

OCULAR EFFECTS FOLLOWING AQUEOCENTESIS IN DOGS USING
VARIABLE NEEDLE SIZES: FLUOROPHOTOMETRIC AND TONOMETRIC
EVALUATION

by

RACHEL A. ALLBAUGH

B.S., Iowa State University, 2000
D.V.M., Iowa State University, 2004

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Clinical Sciences
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2009

Approved by:

Major Professor
Amy J. Rankin, DVM, MS
Diplomate ACVO

Abstract

Objective – To measure blood aqueous-barrier breakdown following aqueocentesis using various needle sizes and to monitor the intraocular pressure (IOP) response.

Animals – 24 healthy, adult dogs received treatment (24 treated eyes, 24 contralateral eyes); 3 dogs were untreated controls (6 control eyes).

Procedures – Dogs receiving treatment were divided into 3 equal groups (25-, 27-, or 30-gauge needle aqueocentesis). In each dog the treated eye was determined randomly, the contralateral eye was untreated. Dogs that did not have aqueocentesis performed in either eye were used as controls. Aqueocentesis at the lateral limbus was performed under sedation and topical anesthesia. Anterior chamber fluorophotometry was performed before and after aqueocentesis on day 1. On days 2-5 sedation and fluorophotometry were repeated. Intraocular pressure was measured with a rebound tonometer at multiple time points.

Results – Aqueocentesis resulted in blood-aqueous barrier breakdown in all treated eyes with barrier reestablishment present by day 5 detected by fluorophotometry. On day 2 the contralateral untreated eyes of all groups also showed statistically significant increased fluorescence ($P < 0.05$) following treatment of the opposite eye, but these values were not statistically significantly greater than untreated controls. In treated eyes there was no statistical difference in fluorescein concentration or IOP between 27- and 30-gauge needles. Use of the 25-gauge needle resulted in a statistically significant increase in anterior chamber fluorescence on days 3 and 5. It also caused a statistically significant increase in IOP 20 minutes following aqueocentesis as compared to the 27- and 30-gauge needles. Aside from this transient ocular hypertension, rapid resolution of ocular hypotony following aqueocentesis was observed in all treatment groups.

Conclusions and Clinical Relevance – Aqueocentesis using a 25-gauge needle resulted in a greater degree of blood-aqueous barrier breakdown and a brief state of intraocular hypertension following paracentesis. Use of a 27- or 30-gauge needle is recommended for aqueous paracentesis. A consensual ocular reaction appeared to occur in dogs following unilateral traumatic blood-aqueous barrier breakdown and may be of clinical significance. Statistical significance was limited in this study due to high variability and large standard deviations.

Table of Contents

List of Figures	v
List of Tables	vi
Acknowledgements	vii
CHAPTER 1 - Literature Review	1
Aqueous Humor	1
Aqueous Humor Dynamics	1
Tonometry	3
Aqueous Humor Centesis	4
Blood-Aqueous Barrier	6
Anatomy	6
Blood-Aqueous Barrier Breakdown	8
Consensual Reactions	12
Species Variations	13
Quantification of Blood-Aqueous Barrier Breakdown	14
Slit-Lamp Assessment	14
Fluorophotometry	15
Laser Flaremetry	19
Aqueous Humor Microprotein Assays	21
Comparison of Techniques	22
CHAPTER 2 - Ocular Effects Following Aqueocentesis in Dogs Using Variable Needle Sizes: Fluorophotometric and Tonometric Evaluation	23
Introduction	23
Materials and Methods	24
Animals	24
Aqueocentesis	24
Fluorophotometry	26
Tonometry	27
Study Time Points	27

Data Analysis	29
Results.....	30
Fluorophotometry	30
Tonometry.....	36
Discussion.....	37
Conclusion	41
Acknowledgements.....	41
References.....	42

List of Figures

Figure 1-1 Tyndall light phenomenon demonstrating aqueous flare.....	15
Figure 1-2 Ocular fluorophotometry being performed on a canine patient.....	16
Figure 1-3 Data generated by the fluorophotometer showing a graphical display of ocular fluorescence and equivalent fluorescein concentrations (ng/ml) with peak plateau levels present in the anterior chamber.....	17
Figure 1-4 Laser flare cell meter.....	19
Figure 1-5 Feline patient positioned in front of the laser flare cell meter.....	20
Figure 2-1 Globe stabilization and needle positioning for performing aqueocentesis.....	25
Figure 2-2 Patient positioning for ocular fluorophotometry with use of the anterior chamber adapter.....	26
Figure 2-3 Use of the Tono-Vet® rebound tonometer for intraocular pressure measurement.....	27
Figure 2-4 Mean anterior chamber fluorescence (ng/ml) in 25-gauge needle treated and contralateral untreated eyes.....	33
Figure 2-5 Mean anterior chamber fluorescence (ng/ml) in 27-gauge needle treated and contralateral untreated eyes.....	33
Figure 2-6 Mean anterior chamber fluorescence (ng/ml) in 30-gauge needle treated and contralateral untreated eyes.....	34
Figure 2-7 Mean anterior chamber fluorescence (ng/ml) in treated eyes of all groups.....	34
Figure 2-8 Mean anterior chamber fluorescence (ng/ml) in untreated eyes of all groups.....	35
Figure 2-9 Individual eyes of control dogs showing anterior chamber fluorescence.....	35
Figure 2-10 Mean intraocular pressure (mm Hg) of treated eyes in all groups.....	36
Figure 2-11 Mean intraocular pressure (mm Hg) of contralateral untreated eyes in all groups...	37

List of Tables

Table 2-1 Experimental schedule time points on Day 1 of the study (hours:minutes).....	28
Table 2-2 Experimental schedule time points on Day 2 of the study (hours:minutes) with subsequent daily evaluations every 24 hours through Day 5.....	29
Table 2-3 Anterior chamber fluorescein concentrations (mean \pm standard deviation in ng/ml) for treated, contralateral untreated, and control eyes at each study time point.	32

Acknowledgements

I would like to take this opportunity to thank the members of my graduate committee, Drs. Amy Rankin, Harriet Davidson, and James Roush for their support and guidance with this Master's thesis. They not only provided me with wonderful mentorship, but also genuine friendship over the past few years. Thanks to Dr. Michael Dryden for providing the dogs for the study, as well as the Animal Resource Facility faculty and staff for their assistance in housing and caring for the animals used in this project. I would also like to thank Amanda Davis and Sandra Donker for their assistance with animal handling and data collection.

Furthermore, I would like to thank the Kansas State University College of Veterinary Medicine Mentored Clinical, Applied or Translational Research Grant for funding the project.

CHAPTER 1 - Literature Review

Aqueous Humor

Aqueous Humor Dynamics

Aqueous humor is the optically clear fluid that fills the anterior and posterior chambers in the anterior portion of the eye.¹ Normal aqueous humor is nearly acellular with very low protein concentration.² Though this transparent liquid occupies spaces in the eye, it is not a static fluid body. It is actually a slowly flowing stream which provides nutrition to the intraocular structures and allows removal of metabolic waste products.³ Proportionately, the eye contains the largest avascular mass found in any organ in the body with blood vessels not normally present in the cornea, lens, vitreous or trabecular meshwork.⁴ Nutrition of these avascular structures is accomplished primarily by aqueous humor.⁴

Aqueous humor is formed by the ciliary body in the posterior chamber of the eye. Ciliary processes are composed of blood vessels embedded in a loose connective tissue stroma with a double layer of epithelial cells lining the inner surface.⁴ Aqueous humor originates from the vascular sinuses within these folds and processes, fills the posterior chamber, flows through the pupil into the anterior chamber, and drains into the iridocorneal angle.⁵ The rate of aqueous humor formation varies among species and is roughly 2 $\mu\text{l}/\text{min}$ in humans⁴ compared to 4.54 $\mu\text{l}/\text{min}$ in dogs.⁶

Aqueous humor is formed by three different mechanisms: diffusion, ultrafiltration, and active secretion by the ciliary process epithelial cells. Diffusion of solutes occurs down a concentration gradient across the ciliary epithelial barrier while ultrafiltration occurs when movement of water or a compound across a cell membrane is increased by a hydrostatic force.⁷ The latter results from differences between the ciliary body capillary pressure and intraocular pressure;¹ however, it has recently been suggested that ultrafiltration has little if any role in aqueous humor formation.⁷ Both diffusion and ultrafiltration are examples of passive processes. In contrast, active transport of certain solutes, most notably Na and Cl, by the ciliary body epithelium is the principal component of aqueous humor formation and drives fluid inflow from the ciliary body stroma to the posterior chamber.^{4,7-11} Present in the ciliary epithelium, the

membrane-bound enzyme complex sodium-potassium-ATPase actively transports sodium ions from blood to the aqueous humor.¹ Chloride ions enter the posterior chamber through electroneutral transporters and chloride channels, and represent the principal anion secreted by the ciliary epithelium.⁸ In addition, the enzyme carbonic anhydrase catalyzes the reversible hydration of carbon dioxide by the reaction: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+$. Both cytosolic carbonic anhydrase isoform II and membrane-bound isoform IV have been identified in the ciliary epithelium with net transepithelial bicarbonate transport thought to result from the two isoenzymes' combined effect.⁷ Entry of sodium, chloride, and to a lesser extent, bicarbonate ions into the posterior chamber generates an osmotic gradient and results in transepithelial fluid secretion across the ciliary epithelium.⁷ Modulating ciliary epithelial enzyme function impacts aqueous humor production with carbonic anhydrase inhibitors causing up to 50-60% reduction in the formation of aqueous.¹²⁻¹⁵

Drainage of aqueous humor from the eye is via the iridocorneal angle using the conventional route as well as the unconventional uveoscleral outflow pathway.⁵ The conventional route of drainage is via the iridocorneal angle, through the trabecular meshwork and into the angular aqueous plexus in most species or Schlemm's canal in primates.¹ Unconventional outflow is used to varying degrees in different species with fluid leaving by diffusion through the iris, ciliary body, and vitreous.¹ Studies in dogs using fluorescein-labeled dextran and different sized microspheres have shown that in uveoscleral outflow aqueous leaks into the interstitial spaces of the uvea to become part of the tissue fluid, with fluid then moving through the ciliary muscle into the supraciliary and suprachoroidal spaces to be absorbed by the choroidal and scleral circulation.^{16,17} In normal dogs, uveoscleral outflow accounts for 15% of aqueous drainage but in glaucomatous eyes uveoscleral outflow is markedly reduced.¹⁸

In the continuous process of aqueous humor formation and drainage intraocular pressure (IOP) is created.⁵ The rate of aqueous humor formation from within the ciliary stromal tissue is influenced by humoral and autonomic innervation so that constant IOP is sustained.¹ A normal IOP is essential for maintaining the shape of the eye, sustaining its refractive properties, and preserving the close association between the retina and choroid.^{5,8}

Tonometry

The balance of aqueous humor formation and drainage helps to maintain a constant normal pressure in the eye. Pressure in the normal human aqueous and vitreous averages 15.5 mm Hg.³ The normal IOP of most animals is usually between 15 and 25 mm Hg due to conservation between species.¹⁹ Intraocular pressure can be measured experimentally by cannulation of the eye, but this is an invasive and complicated technique.³

Tonometry is the indirect measurement or estimation of IOP through the cornea. Historically, tonometers have been used that fall into two categories, those that indent the cornea and those that flatten, or applanate, the cornea.³ Indentation tonometers provide reasonable estimations of IOP but are not considered as accurate as applanation tonometers.¹⁹ Applanation tonometers are easier to employ and the portable, reliable TonoPen® instrument is very popular among veterinary ophthalmologists. Studies using the Tono-Pen® applanation tonometer, report the normal mean canine IOP to be 16.7 +/- 4.0 mm Hg²⁰ and 19.2 +/- 5.9 mm Hg.²¹ Disadvantages of this device are that it has difficulty measuring very low IOPs²⁰ and that it has been reported to overestimate IOP at lower pressures and underestimate IOP at higher pressures.²²

A recently developed intraocular pressure measuring device is the induction-impact, or rebound, tonometer. A rebound tonometer has a magnetized probe propelled to come into contact with and then rebound from the corneal surface, with the rebound motion characteristics detected by a sensing coil and used to calculate IOP.²³ The disposable probe has a round plastic tip 1 mm in diameter to prevent corneal damage. Due to its small size, topical corneal anesthesia is not necessary for IOP measurement as compared to indentation and applanation tonometers used in clinical settings. Results of a study by Baudouin and Gastaud documented a significant decrease in tonometrically-measured IOP following application of oxybuprocaine and betoxycaine topical anesthetics;²⁴ however, in another study no significant difference was present after the application of lidocaine topically.²³ The rebound tonometer is well-tolerated by dogs, provides rapid, reproducible measurements in both normal and glaucomatous animals, and can measure intraocular pressure values from 0 to 99 mm Hg.²³ In a recent study, IOPs were measured in dogs using both the Tono-Pen® applanation tonometer and the TonoVet® rebound tonometer and found to be comparable (mean IOP ± SD: Tono-Pen® 12.9 ± 2.7 mm Hg, TonoVet® 10.8 ± 3.1 mm Hg).²⁵ The two tonometers have also been shown to provide similar measurements following

intraocular surgery in dogs.²⁶ In 2006, Gorig et al used freshly enucleated canine eyes to compare manometric and tonometric measurements and found that the induction-impact tonometer was the most accurate, while with the Tono-Pen® and MacKay-Marg® applanation tonometers were increasingly less accurate as the IOP was elevated.²³

Tonometry is an essential diagnostic procedure used during examinations to evaluate abnormal eyes and to monitor glaucomatous eyes. With glaucoma, elevated pressure levels related to reduced aqueous outflow are present in the eye.⁴ Ocular hypertension is generally associated with the glaucoma disease complex, but can also be present immediately following intraocular surgery. Postoperative ocular hypertension is a transient increase in IOP (>25 mm Hg) that occurs within 72 hours following cataract surgery and may occur in as many as 50% of cases.²⁷ Intraocular pressures remaining greater than normal for a prolonged period of time can lead to irreversible damage to the retina and optic nerve and therefore require prompt treatment. Pressures lower than the normal range, ocular hypotension, may be present in eyes with uveitis. Prolonged hypotension may result in shrinking of the globe, or phthisis bulbi. Monitoring IOP is imperative with ocular disease and it is for this reason that tonometry has become a standard measure during complete ophthalmic examinations in both humans and animals.

