be due to differences in the inherent difficulty working with the feline species or simple statistical variability that can occur from study to study. Variation between cat eyes existed with some of the eyes having low correlation values or slopes that were less than parallel. Investigations utilizing fluorophotometry commonly experience such issues, which can occur due to patient placement, head or ocular movement, or issues with fluorescein reintroduction. Evaluation of our data pool revealed that often one reading stood out as possibly spurious, thereby creating a lower overall correlation. In general, the corneal measurements were often the most affected by these "off" measurements. This is attributed to the thin width of the cornea, which makes accurate measurement of the corneal concentration more difficult than the large anterior chamber and, thus, especially susceptible to motion artifacts from either head or ocular movements. During the study, sedation was used to limit these movements, but it was possible that minor ocular movements went unnoticed during data collection and led to inaccurate readings.

The reported aqueous humor flow rate in cats ranges widely from 3.6-22.7 µl/min. A review of the literature reveals the original, more invasive investigations of flow tend to have higher values (estimating flow in cats to be between 14 and 20 µl/min).[7,30,35-38] These older measurement techniques were often derivations of outflow facility or were performed under constant ocular perfusion and thus, are less likely to accurately depict true aqueous flow. Fluorophotometry was developed as a direct measure of aqueous humor flow that can be performed in a live patient without significant alteration to the natural flow of fluid within the eye and is considered the most accurate method of aqueous humor flow determination. We

calculated the average aqueous humor flow in normal cats to be 5.51 ± 2.20 µl/min. This compares favorably to previous fluorophotometric investigations, where the calculated flow rates range between 5.0 and 6.33 µl/min.[27-29] Examination of the components of flow calculation between these studies reveal that similar values are utilized for corneal volume (150-170 µl) and aqueous humor volume (700-820 µl), despite different approaches to volume calculation. The anterior chamber turnover rate (K_o) between studies was also similar, ranging from 0.007/min (0.7% per minute, the value from this study) to 0.009/min (0.9% per minute). Interestingly, closer examination of another fluorophotometry study by Hayashi *et al.*, reveals a similar K_o of 0.0075 /min (0.75% per minute) despite a reported flow rate of 3.6 µl/min.[26] The anterior chamber volume utilized for calculation was 479 µl, likely the source of the gross underestimation of aqueous humor flow in that study and was addressed by the authors.

Utilizing a similar technique, the aqueous humor flow rate in cats (5.51 µl/min) is similar to that of dogs (5.22 µl/min).[23] This similarity was unexpected. Due to the large size of the feline anterior chamber, 700-800 µl, one would expect the aqueous humor flow rate in cats would be much higher than that of a dog, whose anterior chamber has been reported to be around 400 µl.[4] When evaluating the anterior chamber turnover rate (K_o), the contrast between the two species is illuminated. At 0.7% per minute, the cat has a lower rate of aqueous turnover than the dog at 1.4% per minute, thus a much lower rate of flow per volume. In other species, these anterior chamber turnover rates have been found to be quite variable with humans between 1.25% and 1.5%,[11-15] owl monkeys at 1.0%,[75] cynomolgus monkeys averaging 1.8%,[18,45,47-49,74,76,131] and rabbits measured at 1.9% and 2.1%.[17,19] In previous publications, it has been postulated that aqueous turnover rates were similar due to the similar metabolic needs of internal ocular structures between animals.[23] This assessment was based on the evaluation of dogs, humans, and owl monkeys. With the addition of the feline data, as well as data from other species, the average aqueous humor turnover of all these species ranges around 1-2% of the volume of the anterior chamber per minute, seeming to support this assertion, especially considering the inherent variability present within aqueous humor flow measurement via fluorophotometry.

It is important to note, however, that, within that range, the average aqueous humor turnover rate in cats is half that of dogs and nearly a third of that in rabbits and cynomolgus monkeys. And, although variability exists, the equations utilized for calculation of aqueous flow are fairly sensitive to small changes in the rate of aqueous turnover. When looking across the

spectrum of species with data available, a trend seems to be present. Higher K_o values are present in animals with smaller anterior chamber sizes (rabbits, cynomolgus monkeys), lower K_o values are present in animals with larger anterior chamber sizes (cats), and the other species with medium sized anterior chambers have K_o values that fall in between. Building upon the idea that a basic metabolic requirement is necessary for the mammalian eye, this trend could represent modifications made by species with different anterior chamber sizes to either avoid excessively high aqueous flow rates (animals with larger anterior chambers) or to maintain a minimum aqueous flow rate necessary for proper ocular function (animals with smaller anterior chamber sizes).

Future investigations into the relationship between aqueous humor flow, anterior chamber size, globe size, and the metabolic demands of the internal ocular structures of species with different anterior chamber sizes are necessary to firmly establish if this trend is relevant or simply a product of variability within aqueous flow measurement. If relevant, these studies might also illuminate differences in ocular function that could influence our understanding of ocular disease and strategies toward their treatment.

Footnotes

[a] Liberty Research Inc, Waverly, NY; Sinclair Bio Resources LLC, Auxvasse, MO

[b] Kowa SL-15, Kowa Optimed Inc, Torrance, CA

[c] Bio Glo$_{TM}$, Rose Stone Enterprises, Alta Loma, CA

[d] TonoVet®, Tiolat Oy, Helsinki, Finland

[e] AK-Fluor®10%, Akorn Inc, Lake Forest, Illinois

[f] Domitor®, Orion Corporation, Espoo, Finland

[g] VetaKet®,IVX Animal Health Inc, St. Joseph, Missouri

[h] FM-2 Fluorotron Master, Ocumetrics, Mountain View, CA

Chapter 3 - Effects of Topical 2% Dorzolamide Solution on Aqueous Humor Flow Rate and Intraocular Pressure in Normal Cats

Introduction

Carbonic anhydrase inhibitors are used for the clinical management of glaucoma and ocular hypertension in humans and animals. The enzyme carbonic anhydrase catalyzes the reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid. Carbonic anhydrase within the ciliary body epithelium produces bicarbonate, which is excreted into the posterior chamber of the eye. This bicarbonate, along with actively secreted sodium ions, creates an osmotic diffusion gradient pulling water into the posterior chamber to form aqueous humor.[4] The administration of CAIs suppresses the production of bicarbonate by using a sulfonamide group to attach to the carbonic anhydrase enzyme, thereby preventing carbonic acid attachment.[133] In the ciliary body epithelium, carbonic anhydrase II, [147] III, [4] and possibly IV[148] have been demonstrated to be present; however, carbonic anhydrase II is considered the most important target for CAIs. In order to decrease IOP, 99% inhibition of carbonic anhydrase II is necessary in the ciliary body epithelium.[135] The administration of oral CAIs can be associated with anorexia, gastrointestinal disturbances, increased respiratory rate secondary to metabolic acidosis, hypokalemia, blood dyscrasias, and neurologic abnormalities in humans and other species.[109,149-151] Cats appear to be more susceptible to the side effects of oral CAIs and these medications should not be used in this species.[109,152] Due to the adverse effects associated with systemic CAIs, topical formulations have been developed. The current commercially available topical ophthalmic CAIs include a 2% dorzolamide hydrochloride solution and a 1% brinzolamide suspension.

Previous studies have documented the IOP lowering effects of topical dorzolamide solution in several species. [22,103,104,153-156] Topical dorzolamide has also been demonstrated to decrease aqueous humor flow in dogs,[22] rabbits,[153] and humans.[114,157] The mechanism of action of topical application of 2% dorzolamide solution in humans,[158] monkeys,[159] and dogs[22] is to decrease aqueous humor production, but this has not been documented in cats. We hypothesized

that topical application of 2% dorzolamide solution three times daily in clinically normal cats would significantly decrease aqueous humor flow rate and IOP.