It has been shown that body position can alter intraocular pressure in dogs as measured by tonometry and that the sternal recumbent position may allow for the most consistent and repeatable IOP measurements in research investigations.²⁸ Physiologic variables such as changes in extraocular muscle tone and eyelid contraction may also alter IOP measurements. These factors are possible mechanisms by which systemic anesthetic drugs affect IOP. In one study, dogs administered 5 mg/kg ketamine and 10 mg/kg ketamine with 0.5 mg/kg diazepam had significantly increased IOP over baseline values.²⁹ An unexplained finding in this study was why dogs administered 10 mg/kg ketamine alone did not show a significant IOP change.²⁹ Though both patient positioning and anesthetic drug factors are of consequence, it is important to note that in both studies IOP differences were only a few mm Hg with no values reported to be above the clinically normal canine pressure range.^{28,29}

Aqueous Humor Centesis

Anterior chamber paracentesis is used in clinical practice for diagnostic and therapeutic purposes to remove aqueous humor fluid from the eye. The procedure can be done on

cooperative, awake patients under topical anesthesia, though in some veterinary patients sedation or short-acting general anesthesia may be required. Prior to paracentesis the ocular surfaces are cleansed with dilute (5%) povidone iodine solution, rinsed with normal saline, and topical anesthetic is applied. With the eyelids held open, the bulbar conjunctiva is grasped with small forceps near the site of entry and a small needle is inserted bevel up through the perilimbal cornea or subconjunctival limbus. The needle enters the eye parallel and anterior to the iris avoiding contact with the iris, lens and corneal endothelium. The needle size reported for aqueous paracentesis use may range from 25 to 30-gauge.^{19,30} Once the needle is in the eye, aqueous humor fluid is aspirated by a small syringe. An alternative technique is to let the hub of the needle fill with fluid without a cumbersome syringe attached, allowing greater control over the needle's position.³⁰ An additional approach more commonly used in humans is to use a sterile surgical blade inserted through the peripheral cornea to make a self-sealing stab incision.³¹⁻³³ The technique will vary depending on the indication for paracentesis, the species being treated, and the clinician's preference.

Aqueous paracentesis is used in clinical practice to collect samples from inside of the eyeball for cytological evaluation, culture and sensitivity, antibody determination or other diagnostic purposes.^{2,34-43} Specific ocular diseases where it may be utilized include cases of uveitis or intraocular neoplasia. As long as patients are selected appropriately and aseptically prepared for the procedure, the technique has been found to be safe with minimal risk of complication.^{38,44}

In addition to diagnostic paracentesis, therapeutic paracentesis is also utilized in a clinical setting. Patients with glaucoma or postoperative ocular hypertension may be treated with aqueous paracentesis as emergency therapy to rapidly reduce the IOP and prevent damage to the retina and optic nerve.^{19,31-33,45,46} In a human study, cataract surgery patients experiencing postoperative ocular hypertension were treated with paracentesis, and though it provided immediate reduction in IOP, pressures rebounded to near initial values by one hour after treatment.³³ Recent human glaucoma studies have combined aqueous paracentesis with medical therapy and found that paracentesis provides rapid symptomatic relief, as opposed to medical management alone, and can be considered as adjunctive therapy in the management of acute elevation of IOP.^{31,32,45} Therapeutic paracentesis may also be utilized prior to intraocular

injection of drugs, such as tissue plasminogen activator, to prevent abnormally elevated pressure following drug injection.

For many decades aqueous paracentesis has been used as a model of intraocular inflammation because it causes breakdown of the blood-aqueous barrier with resulting signs of uveitis.⁴⁷⁻⁸¹ Research studies involving paracentesis have allowed detailed study of the blood-aqueous barrier, have improved our understanding of species-specific differences, and have allowed evaluation of therapies to prevent or reduce barrier compromise.

Anterior chamber centesis is also used in experimental studies to collect aqueous humor for analysis of the fluid components. Multiple investigations have measured anterior chamber drug levels of various antimicrobial agents following topical, subconjunctival, or systemic administration.^{74,82-88} Aqueous paracentesis is also used to monitor intraocular inflammation by analyzing protein, cells, and inflammatory mediators in the fluid. It has been documented that aqueous humor in healthy animal species is nearly acellular with low protein concentration and only albumin detectable on electrophoresis.² Increased cellular composition, elevated protein values, and other measurable inflammatory mediators in aqueous humor have been used to quantify blood-aqueous barrier breakdown in numerous clinical cases and research studies.^{49,50,52,53,57-68,70-73,75,76,78-81,89-95}

Blood-Aqueous Barrier

Anatomy

The blood-ocular barriers consist of the blood-aqueous barrier and the blood-retinal barrier, functioning to keep the eye as a privileged site by regulating the contents of the ocular fluids and protecting the internal ocular tissues from variations which occur constantly in the systemic circulation.¹¹ These barriers provide a suitable, highly regulated, chemical environment for the avascular, transparent tissues of the eye.¹¹ It is important for optical clarity that virtually no protein or cells be present in the ocular fluids as these components would result in light scattering and impaired vision.

The blood-aqueous barrier is composed of tight junctions between the apicolateral surfaces of the nonpigmented epithelial cells of the ciliary body processes and between the endothelial cells of the iris vasculature.^{1,11,67} Following intravenous injection of horseradish peroxidase, histopathologic examination of normal rabbit eyes demonstrated marker presence in

iris vessels and ciliary stroma, but horseradish peroxidase was blocked by zonula occludens of the iris endothelial cells and those at the sides of the ciliary process nonpigmented epithelial cells.⁹⁶ It has been shown that the intercellular tight junction proteins occludin and ZO-1 are integral components of the blood-aqueous barrier.^{97,98}

In addition to the nonpigmented ciliary epithelium lateral tight junctions, the morphology of the normal bilayered ciliary body epithelium is a formidable barrier to blood-borne substances with numerous desmosomes and complicated interdigitations between adjoining nonpigmented and pigmented ciliary body epithelial cells.^{67,99} In the healthy eyes this intercellular pathway from the pigment epithelium to the posterior chamber is extremely narrow, long, and tortuous; however, in abnormal eyes this pathway becomes much simpler, shorter, and wider due to separation of the epithelial cells.⁹⁹

A recent review of the blood-aqueous barrier shifts the concept slightly to also include the posterior pigmented iris epithelium with tight junctions analogous to those in the nonpigmented ciliary epithelium.¹⁰⁰ Many years prior, Pedersen observed blockage of horseradish peroxidase at the posterior limit of the iris and surmised the presence of zonula occludens between the posterior iris epithelium considering they are the only type of junctions effective at blocking horseradish peroxidase movement though the intercellular spaces of epithelia.¹⁰¹ This finding of similar tight junctions in the posterior iris and nonpigmented ciliary epithelium is not surprising as both epithelial tissues originate embryologically from the inner layer of the optic cup and are confluent with one another.⁵

Given that the non-pigmented ciliary epithelium, posterior iris epithelium, and iris vessels are impermeable to albumin,^{100,102} yet small amounts of protein are present in normal aqueous humor,^{2,92} the source of this protein is believed to be leakage from the ciliary body stroma via the iris.^{100,103} It is well known that ciliary body blood vessels are highly fenestrated and leak most of the plasma components into the stroma.^{1,100,103} Though proteins are prevented from entering the posterior chamber by the tight junctions of the ciliary epithelium, proteins can diffuse forward along the continuous pathway of loose connective tissue from the ciliary body stroma to that of the iris.¹⁰⁰ With no epithelium present on the anterior surface of the iris, protein reaching the anterior iris surface is able to enter the anterior chamber.¹⁰⁰ A kinetic model of fluorescein diffusion assessing transfer from plasma, into the iris stroma, and then into the anterior chamber closely matched actual findings in rabbit and human eyes.¹⁰⁴ This finding supported the

conclusion that the principal route of normal aqueous humor plasma-derived protein entry was via the iris and not the posterior chamber.¹⁰⁰ Thus in normal eyes the posterior chamber is free of protein due to the tight junctions of the nonpigmented ciliary and posterior pigmented iris epithelium along with the unidirectional flow of aqueous humor forward through the pupil.¹⁰⁰ This modified barrier separates constituents of plasma from the tissues behind the iris and is extremely important as there is no functional barrier present between the aqueous humor and the vitreous humor.¹

Diurnal protein variations have been detected in the aqueous humor of normal human eyes.¹⁰⁵ These variations were found to be due to changes in aqueous humor flow rate and not changes in blood-aqueous barrier protein permeability as the latter was stable over a 24-hour period.¹⁰⁵

Blood-Aqueous Barrier Breakdown

When the anterior segment of the eye becomes traumatized, irritated, or inflamed clinical changes that manifest include conjunctival hyperemia, uveal vasodilation, pupillary constriction, breakdown of the blood-aqueous barrier, and a transient rise in IOP followed by relative hypotony.^{66,71,72,103} Blood-aqueous barrier breakdown results in leakage of plasma proteins into the aqueous humor due to collapse of the epithelial barrier and failure of endothelial cell junctions.⁶⁷

Disruption of the blood-aqueous barrier can occur following antidromic release of endogenous vasodilator substances and from the direct action of prostaglandins.¹⁰³ Response to an irritative stimulus like topical nitrogen mustard depends on intact, sensory innervation and is mediated by pain fibers as opposed to prostaglandins or the adrenergic nervous system.⁶⁶ This is supported by the fact that aspirin treatment did not inhibit aqueous humor protein rise after topical nitrogen mustard application to rabbit eyes.⁷² In addition to nitrogen mustard-induced irritation, antidromic stimulation of the trigeminal nerve and formaldehyde-induced irritation of the eye do not cause prostaglandin release nor are the responses to these stimuli inhibited by prostaglandin synthetase inhibitors.¹⁰⁶ The mediators of blood-aqueous barrier breakdown following trigeminal nerve stimulation are likely sensory neuropeptides like calcitonin gene-related peptide and substance P.¹⁰⁷⁻¹¹⁰ Substance P-like immunoreactivity was documented in rabbit eyes following trigeminal nerve electrical stimulation, and similar ocular signs of miosis

and blood-aqueous barrier breakdown were also observed in rabbit eyes after intracameral injection of substance P, with the nonsteroidal anti-inflammatory agent indomethacin failing to block the effects.¹⁰⁷

Aside from neurogenic stimulation, ocular irritative and inflammatory responses are most notably mediated by prostaglandins. Prostaglandins are formed in vivo from metabolism of cell membrane arachidonic acid via the cyclooxygenase pathway.¹¹¹ PGE and PGF_{2α} are the predominant metabolites present in ocular tissues during inflammatory events.⁸⁰ Rabbits administered various prostanoids showed a rapid, monophasic response of blood-aqueous barrier breakdown to PGE₂ and the EP₂ selective agonist 11-deoxy PGE₁ indicating EP₂ receptor subtype mediation.¹¹² Paracentesis-induced disruption of the blood-aqueous barrier is mediated largely by prostaglandins, most notably E-type, released from the anterior uveal tissues with the response minimized by prostaglandin inhibitor treatment.^{47,49,50,55-57,65,71-73,79,113-115}

As previously discussed, aqueous paracentesis has been used as a model of blood-aqueous barrier breakdown and has contributed significantly to description of the ocular changes. It has been documented that the main site of blood-aqueous barrier disruption is the ciliary processes.^{71,99,106,116,117} Fluorescein angiography has been used to document that after paracentesis the ciliary processes are the origin of the protein and fluorescein that enter the anterior chamber via the pupil.⁵⁹ Scanning electron microscopy images of the ciliary body from monkey eyes following paracentesis have demonstrated swollen ciliary processes with plasma proteins, particularly fibrin, entering the posterior chamber.⁵¹ Light and electron microscopy also showed prominent structural alterations in the ciliary epithelium of the pars plicata in cynomolgus monkey eyes, but these changes were not diffuse with the anterior portion more severely affected and the posterior epithelium less disrupted.⁶⁹ However, following prostaglandin-treatment of rabbit eyes, the tracer horseradish peroxidase was seen penetrating the anterior and posterior ciliary process nonpigmented epithelium intercellular clefts equally.¹¹⁸