Fluorophotometry is an accurate and noninvasive method for measuring aqueous humor flow.[11,13] Anterior segment fluorophotometry has been used to determine the aqueous humor flow rate in clinically normal dogs[23] and cats (see Chapter 2) and in dogs after topical and systemic administration of CAIs.[22,24] The purpose of this study was to investigate aqueous humor flow rate and IOP following topical application of 2% dorzolamide in normal cats using fluorophotometry and rebound tonometry, respectively.

Materials and Methods

Animals

Twenty domestic shorthair cats (8 neutered males and 12 intact females) weighing between 2.5 and 5.2 kg were used in this study. The average age of the cats used in our study was 11 months.The cats were purchased from various commercial suppliers and provided by the Kansas State University Department of Diagnostic Medicine/Pathobiology.[a] An ophthalmic examination including slit lamp biomicroscopy,[b] fluorescein staining,[c] rebound tonometry,[d] and indirect ophthalmoscopy[e] was performed prior to the study. All of the cats had normal ophthalmic and physical examinations prior to inclusion in the study. The cats were housed in a temperature-controlled environment and exposed to an automated 12-hour light/dark cycle (light phase from 7_{AM} to 7_{PM}, dark phase from 7_{PM} to 7_{AM}). This study was approved by the Institutional Animal Care and Use Committee at Kansas State University

Experimental Design

During a 1-week acclimation period, IOP was measured three times daily in all cats using a rebound tonometer without the aid of a topical anesthetic. At the initiation of the experiment, the cats were randomly divided into a control group (5 cats) and a treatment group (15 cats) using a random number generator. The length of the study was 10 days, divided into a pretreatment phase (days 1-3), baseline fluorophotometry measurement (day 4), treatment phase (days 5-9), and treatment fluorophotometry measurement (day 10). IOP was measured at $7_{AM,}$ 10_{AM} , 1_{PM}, 5_{PM}, and 9_{PM} during the pretreatment and treatment phases by a single investigator (AJR). During the treatment phase, the control group received one drop (50 µL) of an artificial

tear preparation[f] in both eyes and the treatment group received one drop (50 µL) of topical 2% dorzolamidesolution[g] in both eyes at 7_{AM}, $3_{PM,}$ and 11_{PM}. A final treatment was administered at 7_{AM} on day 10. A second investigator (WRC) administered the topical medications to each cat from identical, sterile dropper bottles.

The aqueous humor flow rate was measured using anterior segment fluorophotometry to obtain baseline data prior to medication administration and on the final day of the study. The fluorescein administration protocol was similar to that used in previous studies: one drop (50 µL) of 10% fluorescein[h] was applied to each cornea of all cats every 5 minutes until a total of 3 drops had been given. Five minutes after the last fluorescein drop was administered, the eyes of each cat were rinsed thoroughly to ensure that fluorescein did not remain in the preocular tear film. The forelimb paws and other areas of the body were also rinsed thoroughly to prevent fluorescein dye from being reintroduced into the tear film by the cat rubbing its eyes.[22] Fluorescein concentrations were measured in the cornea and mid-central anterior chamber, using a computerized scanning ocular fluorophotometer[i] with an anterior chamber adapter. The cats were sedated with a combination of medetomidine[j] (0.03 mg/kg) and ketamine[k] (5 mg/kg) intramuscularly in order to facilitate proper positioning in front of the fluorophotometer. The scans were performed at 5, 6, 7, and 8 hours after fluorescein administration.

Aqueous Flow Rate Calculation

At each time point the corneal and aqueous humor fluorescein concentrations were determined. The fluorescein concentrations (ng/ml) were natural log transformed, plotted, and regression analysis was performed to derive the slope. The equations used to determine the aqueous humor flow rate were: Flow = K_oV_a . Where K_o = $-A$ x (1 + [$k_{spat.res.}$ V_cC_c/1.2 V_aC_a]), A = average of the slopes of the decreasing cornea and aqueous humor fluorescein levels, V_c= corneal volume, V_a= anterior chamber volume, C_c = corneal fluorescein concentration, C_a = anterior chamber fluorescein concentration, and $k_{spat.res.}$ = a correction factor necessary to compensate for underestimation of corneal fluorescence inherent to fluorophotometry measurements.[11,13,14]This underestimation results because the focal diamond created by the fluorophotometer for measurement of fluorescein concentration is wider than the thickness of the feline cornea.$k_{spat.res.}$ = $1/(1 - Q \text{ x } e^{Bd})$, where Q =0.9622, B = -1.848, and d is the thickness of the cornea in millimeters. A value of 0.565 mm has been reported for the corneal thickness in cats

less than 1 year of age and was utilized for *d* in this study, resulting in $k_{spat.res.} = 1.51$.[146] Published values for the feline anterior chamber volume (V_a = 820 µl) and corneal volume (V_c =165 µl) were used.[27] The denominator value of 1.2 represents a second correction factor designed to account for an inherent difference in fluorescence between the cornea and the aqueous humor.[11]

Data Analysis

Regression analysis of the natural log transformed corneal and aqueous fluorescein concentration was utilized to create linear decay curves. Correlation coefficients were calculated to assess the fit of the four time points to the approximated straight line. The slopes of these curves were then compared to ensure they were decreasing in a reasonably parallel fashion. Eyes were excluded from flow calculation if correlation or slope ratio values represented extreme outliers (defined as greater than three times the interquartile range). Aqueous humor flow rates were calculated for all eyes both before and during the assigned treatment. A repeated measures ANOVA was used to compare aqueous humor flow rates before and during treatment in the dorzolamide and control groups. IOP measurements before and during treatment in the control and dorzolamide groups were analyzed using a hierarchical linear mixed-model ANOVA with repeated measures and a Tukey-Kramer adjustment. IOPs were also analyzed using a repeated measures ANOVA with a Tukey-Kramer adjustment for multiple comparisons to evaluate time-matched IOPs before and after treatment in both the control and dorzolamide groups at each measurement time point. Statistical significance was set at a $P \leq 0.05$.