Even though the ciliary processes are known to be the main site of blood-aqueous barrier breakdown, other tissues have also been studied. Following topical application of PGE₁ or PGE₂ to rabbit eyes, iris vessels become permeable to horseradish peroxidase with notable leakage into the iris stroma.¹⁰¹ On the other hand, posterior inflammation does not appear to result from ocular irritation or trauma to the anterior segment. Aqueous paracentesis of rabbit eyes resulted in increased aqueous humor PGE₂ values; however, PGE₂ was not detected in the vitreous,

indicating that prostaglandins do not diffuse posteriorly and that they are not released locally from posterior segment tissues.¹¹⁹

Early studies identified the importance of prostaglandins following paracentesis; however, given that no drug completely abolished the increase in protein after paracentesis injury, it was hypothesized that an antidromic nervous component existed or that the ciliary epithelium was mechanically damaged during paracentesis.^{65,71} Paracentesis-induced blood-aqueous barrier breakdown has been studied specifically in dogs and results show that prostaglandins are indeed the most important mediators of the ocular irritative response, with sensory neuropeptides less important and leukotrienes playing no role.⁴⁷ Though topical flurbiprofen significantly reduced blood-aqueous barrier breakdown as measured by anterior chamber fluorophotometry,⁴⁷ the inability to completely abolish the response suggests that additional non-prostaglandin, non-sensorineurally-derived mediators may be involved or that the rapid reduction in IOP causes physical damage to the blood-aqueous barrier.^{47,51,54} Similar canine studies evaluating other nonsteroidal anti-inflammatory drugs document reduced but not abolished aqueous protein increases following paracentesis, again suggesting the blood-aqueous barrier breakdown is only partially mediated by prostaglandins.^{75,76,120}

The sequence of events after paracentesis-induced blood-aqueous barrier breakdown involves prostaglandin accumulation followed by anterior chamber protein entry. After paracentesis of rabbit eyes, aqueous humor PGE₂ and 6-keto-PGF_{1α} values rapidly increased, followed by protein increases to maximal levels at 30 minutes.⁷⁸ The increased protein concentration in the reformed, or secondary, aqueous humor has a decreased albumin:globulin ratio and increased percent of α- and γ- globulins as opposed to the larger β-globulins.⁸⁹ An early paracentesis study showed that marked hypotony significantly affected the protein content of the reformed aqueous, but speed of aspiration and grasping of the conjunctiva with forceps had no effect on protein content.¹²¹ Neupert and Lawrence also found that final IOP (<12.5 mm Hg) after paracentesis may be more important in determining aqueous protein concentration than rate of IOP change.⁸⁹

Blood-aqueous barrier breakdown is accompanied by a rapid rise in IOP, a response also induced by prostaglandins. Various prostaglandins administered topically and systemically have been shown to cause transient IOP elevation in animals.¹²²⁻¹²⁴ Paracentesis-induced ocular hypertension follows blood-aqueous barrier breakdown and is variable between species. Unger et

al found rebound hypertension was maximal in treated eyes of rabbits 15-20 minutes following paracentesis with pressures 13 mm Hg greater than control values.⁷¹ A separate rabbit study documented elevated IOP within 15 minutes and lasting 2 hours after paracentesis.⁶⁷ The ocular hypertension is likely due to a sudden rise in the anterior uveal blood volume with a subsequent increase in ultrafiltration and plasma extravasion, though blepharospasm and reflex contraction of the extraocular muscles may also confound the effect.^{103,125} In addition to inflow of plasmoid aqueous, the role of pupillary block associated with severe miosis was also suggested as rabbit eyes pretreated with sector iridectomy had less profound (50% less) pressure elevation after paracentesis;⁶⁷ however, in the study by Unger et al only slight pupillary constriction was noted.⁷¹ Following paracentesis in cynomolgus monkeys IOP increased rapidly reaching its highest value (20 mm Hg) at 3 hours, then returned to normal at 6-9 hours.⁶⁹

Though prostaglandins are known to cause increased IOP, other mediators of ocular inflammation may also be involved or have similar effects, with variation noted between species.¹²⁶ This is suggested by studies in which nonsteroidal anti-inflammatory agents were unable to block the hypertensive response following ocular irritation.^{72,127}

After blood-aqueous barrier breakdown ocular hypertension persists until aqueous outflow increases as compensation, or congestion and edema of the ciliary processes decrease aqueous formation.^{103,109} Prostaglandins are believed to increase aqueous humor drainage via uveoscleral outflow, though other mediators may also be associated with the subsequent fall in IOP.^{109,126} Thereafter ocular hypotony ensues, with decreased IOP a common clinical finding in uveitis.¹²⁸

It is uncertain exactly how long the blood-aqueous barrier takes to recover its function after damage with variability likely dependent on type and severity of trauma as well as the species affected. After paracentesis of rabbit eyes, increased aqueous humor prostaglandin and protein levels decreased to near baseline values 48 hours after trauma indicating almost complete resolution.⁷⁸ In a monkey study involving paracentesis and intravenous horseradish peroxidase, breakdown of the blood-aqueous barrier was not functionally repaired even seven days after the operation with marker molecules noted in the intercellular spaces beyond the nonpigmented ciliary epithelium tight junctions.⁷⁷ A separate study using cynomolgus monkeys noted persistent morphologic differences present in the anterior portion of the pars plicata seven days following paracentesis.⁶⁹

In addition to the numerous studies involving paracentesis, other research models have also been used to study blood-aqueous barrier breakdown and therapeutic modalities. Investigations have utilized topical irritants,⁴⁸ topical pilocarpine,^{81,129-131} ocular surgery,¹³² anterior segment ischemia,¹³³ laser treatment of the iris^{59,134} or lens,^{90,91,127,135} intraocular injection of endotoxin,^{80,93,115,136-138} intravitreal injection of vascular endothelial growth factor,¹³⁹ intravitreal injection of endothelin-1,⁹⁶ intravitreal injection of adjuvant,¹¹⁵ and a more recent ocular perfusion model that does not have to be performed on live animals.¹⁴⁰

Blood-aqueous barrier breakdown associated with anterior uveitis results in the clinical changes previously discussed, and if severe may be accompanied by signs of ocular pain, epiphora, photophobia, corneal changes, hypopyon, hyphema, and iris changes.^{141,142} Consequences of severe or prolonged blood-aqueous barrier breakdown include iris adhesions (synechiae), pre-iridofibrovascular membranes, loss of corneal transparency, cataract, lens subluxation, glaucoma, and phthisis bulbi.¹⁴² Treatment of ocular inflammation utilizing both corticosteroids and nonsteroidal anti-inflammatory agents to block production of prostaglandins and other metabolites of arachidonic acid is imperative so that vision-threatening sequelae of ocular inflammation can be avoided.^{111,143,144}

Consensual Reactions

Not only does ocular trauma affect the treated eye, but a consensual reaction has also been documented in the contralateral untreated eye of rabbits^{117,119,145-147} and humans.¹⁴⁸ Following anterior chamber paracentesis of rabbit eyes a rapid rise in PGE₂ levels was documented in treated eyes as well as contralateral untreated eyes.¹¹⁹ Scanning electron microscopy of rabbit eyes treated with paracentesis and contralateral control eyes demonstrated changes in ciliary body processes consistent with both a direct and consensual reaction.¹¹⁷ The researchers hypothesized the consensual response was carried from the traumatized to non-traumatized eye by a neural reflex arc but could not rule out a modulating or mediating role for prostaglandins.¹¹⁷ Early studies involving intracameral injections of prostaglandins in rabbit eyes resulted in elevated IOP in both the treated and contralateral untreated eye.^{145,146} The authors concluded the consensual reaction was due to a transfer of prostaglandin from the injected eye to the opposite eye via systemic blood circulation.^{145,146} However, a later study involving rabbit paracentesis and anterior segment fluorescein angiography found that the consensual responses

were more effectively inhibited by nerve-blocking agents than prostaglandin inhibitors and therefore the interocular pathway mediating the response was probably neural.¹⁴⁷ This is supported by the fact that prostaglandins released into the general circulation are rapidly inactivated by the liver and lungs so that only scant amounts may reach arterial circulation.¹⁴⁷ The consensual ocular reaction is an important biological finding and is clinically noteworthy as it was documented immediately following and then up to one month following cataract surgery in humans.¹⁴⁸ A study using paracentesis and fluorophotometry in cynomolgus monkeys did not find increased fluorescein concentration in the contralateral eye though.⁷⁰

Species Variations

Blood-aqueous barrier breakdown has been studied extensively over the years but there is considerable species variation in the responsiveness of the eye to acute insults.¹²⁵ This variability is important to note, especially in regard to research models and when evaluating therapeutic strategies. Rabbit eyes are much more responsive to injury than primates and the physiologic response is somewhat altered.¹⁰³ For example, paracentesis in monkeys causes only a mild and short-lived breakdown of the blood-aqueous barrier,⁷⁰ and protein accumulation in the anterior chamber following paracentesis in monkeys does not originate from the posterior chamber, instead entering the aqueous humor by reflux through Schlemm's canal.^{51,69,149} This variability is not surprising as a mechanism facilitating blood-aqueous barrier breakdown is advantageous to some species but disadvantageous to others.

A paper by Bito extensively discusses evolutionary divergence in ocular defense mechanisms.¹⁵⁰ On a physiologic basis, the most pronounced effect of acute blood-aqueous barrier breakdown is protein entry into the aqueous humor. This is a primary ocular defense mechanism necessary for the rapid delivery of clotting factors into the anterior chamber so that penetrating corneal wounds can be rapidly sealed and repaired. It has been shown that those animals with more labile blood-aqueous barriers are the most vulnerable to corneal perforation because of their morphological, behavioral, and environmental adaptations. These species, such as rabbits, rely on monitoring visual systems with laterally placed, somewhat protruding, and hence relatively unprotected globes. In contrast, primates have searching type visual systems that require visual acuity and stereopsis. These species have well-protected globes and more stable blood-aqueous barriers given the obvious disadvantage aqueous humor protein has on visual

acuity. There is a positive correlation among species between dependence on visual acuity and blood-aqueous barrier stability and a negative correlation between corneal vulnerability and blood-aqueous barrier stability.¹⁵⁰

For this reason, extrapolation from rabbits or other species commonly used in ocular research is not always appropriate. Comparative studies are valuable, but ultimately documentation of a response in species of interest is most important and necessary for evidence based medicine.

Quantification of Blood-Aqueous Barrier Breakdown

Slit-Lamp Assessment

Aqueous flare is the result of proteins leaking from systemic circulation into the relatively protein-free aqueous humor when the anterior uveal blood vessel integrity and epithelial cell junctions are compromised.⁹⁴ Though the normal aqueous humor is acellular, cells can also be observed in the anterior chamber during intraocular inflammation. Cells in the aqueous humor originate locally from the uveal tissues or enter the aqueous humor after passing through compromised capillary walls and epithelial barriers.¹⁴¹

Subjective grading of changes in aqueous humor composition with blood-aqueous barrier breakdown by slit-lamp examination has been widely employed to quantify aqueous flare intensity and cell number in uveitis.¹⁴¹ Normal aqueous humor is optically clear; however, if blood-aqueous barrier breakdown occurs protein and cells leak into the aqueous and can be visualized in a slit-lamp beam of light passing through the anterior chamber (Tyndall light phenomenon).¹⁴¹ Evaluation is performed in a very dark room with intensity of the beam passing through the protein-rich aqueous humor subjectively quantified (Figure 1-1).¹⁴¹ Grades for aqueous flare include 0 for complete absence, 1+ for faint flare (barely detectable), 2+ for moderate flare (iris and lens details clear), 3+ for marked flare (iris and lens details hazy), and 4+ for intense flare (fixed, coagulated aqueous humor with considerable fibrin). Aqueous humor cell levels are quantified on a similar scale based on cellular density in the beam of light (wide beam with narrow slit) with a grading of 0 for no cells, 1+ for 5-10 cells per field, 2+ for 10-20 cells per field, 3+ for 20-50 cells per field, and 4+ for 50+ cells per field. Though this method is simple, convenient, repeatable and clinically useful, it is subjective, not sensitive, and not standardized. Slit-lamp assessment is most applicable in a clinical setting. In research studies,

this type of subjective examination has low sensitivity, poor reproducibility, a lack of standardization values between different instruments, and suffers from observer bias.¹⁵¹

Figure 1-1 Tyndall light phenomenon demonstrating aqueous flare.



Image courtesy of Dr. Paul Miller

Fluorophotometry

Increased concentration of fluorescein in the eye following systemic administration has been shown to correlate well with increased protein concentration in the eye, suggesting protein and fluorescein pass into the aqueous at the same place (ciliary body) and from the same source (blood plasma).⁷³ This basis allows fluorophotometry to be used to quantitatively assess anterior chamber inflammation and is an objective means of assessing blood-aqueous barrier breakdown.

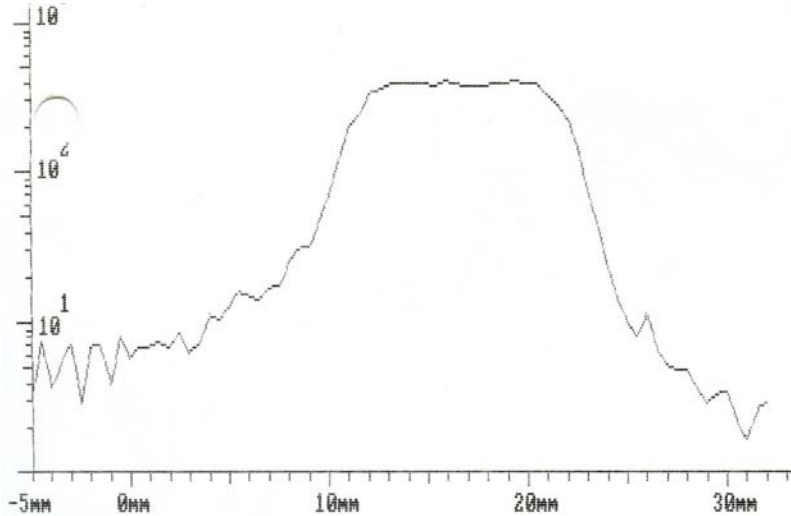
Fluorophotometric evaluation of the blood-aqueous barrier measures the diffusion of small fluorescein molecules (MW 376) into the anterior chamber and allows detection of even subclinical alterations.¹⁵² The amount of fluorescein that enters the anterior chamber following systemic administration is proportional to the degree of blood-aqueous barrier disruption, allowing anterior chamber fluorophotometry to provide a reliable and noninvasive method of evaluating the integrity of the blood-aqueous barrier.⁵⁴ Following administration of intravenous fluorescein, a patient is placed in front of the fluorophotometer and a blue excitation beam scans the eye along the optical axis (Figure 1-2). The machine's optic head receives green fluorescence

readings, which are processed by the photodetector and associated computer. Results are reported in fluorescein ng/ml and displayed graphically (Figure 1-3). Each scan takes only a few seconds, but some animal patients may need sedation or anesthesia to allow proper positioning. The cited disadvantages of fluorophotometry are that it requires administration of systemic fluorescein with potential adverse reactions^{153,154} and a short time delay (30-90 minutes) necessary before readings can be performed.

Figure 1-2 Ocular fluorophotometry being performed on a canine patient.



Figure 1-3 Data generated by the fluorophotometer showing a graphical display of ocular fluorescence and equivalent fluorescein concentrations (ng/ml) with peak plateau levels present in the anterior chamber.



NAME: DRYDEN, 13-06 LINUS
 DATE: 09-05-2006 TIME: 10:28:47 EYE: LEFT
 P/N 0611-279-XX REV A.2.2.3.5.7
 DARK: 15 REF: 134041
 GATE 100 STEP 2 CAL# 1825
 DAY 1 30 MIN POST AQUEO

POSITION:	.00 mm	.50 mm
-5	3.4	7.4
-4	3.8	5.6
-3	7.1	2.9
-2	6.8	7.2
-1	3.9	8
0	5.9	6.7
1	6.9	7.5
2	6.9	8.4
3	6.3	7.2
4	11.6	10.3
5	12.4	16.1
6	15.2	14
7	16.9	17.6
8	24.8	30.2
9	31.7	46.3
10	70.5	117.5
11	192	239.2
12	325.9	346.9
13	380	390.1
14	386.8	384.6
15	388.9	374.8
16	401	385
17	370.7	381.2
18	380	391.4
19	398.7	402.3
20	399.7	388.7
21	328.9	274.6
22	220.8	138.5
23	72.8	44.7
24	22.3	14
25	10.2	8
26	11.2	6.5
27	5.3	4.8
28	4.8	3.8
29	2.9	3.3
30	3.4	2.2
31	1.6	2.7

Data reporting and analysis of fluorophotometry results has been performed in a variety of ways over the years with methods that include actual fluorescein concentrations, percent increase in the treated versus the contralateral eye, percent increase as compared to baseline, and calculation of a diffusion coefficient. No one method has shown to be superior. Studies that utilize mean anterior chamber fluorescence report results in ng/ml.^{70,90,135,136,155-159} Investigations that report results in percent increase in the treated versus the contralateral eye use the formula: $\%INC\ FL = \{(FL_{tx} - FL_{untx}) / (FL_{untx})\} \times 100$.^{47,54-56,132,160,161} This method has been used for calculation because both eyes equilibrate against the same serum concentration; however, it has been stated that this ratio may be deceptive due to a consensual reaction in the contralateral unoperated eye.^{148,152,157} A 2008 study reported results as a percentage increase in the post-treatment fluorescein concentration over the baseline concentration using the following formula: $\%INC\ FL = \{(FL_{post} - FL_{baseline}) / (FL_{baseline})\} \times 100$.¹⁶² A diffusion coefficient for fluorescein can also be calculated by fluorophotometry, providing a physical value for the leakage of fluorescein molecules through the blood-aqueous barrier. This method is reliable and reproducible as long as a strict protocol is followed, which involves multiple blood samples and numerous fluorophotometric measurements per eye.¹⁶³ This method is less commonly used for investigations due to the conclusion that measurement of plasma fluorescence and calculation of a diffusion coefficient does not improve the clinical accuracy of anterior chamber fluorophotometry.¹⁵⁶ Shah et al suggest that the concentration of anterior chamber fluorescence (ng/ml) is appropriate for quantification provided that patients are systemically well and are given the same dose of fluorescein by the same route.¹⁵⁶ Further studies are warranted to determine which method of analysis is most appropriate in dogs.

Anterior chamber fluorophotometry can be used to assess the integrity of the blood-aqueous barrier in research studies, after intraocular surgery, and during clinical disease (uveitis).^{47,54-56,70,90,93,132,135,139,152,155,157,159-162,164} This method of assessing the functional status of the blood-aqueous barrier is simple, sensitive, objective, reproducible, and provides observer-independent results.^{155,157,164} Fluorophotometry is able to detect differences that cannot be clinically assessed^{155,160} and provides a longer-lasting indicator of permeability barrier disruption than macromolecule leakage.¹³²

Laser Flaremetry

Aqueous humor protein and cells can be noninvasively and objectively quantified by a laser flare cell meter.¹⁶⁵ The system consists of a helium-neon laser slit lamp, a binocular microscope fitted with a photomultiplier, and a personal computer that controls the system and analyzes the data (Figures 1-4 and 1-5). The 25 μ W helium-neon laser beam has a focused diameter of 20 μ m and is projected into the anterior chamber with beam scattering detected in a sampling window (0.3 x 0.5 mm) by the photomultiplier. Scans lasting only 0.5 seconds are performed sequentially for protein concentration and cell count with the total scan time taking one second. The laser beam light scattering intensity in the anterior chamber is analyzed and results are reported as photon counts of scattered light per millisecond (pc/ms). The test is rapidly repeatable and therefore allows demonstration of dynamic changes.

Figure 1-4 Laser flare cell meter.

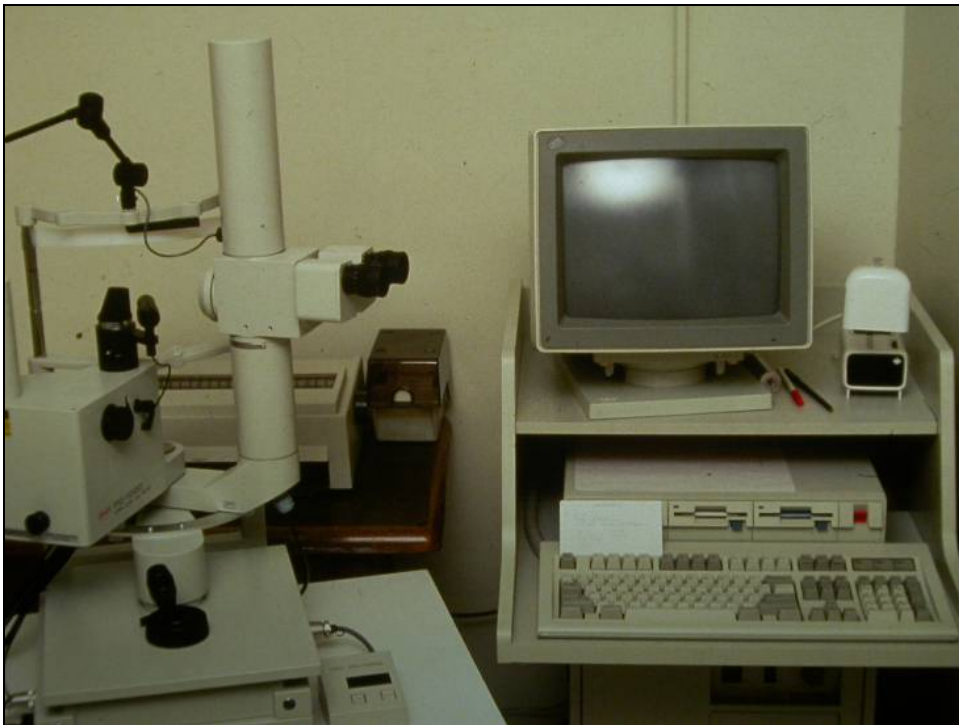


Image courtesy of Dr. Amy Rankin

Figure 1-5 Feline patient positioned in front of the laser flare cell meter.



Image courtesy of Dr. Amy Rankin

Albumin is the major protein constituent of normal aqueous humor and laser flaremetry has been shown to reliably predict albumin protein concentration in noninflamed eyes.¹⁶⁶ However, as eyes become progressively more inflamed there is an increase in both the concentration and proportion of higher molecular weight molecules, both of which increase light scattering and preclude use of a calibration curve based on albumin alone.¹⁶⁷ To avoid protein overestimation, it is recommended that laser flare results be expressed in either pc/ms or converted into an equivalent protein concentration using a calibration curve based on actual anterior chamber protein measurements.^{166,167} Investigation of canine patients confirms that laser flaremetry results should be expressed as pc/ms or converted to protein concentration by using the dog *in vivo* calibration curve for comparisons with data of other studies to avoid overestimation from use of the albumin curve.⁹² Others recognize that macromolecules, like globulin and lipids, generate stronger scattering effects than albumin, but feel that except in cases of very strong inflammation the intensity measured with the laser flare cell meter parallels the actual aqueous protein concentration.¹⁵¹

Aqueous humor cell count can also be quantified with the laser flare cell meter. A study by Krohne et al determined that the cell measuring function was accurate and useful, but did cite certain limitations.¹⁶⁸ The authors identified a difference between cell sizes and counting accuracy and also noted that the instrument counted flare if beads or cells were present, even when protein was not present.¹⁶⁸ The artifactual flare was attributed to scatter from the cells or beads studied, and would not likely cause a problem in clinical or research cases with uveitis due to higher expected protein concentrations in diseased eyes.¹⁶⁸ One study in humans also recognized a limitation in cell counting with the laser flare cell meter as 16.8% of clinical uveitis cases graded to have 1+ cells by slit-lamp examination were not detected by the laser flare cell meter.¹⁵¹ This was attributed to the limited sampling window and short sampling time of the laser flare cell meter as compared to the larger observation volume and arbitrary time of slit-lamp examination.¹⁵¹

Aside from these minor limitations, the laser flare cell meter has been deemed a useful clinical and investigative tool for noninvasive, repeatable, quantitative assessment of the blood-aqueous barrier and has been used in numerous studies.^{81,92,96,129-131,133,151,152,164,168-170} Laser flare and cell measurement is relatively easy to perform and reproducibility is within a reliable range for biological systems.¹⁶⁴

Aqueous Humor Microprotein Assays

Given that aqueous humor protein concentration increases are directly proportional to the severity of blood-aqueous barrier breakdown actual protein values can be evaluated.¹⁷¹ In order to determine aqueous humor protein a sample of aqueous fluid is collected by paracentesis and protein concentration is quantified in mg/dl typically using the Coomassie blue technique for microprotein analysis.¹⁷² In one study healthy dogs averaged 15.1 mg/dl aqueous protein with a range of 5-28 mg/dl,⁹² while another canine study reported slightly higher normal values averaging 36.4 mg/dl and ranging from 21 to 65 mg/dl.² Though calculation of aqueous humor protein has been utilized in numerous studies as an objective measure of blood-aqueous barrier breakdown,^{49,50,57-59,66,68,72,73,75,76,79,81,92,95,138} collection of the sample is invasive and further blood-aqueous barrier breakdown complicates sequential measurements.