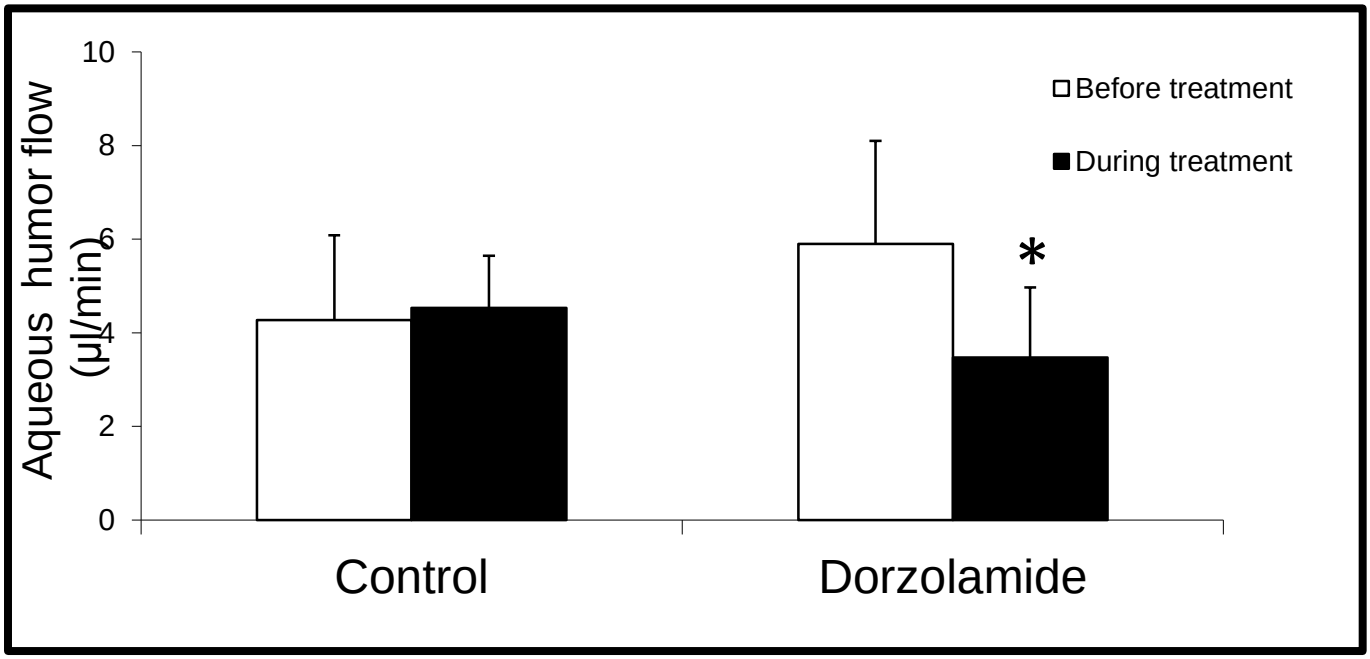
Results

The calculated correlation coefficients and slope ratios for the logarithmically transformed fluorescein concentration data utilized for aqueous humor flow calculation (for the cornea and aqueous humor) were acceptable, indicating the calculated aqueous humor flow values are accurate.

The average aqueous humor flow rate for normal cats prior to treatment was 5.90 ± 2.2 µl/min in the dorzolamide group and 4.27 ± 1.8 µl/min in the control group. There was no statistically significant difference between the two groups (P = 0.1308). Aqueous humor flow values after treatment for the dorzolamide and control groups were 3.47 ± 1.5 µl/min and 4.53 ±

1.1 µl/min, respectively. The difference between aqueous humor flow values before and after treatment was statistically significant for the dorzolamide group (P = 0.0002) and was not significant for the control group (P = 0.9922). These findings are summarized in Figure 3.1. The average decrease in aqueous humor flow in normal cats receiving topical 2% dorzolamide was 2.43 µl/min, a decrease of 41%.