Comparison of Techniques

In a clinical setting, slit-lamp assessment is the most practical method for evaluating blood-aqueous barrier breakdown. The semi-quantitative method of grading aqueous humor flare and cells can be performed repeatedly and by different observers, but is therefore subjective and insensitive. On the other end of the spectrum, microprotein assays are very sensitive and specific, but due to the nature of aqueous collection are invasive and preclude repeated monitoring over short periods of time. For these reasons, the fluorophotometer and laser flare cell meter have become the primary means of assessing blood-aqueous barrier breakdown in research studies. There is widespread debate as to which method is superior, and it has been stated that the two techniques may measure different and not identical parameters of blood-aqueous barrier function.¹⁵⁶

It is known that the laser flare cell meter measures the barrier function to protein and cells, while the fluorophotometer measures the influx of small fluorescein molecules. A 1983 study in rabbits documented selective barrier reestablishment for different-sized molecules and found fluorescein leakage to be a more sensitive and longer-lasting indicator of loss of integrity of permeability barriers between the blood and aqueous than macromolecule leakage.¹³² In a 1992 human study, fluorophotometry was found to be more sensitive for early blood-aqueous barrier changes as compared to laser flaremetry.¹⁶⁴ It was stated that in cases of moderate blood-aqueous barrier dysfunction increased permeation of fluorescein possibly precedes that of bigger molecules, whereas albumin passes into the eye in higher quantities with more distinct failure of the blood-aqueous barrier.¹⁶⁴ Another human study measuring blood-aqueous barrier function following cataract surgery found fluorophotometry to be more sensitive than flaremetry in detecting small alterations in barrier permeability.¹⁵² The need to administer systemic fluorescein, wait for steady-state aqueous humor levels, and allow adequate wash-out of fluorescein between repeated fluorophotometry measurements has been cited as the main benefit to using laser flaremetry.⁹² Despite these minor differences, both fluorophotometry and laser flaremetry are able to provide sensitive, reliable measures of ocular inflammation and both are of use in the quantitative assessment of damage to the blood-aqueous barrier.^{152,156}

CHAPTER 2 - Ocular Effects Following Aqueocentesis in Dogs Using Variable Needle Sizes: Fluorophotometric and Tonometric Evaluation

Introduction

Ocular anterior chamber paracentesis, or aqueocentesis, is a commonly performed procedure for both diagnostic and therapeutic purposes in veterinary medicine. In a clinical setting it may be performed in the face of anterior chamber disease to collect a sample of material for diagnostic purposes. It is also employed as therapy in emergency management of glaucoma to protect the retina from the deleterious effects of elevated intraocular pressure (IOP) when refractive to medical means of pressure control. Aqueocentesis is accomplished by inserting a 25- to 30-gauge hypodermic needle through the limbal cornea into the anterior chamber, with the needle passing parallel to the iris.¹⁹ This is commonly performed in a clinical setting on awake patients under topical anesthesia alone, though general anesthesia may be employed in select cases. It is important to note that aqueocentesis has been shown to cause intraocular inflammation by inducing breakdown of the blood-aqueous barrier. For this reason anterior chamber paracentesis has been used as a model of intraocular inflammation for research investigations in a variety of species.^{47-76,81}

The ocular blood-aqueous barrier is formed by the endothelium of the iris blood vessels, the non-pigmented layer of the ciliary epithelium, and the posterior pigmented epithelium of the iris.¹⁰⁰ These structures normally prevent substances present in the blood from entering the eye. When the barrier is disrupted the blood vessels dilate and plasma proteins leak into the aqueous humor.¹⁰³ Common causes of blood-aqueous barrier breakdown are anterior uveitis, ocular surgery, trauma, aqueous paracentesis and ocular irritants. Blood-aqueous barrier breakdown can be assessed by subjective ophthalmic examination using a time-honored semiquantitative grading scheme, with aqueous flare indicating protein-rich aqueous humor.¹⁴¹ Objective techniques that allow for more accurate comparison of blood-aqueous barrier compromise include fluorophotometry, laser flaremetry, and aqueous humor protein assays. Anterior chamber fluorophotometry noninvasively measures the fluorescein concentration in the anterior chamber

following systemic administration of fluorescein. Greater levels of fluorescein entering the anterior chamber indicate greater permeability of the blood-aqueous barrier; therefore fluorophotometry can be used to quantify the degree of blood-aqueous barrier disruption.

Considering aqueocentesis is a common diagnostic and therapeutic practice with known adverse effects, the question remains whether those adverse effects can be minimized with use of a smaller gauge needle. This variable has not been previously studied so no scientifically-based recommendations for aqueocentesis needle size are currently reported in the literature. The purposes of this study were to use anterior chamber fluorophotometry to evaluate the degree of blood-aqueous barrier breakdown following aqueocentesis using variable needle sizes and tonometry to track the IOP response.

Materials and Methods

Animals

The use of dogs and all procedures in this study were approved by the Institutional Animal Care and Use Committee at Kansas State University. Beagle dogs were obtained following use in prior studies unrelated to ophthalmic research, and after completion of our study were returned for eventual adoption. Dogs were housed individually in a temperature-controlled environment illuminated by fluorescent lights that were automatically turned on (from 8 a.m. to 8 p.m.) and off. Prior to their inclusion in the study, individual physical and ophthalmologic examinations were performed and all dogs were deemed healthy with no confounding conditions. Ocular examination included rebound tonometry,^a slit-lamp biomicroscopy,^b and indirect ophthalmoscopy.^c Animals were adapted to human contact for a minimum of 3-6 weeks during their previous research investigations. Three dogs (two intact males and one intact female) were used for preliminary work to determine ideal time points for study design. Twenty-eight dogs (13 intact male dogs, two neutered male dogs, and 13 intact female dogs) were used for the research study with 24 dogs in the treatment groups and 4 dogs in the control group; however, one control dog developed a corneal ulcer in one eye and was removed from the study.

Aqueocentesis

Twenty-four healthy, adult beagles were divided into 3 equal treatment groups (25-, 27-, or 30-gauge needle) by permuted block randomization. In each dog the treated eye was

determined randomly by the flip of a coin and the contralateral eye remained untreated. Three healthy, adult beagles did not receive treatment in either eye but participated in all other aspects of the study and were used as controls with each eye treated as an independent variable.

Aqueous paracentesis, anterior chamber fluorophotometry, and tonometric measurements were all performed by a single investigator (RAA). Animals were sedated with ketamine^d (8.8 mg/kg of body weight, IM) and xylazine^e (0.88 mg/kg of body weight, IM) prior to aqueocentesis and fluorophotometer scans for optimal patient positioning and accurate measurements. Topical anesthetic^f (0.5% proparacaine) and 5% povidone iodine were applied to the eye prior to aqueocentesis. Bishop-Harmon forceps grasped the bulbar conjunctiva to stabilize the eye and a needle was inserted through the lateral perlimbal cornea parallel to the iris (Figure 2-1). Care was taken to avoid the iris, lens, and corneal endothelium. The needle hub was allowed to fill half-way and then was rapidly removed from the eye. No effort was made to prevent regurgitation of aqueous humor through the corneal puncture site. The aim of our study was to evaluate the clinical practice of therapeutic aqueocentesis, therefore uncontrolled paracentesis was performed.

Figure 2-1 Globe stabilization and needle positioning for performing aqueocentesis.



Fluorophotometry

A computerized scanning ocular fluorophotometer^g with an anterior chamber adapter was used to measure fluorescein concentrations in the central anterior chamber of each eye following administration of 10% fluorescein^h (20 mg/kg of body weight, IV). Each dog was placed in sternal recumbency, the head was stabilized, the eyelids were held open, and the eye was positioned in front of the scanner (Figure 2-2). For consistency the left eye was always scanned first, followed immediately by the right eye with no more than 2 minutes elapsing between measurements at each time point. Aqueous humor fluorescein values are reportedly maximal and stable in dogs between approximately 30 and 90 minutes after intravenous injection of fluorescein.⁵⁴ Results from our preliminary work with three dogs confirmed this finding and for the research study all fluorophotometry readings were scheduled during this appropriate post-injection period. Fluorophotometry was performed on sedated dogs prior to and following aqueocentesis on day 1, then daily through day 5 (and at equal time points in control dogs). To minimize motion during fluorophotometric readings chemical restraint is commonly needed in dogs. It has been previously shown that the administration of ketamine and xylazine does not alter blood-aqueous barrier permeability.⁵⁴

Figure 2-2 Patient positioning for ocular fluorophotometry with use of the anterior chamber adapter.



Tonometry

All IOP measurements were performed using a rebound tonometer as previously described.^{23,25,173} The measurements were taken with each dog manually restrained sternally, the head maintained in an upright position and the eyelids gently held open while avoiding pressure on the globe (Figure 2-3). Three consecutive IOP readings were obtained on each eye according to manufacturer specifications and IOP was determined as the mean of these readings. Given that the cornea would be anesthetized for IOP readings immediately following aqueocentesis, initial tonometric readings were taken both prior to and after application of topical anesthetic to evaluate for significant variation. One drop of topical anesthetic (0.5% proparacaine) was applied to the cornea and tonometry was repeated in the same manner described above. In order to maintain consistent and comparable IOP values throughout the study topical anesthesia was utilized for every tonometric measurement.

Figure 2-3 Use of the Tono-Vet® rebound tonometer for intraocular pressure measurement.



Study Time Points

The experimental schedule was based on reports from previous studies⁵⁴ and results of preliminary testing on three dogs. Time points for the 24 treated dogs were as follows

(hours:minutes): Day 1 – initial examination and IOP; time 0 IV fluorescein; 0:25 IM sedation; 0:30 pre-aqueocentesis fluorophotometer scan (baseline); 0:33 pre-aqueocentesis IOP; 0:35 aqueocentesis; 0:36 1 minute post-aqueocentesis IOP; 0:55 20 minutes post-aqueocentesis IOP; 1:05 post-aqueocentesis fluorophotometer scan; 1:15 40 minutes post-aqueocentesis IOP; 1:35 60 minutes post-aqueocentesis IOP; IOP measurements continued every 60 minutes until 8 hours following aqueocentesis (Table 2-1). Follow-up ocular examinations were performed 6 hours after aqueocentesis. Days 2-5 – examination and IOP; IV fluorescein 1 hour prior to fluorophotometry; IM sedation 10 minutes prior to fluorophotometry; fluorophotometer scans every 24 hours post aqueocentesis (Table 2-2). Control dogs were studied similarly; however, aqueocentesis was not performed and only topical betadine and ophthalmic anesthetic were applied to the eyes at 0:35 on Day 1.

Table 2-1 Experimental schedule time points on Day 1 of the study (hours:minutes)

Time Point	Measurement
Baseline data	Initial exam and IOP
0:00	Intravenous fluorescein
0:25	Intramuscular sedation
0:30	Pre-aqueocentesis fluorophotometer scan
0:33	Pre-aqueocentesis IOP
0:35	Aqueocentesis
0:36	1 minute post-aqueocentesis IOP
0:55	20 minutes post-aqueocentesis IOP
1:05	Post-aqueocentesis fluorophotometer scan
1:15	40 minutes post-aqueocentesis IOP
1:35	60 minutes post-aqueocentesis IOP
+1:00	Hourly post-aqueocentesis IOPs
6:35	6 hours post-aqueocentesis ocular examination

Table 2-2 Experimental schedule time points on Day 2 of the study (hours:minutes) with subsequent daily evaluations every 24 hours through Day 5.

Time Point	Measurement
23:00	Ocular exam and IOP
23:35	Intravenous fluorescein
24:25	Intramuscular sedation
24:35	Fluorophotometer scan
End of day	Ocular examination

Data Analysis

Results from this study are reported as mean anterior chamber fluorescence in ng/ml as has been used in previous investigations.^{70,90,135,136,155-159} Additional methods of analyzing fluorophotometer results include percent increase in the treated versus the contralateral eye using the formula: $\%INC FL = \{(FL_{tx} - FL_{untx}) / (FL_{untx})\} \times 100$ ^{47,54-56,132,160,161} and percentage increase in the post-treatment fluorescein concentration over the baseline concentration using the formula: $\%INC FL = \{(FL_{post} - FL_{baseline}) / (FL_{baseline})\} \times 100$.¹⁶² Though mean fluorescence in ng/ml was used as the primary method of analysis in our study, the additional percentage increase methods were also employed when comparing between treatment groups.

A commercial software programⁱ was used for all statistical analyses. Values of $P < 0.05$ were considered significant. Intraocular pressure values obtained prior to or following application of topical anesthetic were compared using a paired T-test. Anterior chamber fluorescein values in the treated versus the contralateral untreated eyes were compared within each treatment group using a paired T-test. Treatment groups were compared by repeated measures analysis of variance followed by a Newman-Keuls posthoc multiple comparisons test to discern individual differences. This method was utilized for both gross anterior chamber fluorescein values and percent increase in fluorescence evaluation. Anterior chamber fluorescein values in treated or contralateral untreated eyes were compared over time by repeated measures analysis of variance followed by a Newman-Keuls posthoc multiple comparisons test to discern individual differences. Multiple linear regression was used to evaluate the effect of treatment

group on IOP over time and analysis of variance was performed to determine whether there were significant differences in IOP measurements at specific time points between treatment groups.

Results

Fluorophotometry

Aqueocentesis caused blood-aqueous barrier disruption using all needle sizes. Evaluating all treated eyes as a group, significant increased anterior chamber fluorescence was present at the post-aqueocentesis ($P < 0.001$), day 2 ($P < 0.001$), day 3 ($P = 0.0014$) and day 4 ($P = 0.0011$) time points as compared to contralateral untreated eyes (Table 2-3). In the 25-gauge needle group a significant difference was present between the treated and contralateral untreated eyes at the post-aqueocentesis ($P = 0.0163$), day 2 ($P = 0.0387$), and day 3 ($P = 0.0428$) time points with significantly greater anterior chamber fluorescence in the treated eyes (Figure 2-4). In the 27-gauge needle group a significant difference was present between the treated and contralateral untreated eyes at the post-aqueocentesis ($P = 0.0017$), day 2 ($P = 0.001$), day 3 ($P = 0.001$) and day 4 ($P = 0.002$) time points with significantly greater anterior chamber fluorescence in the treated eyes (Figure 2-5). In the 30-gauge needle group a significant difference was present between the treated and contralateral untreated eyes at the post-aqueocentesis ($P = 0.0333$), day 2 ($P = 0.0173$), and day 3 ($P = 0.0078$) time points with significantly greater anterior chamber fluorescence in the treated eyes (Figure 2-6).

Fluorophotometry values for treated eyes showed statistically significant mean increased fluorescence in the 25-gauge treatment group as compared to the 27- and 30-gauge treatment groups on day 3 ($P = 0.0166$) and as compared to the 30-gauge treatment group on day 5 ($P = 0.0478$) (Figure 2-7). When percentage increase in fluorescein concentration in the treated versus untreated eye is calculated no significant difference is noted between treatment groups at any time point (day 3 $P = 0.0633$ and day 5 $P = 0.1919$); however, when analyzed based on the percentage increase in the post-treatment fluorescein concentration over the baseline concentration a statistically significant difference remains on day 3 ($P = 0.0383$) with 25-gauge needle treated eyes having increased fluorescence as compared to 27- and 30-gauge needle treated eyes, but there is no significant difference on day 5 ($P = 0.0978$).

There were significant differences present over time within all treatment and contralateral untreated eye groups ($P < 0.001$ by repeated measures ANOVA and Newman-Keuls posthoc;

Table 2-3). In the 25-gauge needle treated eyes the fluorescein concentration on day 2 was significantly greater than prior to aqueocentesis, day 4 and day 5. In addition, the post-aqueocentesis and day 3 fluorescein values were greater than prior to aqueocentesis. In the 25-gauge needle contralateral untreated eyes significantly greater fluorescence was present on days 2 and 3 as compared to prior to and post-aqueocentesis. In the 27-gauge needle treated eyes the post-aqueocentesis and day 2 fluorescein values were greater than prior to aqueocentesis, day 3, day 4, and day 5. In addition, days 3 and 4 fluorescence was also greater than prior to aqueocentesis and on day 5. In the 27-gauge needle contralateral untreated eyes the fluorescein value on day 2 was greater than at all other time points. The day 4 fluorescein value was also greater than prior to aqueocentesis. In the 30-gauge needle treated eyes day 2 fluorescence was greater than all other time points. In the 30-gauge needle contralateral untreated eyes the fluorescein value on day 2 was greater than prior to aqueocentesis, post-aqueocentesis, and on day 5. Days 3 and 4 fluorescence were greater than prior to and post-aqueocentesis. The day 5 fluorescein value was greater than prior to aqueocentesis.

Though fluorescein changes were noted over time in the contralateral untreated eyes of all groups, no significant difference in anterior chamber fluorescence was found at any time point when compared to the control group (Figure 2-8). An unexpected finding was noted in the one control dog pulled from the study due to development of a corneal ulcer. In this dog data values were still collected over the 5 days and a notable rise in anterior chamber fluorescein was present on day 2 in the ulcerated eye, with a mild increase in fluorescence also present in the contralateral healthy eye (Figure 2-9).

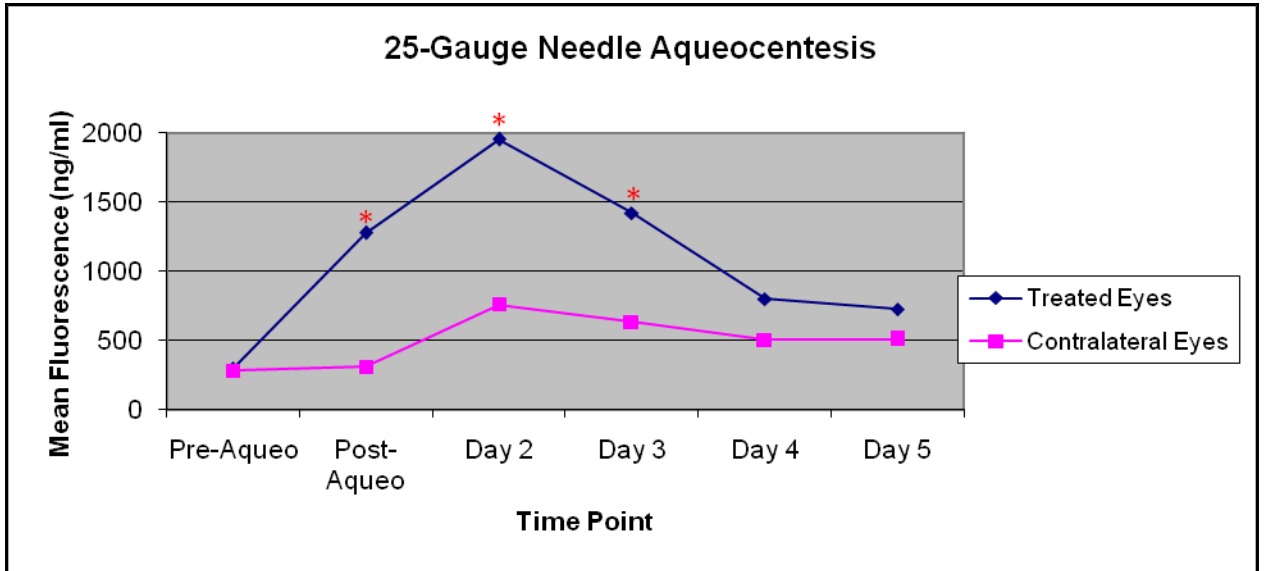
Table 2-3 Anterior chamber fluorescein concentrations (mean \pm standard deviation in ng/ml) for treated, contralateral untreated, and control eyes at each study time point.

	Pre-aqueo	Post-aqueo	Day 2	Day 3	Day 4	Day 5
25-gauge treated	298.31 \pm 140.94 ^{bd}	1274.96 \pm 942.66 ^{*c}	1953.65 \pm 1360.54 ^{*a}	1419.71 \pm 778.85 ^{*c}	800.50 \pm 309.61 ^b	723.71 \pm 278.82 ^b
25-gauge contralateral	282.64 \pm 116.72 ^b	310.53 \pm 128.66 ^b	760.01 \pm 518.49 ^a	631.79 \pm 313.80 ^a	504.16 \pm 250.46	509.15 \pm 221.69
27-gauge treated	355.11 \pm 113.24 ^{bd}	1087.4 \pm 421.49 ^{*a}	1319.06 \pm 348.89 ^{*a}	758.02 \pm 190.34 ^{*bc}	662.31 \pm 192.47 ^{*bc}	545.18 \pm 120.72 ^{bd}
27-gauge contralateral	338.15 \pm 127.35 ^{bd}	358.34 \pm 111.69 ^b	567.73 \pm 190.72 ^a	433.90 \pm 101.22 ^b	459.29 \pm 137.72 ^{bc}	446.44 \pm 110.06 ^b
30-gauge treated	290.29 \pm 95.18 ^b	761.20 \pm 418.01 ^{*b}	1395.36 \pm 1064.39 ^{*a}	706.45 \pm 338.09 ^{*b}	594.96 \pm 238.46 ^b	483.13 \pm 117.62 ^b
30-gauge contralateral	265.44 \pm 90.74 ^{bdf}	313.71 \pm 96.10 ^{bd}	651.28 \pm 414.23 ^a	540.34 \pm 243.32 ^c	521.43 \pm 252.23 ^c	442.15 \pm 157.54 ^{bc}
All treated eyes	314.57 \pm 116.51	1041.19 \pm 651.70 [*]	1556.03 \pm 1014.26 [*]	964.40 \pm 583.51 [*]	685.93 \pm 255.72 [*]	584.00 \pm 207.75
All contralateral eyes	295.41 \pm 112.22	327.53 \pm 110.20	659.67 \pm 389.33	535.34 \pm 240.68	494.96 \pm 212.00	465.91 \pm 164.85
Control eyes	286.57 \pm 122.74	323.72 \pm 119.42	372.83 \pm 113.06	400.77 \pm 125.82	349.65 \pm 135.59	442.90 \pm 129.31

* Indicates statistically significant difference at each time point between treated and contralateral untreated eyes

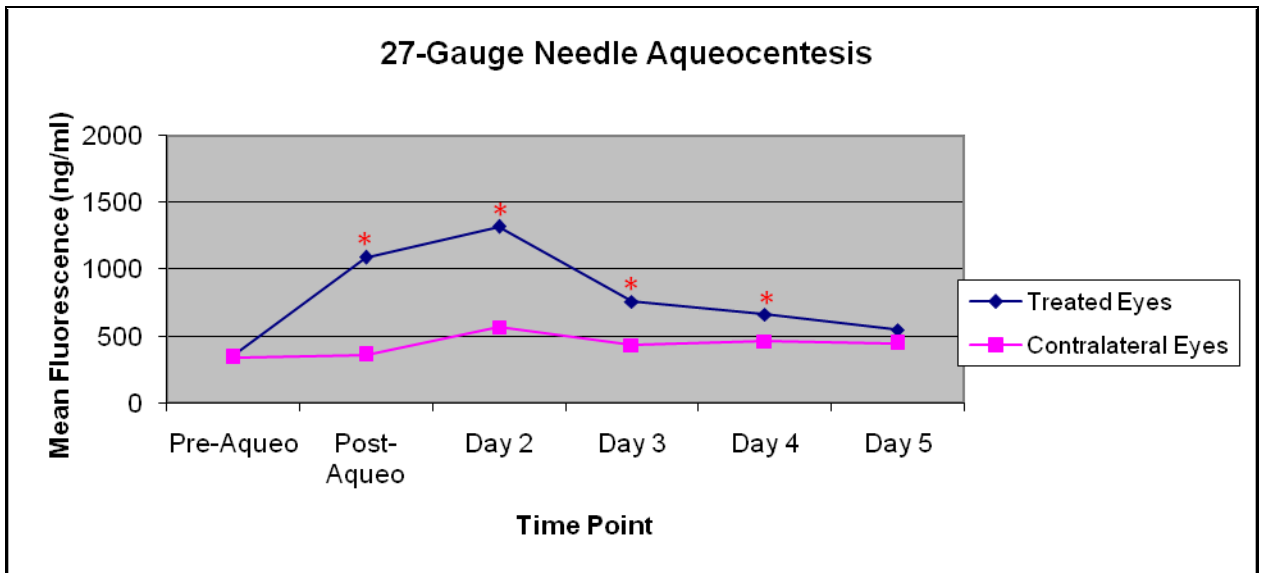
Statistically significant differences over time within each treated or contralateral untreated eye group: a > b, c > d, e > f

Figure 2-4 Mean anterior chamber fluorescence (ng/ml) in 25-gauge needle treated and contralateral untreated eyes.



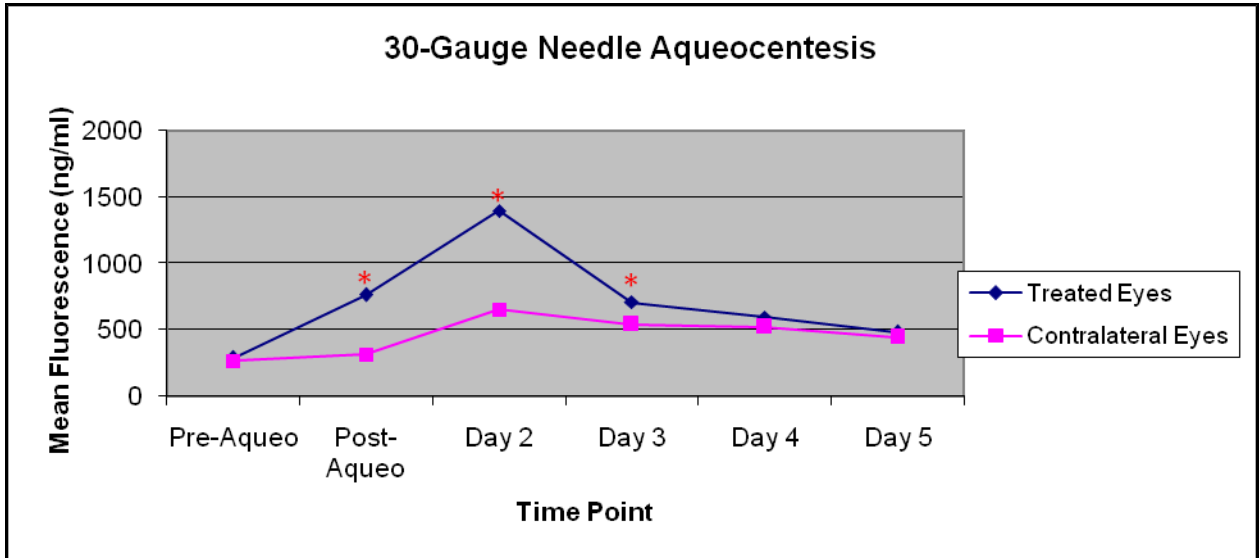
* Indicates statistically significant difference at each time point between treated and contralateral untreated eyes

Figure 2-5 Mean anterior chamber fluorescence (ng/ml) in 27-gauge needle treated and contralateral untreated eyes.