Figure 3.1 Aqueous Humor Flow: Before and During Treatment

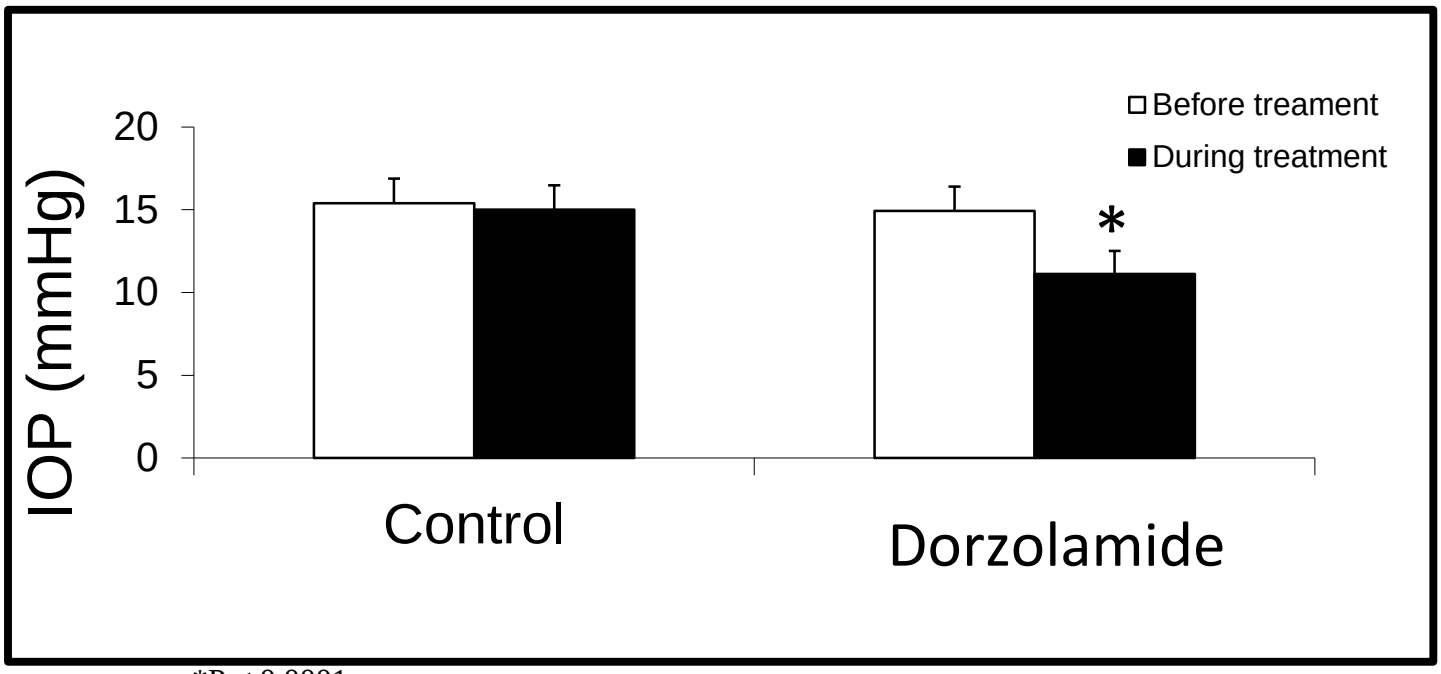


*P = 0.0002

The average IOP for normal cats prior to treatment was 14.9 ± 1.0 mmHg in the dorzolamide group and 15.5 ± 1.1 mmHg in the control group. There was no statistically significant difference between the two groups (P = 0.9288). IOP values after treatment for the dorzolamide and treatment groups were 11.1 ± 1.0 mmHg and 15 ± 1.0 mmHg. The difference between IOP before and after treatment was statistically significant for the dorzolamide group (P < 0.0001) and was not significant for the control group (P = 0.7198). These findings are summarized in Figure 3.2. The average decrease in IOP in normal cats receiving topical 2% dorzolamide was 3.9 mmHg, a decrease of 26%.IOPs were noted to be significantly reduced at all time points in dorzolamide treated cats when compared to their baseline values (P < 0.0001).

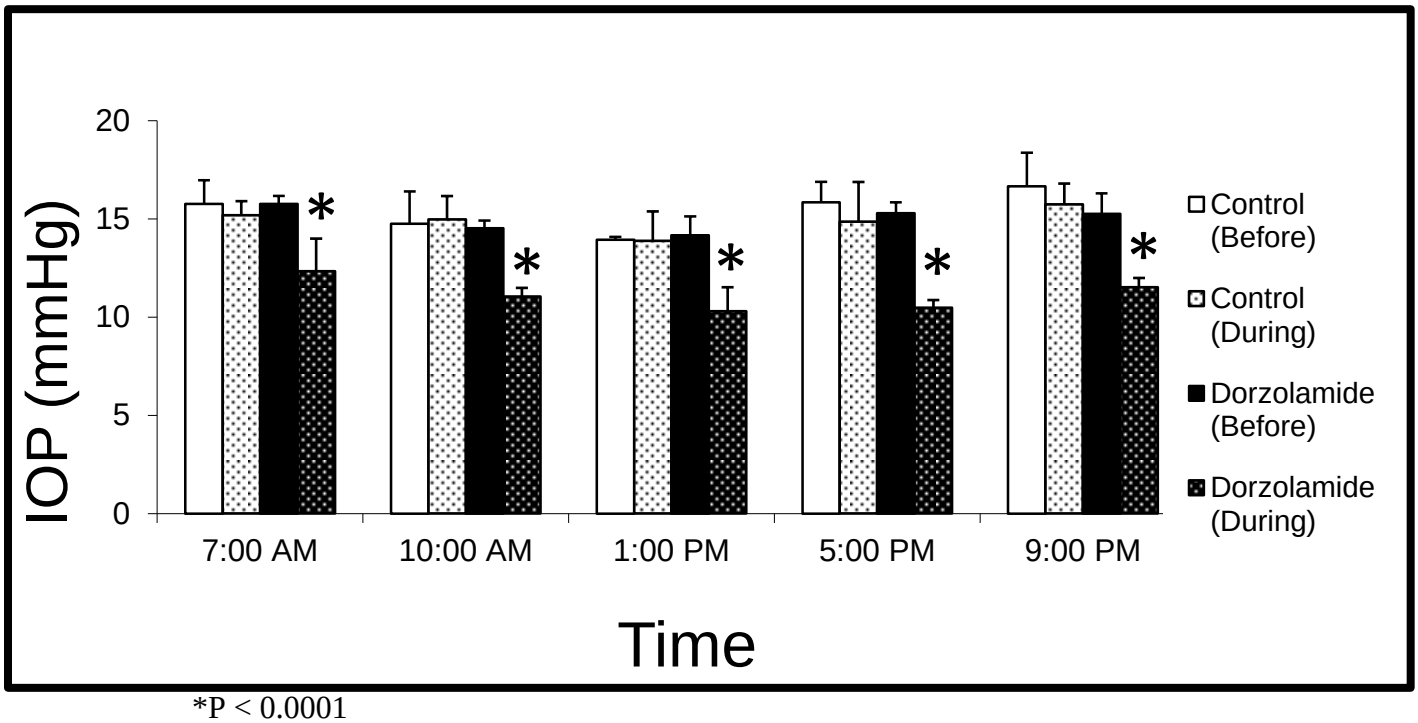
No statistical significance was seen at any time point in the control group ($P \geq 0.9680$). These findings are summarized in Figure 3.3.

Figure 3.2 Intraocular Pressure (Averaged): Before and During Treatment



*P < 0.0001

Figure 3.3 Intraocular Pressure (Time-Matched): Before and During Treatment



*P < 0.0001

Discussion

Fluorophotometry estimates the aqueous humor flow rate based on serial measurements of corneal and aqueous humor fluorescein concentrations following corneal fluorescein loading. The fluorophotometer is essentially a modified slit lamp with a fiber optic probe that emits a focused beam of blue (480 nm) light. A barrier filter allows only the green (520 nm) light that indicates peak fluorescence to be evaluated, thereby reducing the effect of scatter. The digital radiometer then compares the emitted fluorescein to known fluorescein curves to determine the concentration seen at each measurement point.[66] The decline of fluorescein concentration over time is attributed to drainage of the fluorescein-aqueous mix and further dilution as it is replaced by new aqueous humor that is being produced.

The results of this study document that topical 2% dorzolamide administered to normal cats three times daily reduced aqueous humor flow by 2.63 µl/min, which corresponds to a 41% reduction. The reduction in aqueous humor flow in the cats in this study is similar to the results in normal dogs (43%)[22] and monkeys (29%),[160\\59] and higher than the values reported for rabbits (17%)[153] and humans (13-19%).[114,157,160] In the rabbit study,[153] one drop of 2% dorzolamide was administered only once 2 hours prior to fluorophotometry, which may account for the less dramatic reduction in aqueous humor flow compared with the other studies. A wide variability in aqueous humor flow rates has been reported in ocularly normotensive humans with reported ranges of 0.2 to 32 µl/min,[161] 1.5 to 4.6 µl/min,[12] and 1.61 to 5.27 µl/min.[162] In dogs, variability in baseline aqueous humor flow rate has also been reported with ranges of 2.2-9.8 µl/min[22] and 1.47 to 10.69 µl/min.[23] Similarly, in our study there was a range of baseline aqueous humor flow rates from 1.17 to 10.67 µl/min. Despite the variability noted in aqueous humor flow rates, the difference in baseline aqueous humor flow rate for the dorzolamide group (5.90 $\pm$ 2.2 µl/min) and the control group (4.27 $\pm$ 1.8 µl/min) was not statistically significant.