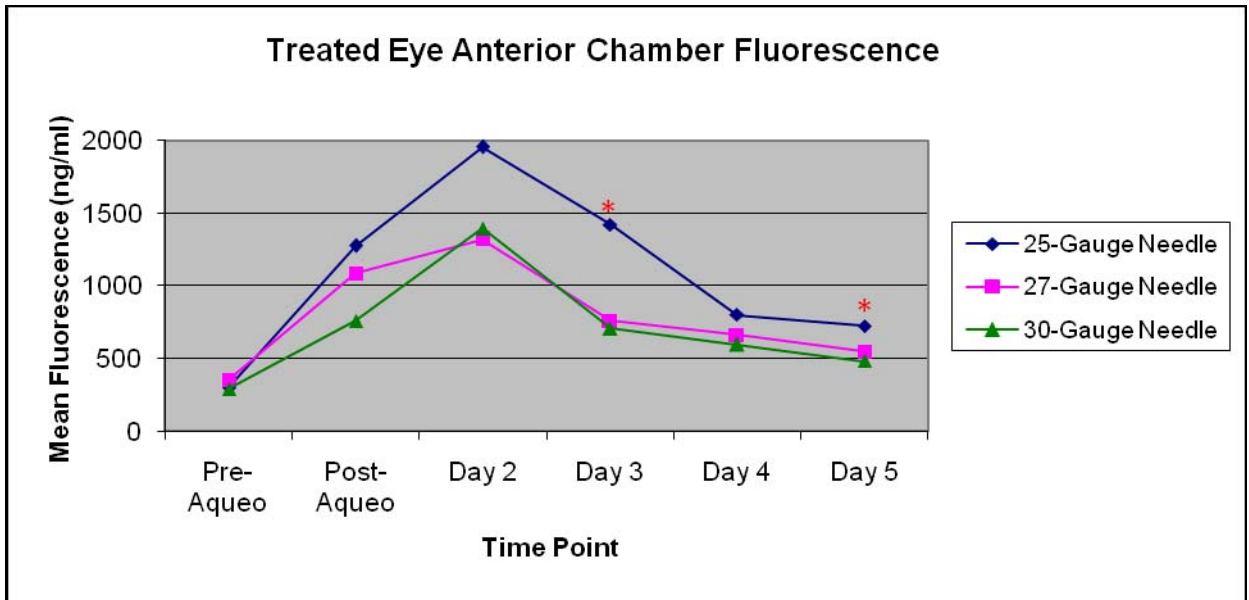
* Indicates statistically significant difference at each time point between treated and contralateral untreated eyes

Figure 2-6 Mean anterior chamber fluorescence (ng/ml) in 30-gauge needle treated and contralateral untreated eyes.



* Indicates statistically significant difference at each time point between treated and contralateral untreated eyes

Figure 2-7 Mean anterior chamber fluorescence (ng/ml) in treated eyes of all groups.



* Indicates statistically significant difference between the 25-gauge treatment group as compared to the 27-gauge and 30-gauge treatment groups on Day 3, and between the 25-gauge treatment group and the 30-gauge treatment group on Day 5

Figure 2-8 Mean anterior chamber fluorescence (ng/ml) in untreated eyes of all groups.

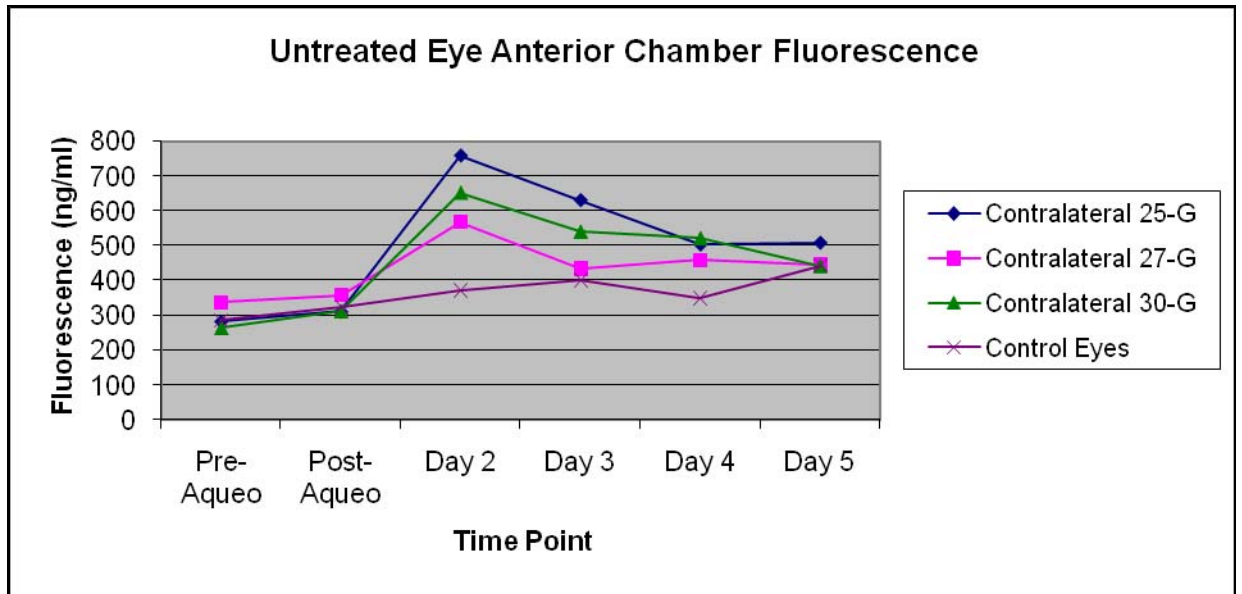
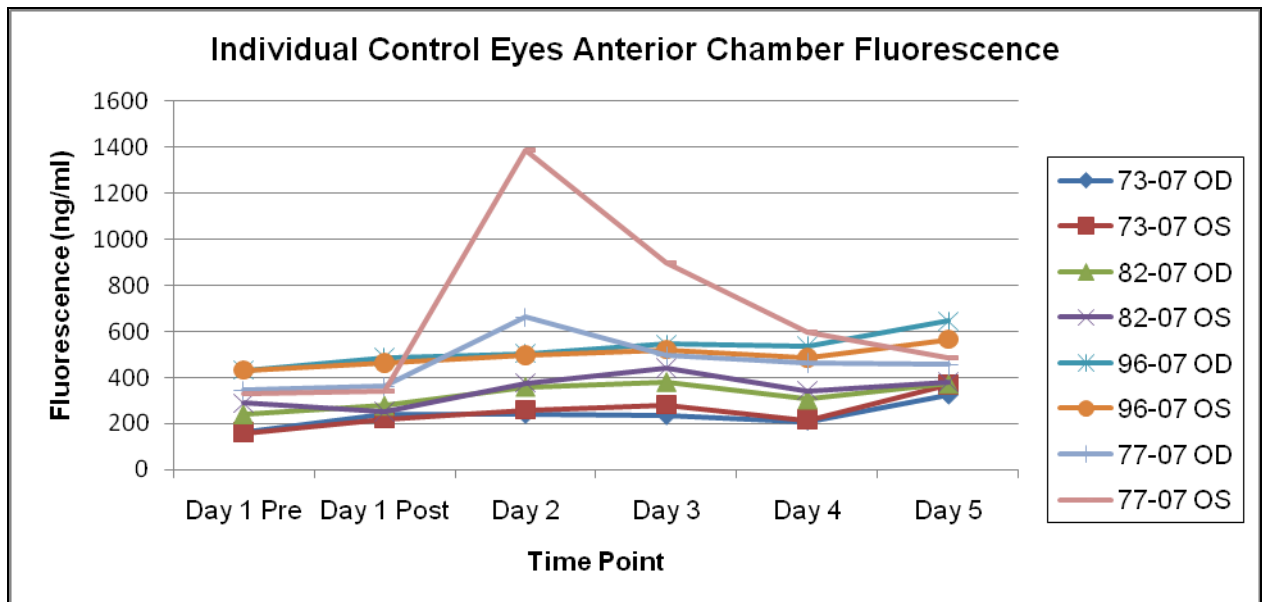


Figure 2-9 Individual eyes of control dogs showing anterior chamber fluorescence.

Note Dog 77-07 (pulled from control study) with increased fluorescence in the OS on Day 2 following development of a corneal ulcer and also notable fluorescein increase in the contralateral nonulcerated OD.

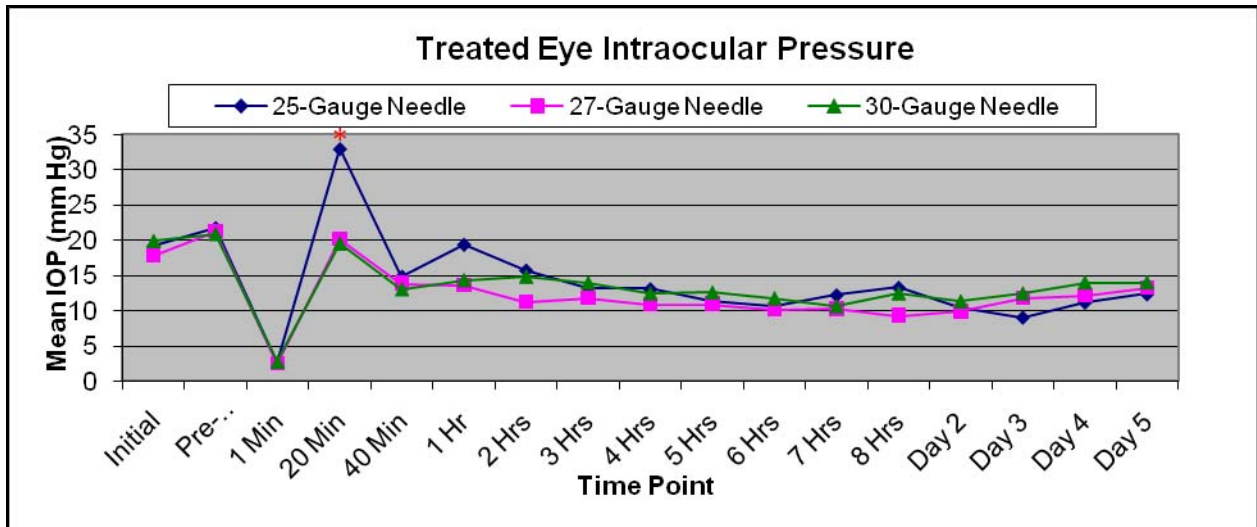


Tonometry

Initial tonometric readings taken on each dog were obtained both prior to and after application of topical anesthetic and a statistically significant difference in IOP was observed following topical anesthesia (no topical anesthesia, mean IOP \pm s.d. = 21.20 ± 4.36 mm Hg; after topical anesthesia, mean IOP \pm s.d. = 19.17 ± 3.60 mm Hg, $P = 0.0013$). This result was relevant for design of the study but did not have clinical significance. In order to maintain consistent and comparable IOP values throughout the study topical anesthesia was utilized for every tonometric measurement.

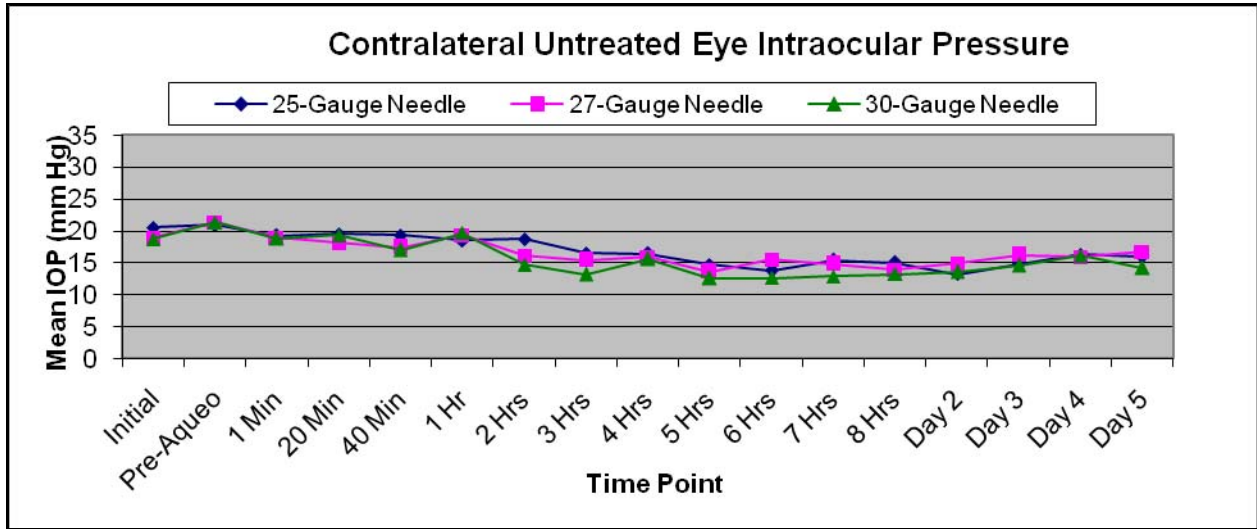
When comparing the IOP between treatment groups a statistically significant difference was present at 20 minutes post-aqueocentesis with the 25-gauge needle treatment group significantly higher (32.96 ± 13.03 mm Hg) than the 27-gauge (20.15 ± 8.07 mm Hg) or 30-gauge (19.54 ± 9.77) treatment groups ($P = 0.0297$) (Figure 2-10). Aside from transient ocular hypertension in the 25-gauge treatment group, IOP rapidly normalized in treated eyes and the IOP of contralateral untreated eyes showed no clinically significant changes (Figure 2-11).

Figure 2-10 Mean intraocular pressure (mm Hg) of treated eyes in all groups.



* Indicates statistically significant difference between the 25-gauge treatment group as compared to the 27-gauge and 30-gauge treatment groups

Figure 2-11 Mean intraocular pressure (mm Hg) of contralateral untreated eyes in all groups.