Intraocular pressure was measured in this study using a rebound tonometer,[c] which estimates the IOP based on the deceleration of the probe as it rebounds from the corneal surface. The tonometer has an internal calibration curve for small animals (dogs and cats). In a recent study the rebound tonometer was compared with direct manometry and applanation tonometry in enucleated feline eyes.[87] The rebound tonometer correlated well with direct manometry in the 25-50 mmHg range. The mean IOP in clinically normal cats has been reported to be 20.74 mmHg (range 11-33mmHg) using the rebound tonometer.[87] Topical anesthetic was not used to

obtain IOP readings in this study. The use of topical anesthetic with the rebound tonometer did not significantly affect the IOP readings in normal cats[87] and dogs.[88] The mean pretreatment IOP values for both groups (14.9 and 15.4 mmHg) were within the reported normal range for cats using the rebound tonometer.

Topical administration of 2% dorzolamide three times daily for five days in normotensive cats caused an average decrease in IOP of 3.9 mmHg compared to pretreatment values, which corresponds to a 26% decrease. In the dorzolamide treated group the IOP at all 5 of the time points was significantly different from the pretreatment values ($P < 0.0001$). In the control group there was no statistically significant difference in IOP at any of the time points evaluated compared to pretreatment values ($P \geq 0.97$). Previous studies evaluating the effects of topically administered 2% dorzolamide solution in normotensive cats also demonstrated a statistically significant decrease in IOP. Twice daily administration of 2% dorzolamide in normotensive cats for 5 days resulted in a 2.9 mmHg (24.2%) decrease in IOP.[104] Another recent study also documented a statistically significant decrease in IOP in normal cats treated with topical 2% dorzolamide either twice daily (1.6 mmHg or 8.8%) or three times daily (2.2 mmHg or 12.1%).[103] Concomitant administration of 2% dorzolamide and 0.5 % timolol twice daily in that same study did not decrease the IOP greater than the three times daily administration of dorzolamide alone. The time points for intraocular pressure measurement were selected to allow for direct comparison to these previously published studies investigating the effect of topical 2% dorzolamide on feline IOP. In comparison, the application of 2% dorzolamide to normotensive eyes reduced IOP by 11% in monkeys,[159] 8.5-13% in humans,[113,140] and by 24.3% in dogs.[22]

The magnitude of the effect of dorzolamide on IOPs in normotensive cats may not be an accurate representation of the effect of this medication on cats with glaucoma. Dogs with glaucoma typically have a greater decrease in IOP compared to normotensive dogs when treated with topical and systemically administered CAIs.[139,163] A recent study demonstrated a dramatic decrease in IOP in cats with primary congenital glaucoma that were treated three times daily with topical 2% dorzolamide solution.[156] In that study, IOP decreased 16.8 mmHg in the left eye and 14 mmHg in the right eye, and the diurnal elevations in IOP were also diminished.

Our study suggests that the mechanism of action of topical 2% dorzolamide solution in normotensive cats is the suppression of aqueous humor production on the basis of decreases in

aqueous humor flow rate and IOP. Topical 2% dorzolamide may be a useful drug in treating cats with glaucoma or ocular hypertension.

Footnotes

[a] Liberty Research Inc, Waverly, NY; Sinclair Bio Resources LLC, Auxvasse, MO

[b] SL-14 Biomicroscope, Kowa Company, Ltd, Tokyo, Japan

[c] BioGlo™ HUB Pharmaceuticals LLC, Rancho Cucamonga, California

[d] TonoVet®, Tiolat Ltd, Helsinki, Finland

[e] HEINE Omega 180® Ophthalmoscope, HEINE Optotechnik, Herrsching, Germany

[f] LiquiTears, Major Pharmaceuticals, Livonia, Michigan

[g] Trusopt®, Bausch & Lomb Incorporated, Tampa, Florida

[h] AK-Fluor®10%,Akorn Inc, Lake Forest, Illinois

[i] FM-2 Fluorotron Master, OcuMetrics, Inc, Mountain View, California

[j] Domitor®, Orion Corporation, Espoo, Finland

[k] VetaKet®,IVX Animal Health Inc, St. Joseph, Missouri

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