Discussion

Consistent with previous reports, aqueocentesis caused blood-aqueous barrier disruption in all treated eyes of this study. Anterior chamber fluorophotometry allowed noninvasive, repeated daily assessment of the breakdown and reestablishment of the blood-aqueous barrier. Maximal fluorescence was noted in the treated eyes of all groups on day 2 (24 hours post-aqueocentesis), with declining values thereafter (Figure 2-7). By day 5 in all treatment groups the mean anterior chamber fluorescein value of the treated eyes was not significantly different than the contralateral untreated eyes (Table 2-3 and Figures 2-4 to 2-6). Though statistically significant differences were present at additional time points in some groups, values between the treated and contralateral untreated eyes that did not differ by more than 25% were considered within normal limits as has been previously reported.⁵⁴

It was of great interest to note that the contralateral untreated eyes of all groups showed maximal and statistically significant anterior chamber fluorescence on day 2 (Table 2-3 and Figure 2-8). Values declined subsequently, but no statistically significant difference was found between the contralateral untreated eyes and control eyes at any point in time. There are two possible explanations for this finding. The first supports the statistical analysis, that aqueocentesis treatment did not affect the contralateral eye and the value disparities over time are due to random variation. The second explanation is that aqueocentesis treatment did indeed

affect the contralateral untreated eye by causing a subtle degree of blood-aqueous barrier disruption as measured by fluorophotometry, but due to limited animal numbers and high variability (large standard deviations) a significant difference was not documented. This latter theory is supported by an inadvertent finding in the control dog (Dog 77-07) pulled from the study due to corneal ulcer development. In this dog a corneal ulcer was present in the OS on day 2, and at that time increased anterior chamber fluorescein was measured. In addition, a mild spike in fluorescence was present on day 2 in the same dog's healthy OD. These fluorescence changes were not noted in any other eye of the control dogs (Figure 2-9). Over days 3 through 5 anterior chamber fluorescence in Dog 77-07 declined in both eyes as the ulcer healed. The increased fluorescence in the ulcerated eye of this dog is not surprising and can be attributed to axonal reflex causing blood-aqueous barrier breakdown.^{107,110} It is the increased fluorescence noted in this dog's contralateral healthy eye that parallels the mean response seen in the contralateral eyes of treated dogs in this study. Though this finding cannot be statistically evaluated, it is of pertinent interest given the other study findings that suggest presence of a consensual ocular reaction in dog eyes.

Consensual ocular reactions have been reported in humans and rabbits,^{119,145-148,174} but have not previously been documented in dogs. Scanning electron microscopy of rabbit eyes treated with paracentesis and contralateral control eyes demonstrated changes in ciliary body processes consistent with both a direct and consensual reaction.¹¹⁷ The mechanism for this reaction is hypothesized to be a neural reflex arc,^{117,147,148} but others suggest it is due to a transfer of prostaglandins via systemic circulation.^{145,146} The consensual ocular reaction is an important biological finding and is clinically noteworthy as it was documented immediately following and then up to one month following cataract surgery in humans.¹⁴⁸ Though it is commonly recognized that drugs applied topically to one eye can result in effects in the opposite eye due to likely systemic absorption of the medication,^{73,129,175-178} our study is the first to suggest a consensual blood aqueous-barrier breakdown reaction in dogs.

The findings in this study confirm that aqueocentesis using a 25-gauge needle caused greater blood-aqueous barrier breakdown than aqueocentesis with 27- or 30-gauge needles. Statistically significant differences were documented by anterior chamber fluorophotometry on days 3 and 5. Though statistically significant differences between groups were not present at the other time points, likely due to large standard deviations and small sample sizes, the trend of

increased fluorescence over time is apparent in the 25-gauge needle treatment group (Figure 2-7). The reason for large individual variance is likely due to the uncontrolled aqueous paracentesis performed in this study. Though this method has been previously shown to induce large variance as compared to controlled, timed paracentesis,⁵⁴ the former is the technique commonly employed during therapeutic aqueocentesis. The aim of our study was to evaluate the clinical practice of aqueocentesis and its effect on blood-aqueous barrier breakdown, therefore uncontrolled paracentesis using various needle sizes was performed.

In this study the cause of increased blood-aqueous barrier breakdown is not clear, but appears to be needle size. Though the degree of ocular hypotony does affect blood-aqueous barrier breakdown and causes increased protein content in the reformed aqueous,¹²¹ in our study the average IOP immediately after aqueocentesis was not significantly different between treatment groups (Figure 2-10). The speed of fluid flow into the needle could also be considered as a cause; however, it has been shown that speed of aspiration during aqueous paracentesis had no effect on protein content of the reformed aqueous humor.¹²¹ Therefore, more rapid fluid outflow is not likely the cause of the greater blood-aqueous barrier breakdown identified in this study.

Not only did 25-gauge needle aqueocentesis induce greater blood-aqueous barrier breakdown, but it also resulted in transient ocular hypertension 20 minutes following treatment. This point of elevated IOP was an unexpected finding given that in all treatment group eyes the IOP immediately after aqueocentesis averaged 2 mm Hg. Elevated IOP in the 25-gauge needle treatment group is consistent with a greater degree of blood-aqueous barrier breakdown as initial ocular hypertension is found in uveitis due to prostaglandin release.^{67,71} Paracentesis-induced ocular hypertension is likely due to a sudden rise in the anterior uveal blood volume with a subsequent increase in ultrafiltration and plasma extravasion.^{103,125} Paracentesis-induced blood-aqueous barrier breakdown has been studied in dogs and results show that prostaglandins are indeed the most important mediators of the ocular irritative response.⁴⁷ While topical flurbiprofen significantly reduced blood-aqueous barrier breakdown as measured by anterior chamber fluorophotometry,⁴⁷ the inability of flurbiprofen and proparacaine to completely abolish the response suggests that additional non-prostaglandin, non-sensorineurally-derived mediators may be involved or that the rapid reduction in IOP causes physical damage to the blood-aqueous barrier.^{47,51,54}

Though patients with glaucoma were not evaluated in the current study, the rapid resolution of ocular hypotony in all groups confirms the assumption that aqueocentesis alone is insufficient therapy for elevated IOP in dogs. This is consistent with a previous human study where cataract surgery patients experiencing postoperative ocular hypertension treated with paracentesis experienced immediate reduction in IOP followed by rebounding pressures to near initial values one hour after treatment.³³ On the other hand, aqueous paracentesis combined with medical therapy provides rapid symptomatic relief of acutely elevated IOP and could be considered as adjunctive therapy in the management of acute elevation of IOP.^{31-33,45}

Data reporting and analysis of fluorophotometry studies has varied over the years with reports that utilize actual fluorescein concentrations, percent increase in the treated versus the contralateral eye, percent increase as compared to baseline, and calculation of a diffusion coefficient. No one method has shown to be superior. In this study actual fluorescein concentrations were used in an effort to avoid confounding effects by the other methods. Specifically, with the possibility that treatment of one eye affected anterior chamber fluorescence in the contralateral eye we felt it was suboptimal to use the percent increase in fluorescence in the treated versus contralateral eye as the means for analyzing fluorophotometry results. Laurell et al cautions that use of this ratio may indeed be deceptive due to a consensual reaction in the opposite (unoperated) eye.¹⁵² It has been reported that 5-6 hours after fluorescein injection aqueous humor levels fall to low or undetectable values;⁵⁴ however, a trend toward increased anterior chamber fluorescence was noted even in control eyes over the five days of the study (Table 2-3 and Figure 2-9) so comparing percent increase in fluorescence to baseline may also be suboptimal. A diffusion coefficient for fluorescein can be calculated by fluorophotometry, providing a physical value for the leakage of fluorescein molecules through the blood-aqueous barrier. This method is reliable and reproducible as long as a strict protocol is followed, which involves multiple blood samples and numerous fluorophotometric measurements per eye.¹⁶³ This method is less commonly used for investigations due to the conclusion that measurement of plasma fluorescence and calculation of a diffusion coefficient does not improve the clinical accuracy of anterior chamber fluorophotometry.¹⁵⁶ Shah et al suggest that the concentration of anterior chamber fluorescence (ng/ml) is appropriate for quantification provided that patients are systemically well and are given the same dose of fluorescein by the same route.¹⁵⁶ Further studies are warranted to determine which method of analysis is most appropriate in dogs.

Conclusion

Uncontrolled aqueocentesis using 25-, 27-, and 30-gauge needles was performed in dogs to evaluate the degree of blood-aqueous barrier breakdown. There was no statistically significant difference in fluorescein concentration or IOP between 27- and 30- gauge needle treatment groups at any time point. Use of the 25-gauge needle resulted in a statistically significant increase ($P < 0.05$) in anterior chamber fluorescence on days 3 and 5. It also caused a statistically significant increase in intraocular pressure at 20 minutes post-aqueocentesis as compared to the 27- and 30- gauge needle treatment groups. Peak anterior chamber fluorescence was documented in the contralateral untreated eyes of all treatment groups on day 2 suggesting a consensual ocular reaction in dogs; however, values were not significantly greater than control eyes. Substantial variability common in biological systems complicates research studies, and as in this investigation, high variability and large standard deviations were found to be a problem with statistical analysis.

Acknowledgements

We would like to thank Dr. Michael Dryden, Kansas State University, for providing the dogs for the study as well as Amanda Davis and Sandra Donker for their assistance with data collection. Supported by Kansas State University College of Veterinary Medicine Intramural Grant.

Footnotes

- a. TonoVet®, Tiolat Ltd, Helsinki, Finland
- b. SL-14 Biomicroscope, Kowa Company, Ltd, Tokyo, Japan
- c. HEINE Omega 180® Ophthalmoscope, HEINE Optotechnik, Herrsching, Germany
- d. VetaKet®, IVX Animal Health, Inc, St. Joseph, Missouri
- e. AnaSed®, Ben Venue Laboratories, Bedford, Ohio
- f. 0.5% proparacaine hydrochloride ophthalmic solution, Akorn, Inc, Buffalo Grove, Illinois
- g. FM-2 Fluorotron Master, OcuMetrics, Inc, Mountain View, California
- h. AK-FLUOR®, Akorn, Inc, Buffalo Grove, Illinois
- i. WINKS 4.8 5th Ed. Statistical Analysis System, TexaSoft, Cedar Hill, TX

References

1. Gum GG, Gelatt KN, Esson DW. Physiology of the eye In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;149-182.
2. Hazel SJ, Thrall MA, Severin GA, et al. Laboratory evaluation of aqueous humor in the healthy dog, cat, horse, and cow. *Am J Vet Res* 1985;46:657-659.
3. Moses RA. Intraocular pressure In: Moses RA, ed. *Adler's Physiology of the Eye: Clinical Application*. 6th ed. Saint Louis: The C. V. Mosby Company, 1975;179-209.
4. Sears ML. The aqueous In: Moses RA, ed. *Adler's Physiology of the Eye: Clinical Application*. 6th ed. Saint Louis: The C. V. Mosby Company, 1975;232-251.
5. Samuelson DA. Ophthalmic anatomy In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;37-148.
6. Ward DA, Cawrse MA, Hendrix DVH. Fluorophotometric determination of aqueous humor flow rate in clinically normal dogs. *Am J Vet Res* 2001;62:853-858.
7. Do CW, Civan MM. Species variation in biology and physiology of the ciliary epithelium: Similarities and differences. *Exp Eye Res* 2008.
8. Civan MM, Macknight AD. The ins and outs of aqueous humour secretion. *Exp Eye Res* 2004;78:625-631.
9. Pederson JE, Green K. Aqueous humor dynamics: experimental studies. *Exp Eye Res* 1973;15:277-297.
10. Cole DF. Secretion of the aqueous humour. *Exp Eye Res* 1977;25 Suppl:161-176.
11. Cunha-Vaz JG. The blood-ocular barriers: past, present, and future. *Doc Ophthalmol* 1997;93:149-157.
12. Plummer CE, MacKay EO, Gelatt KN. Comparison of the effects of topical administration of a fixed combination of dorzolamide-timolol to monotherapy with timolol or dorzolamide on IOP, pupil size, and heart rate in glaucomatous dogs. *Vet Ophthalmol* 2006;9:245-249.
13. Becker B. Carbonic anhydrase and the formation of aqueous humor. *Am J Ophthalmol* 1959;47:342-361.
14. Cawrse MA, Ward DA, Hendrix DVH. Effects of topical application of a 2% solution of dorzolamide on intraocular pressure and aqueous humor flow rate in clinically normal dogs. *Am J Vet Res* 2001;62:859-863.
15. Gelatt KN, MacKay EO. Changes in intraocular pressure associated with topical dorzolamide and oral methazolamide in glaucomatous dogs. *Vet Ophthalmol* 2001;4:61-67.
16. Samuelson DA, Gum GG, Gelatt KN, et al. Aqueous outflow in the beagle: unconventional outflow, using different-sized microspheres. *Am J Vet Res* 1985;46:242-248.
17. Barrie KP, Gum GG, Samuelson DA, et al. Morphologic studies of uveoscleral outflow in normotensive and glaucomatous beagles with fluorescein-labeled dextran. *Am J Vet Res* 1985;46:89-97.
18. Barrie KP, Gum GG, Samuelson DA, et al. Quantitation of uveoscleral outflow in normotensive and glaucomatous Beagles by 3H-labeled dextran. *Am J Vet Res* 1985;46:84-88.
19. Ollivier FJ, Plummer CE, Barrie KP. Ophthalmic examination and diagnostics Part 1: the eye examination and diagnostic procedures In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;438-483.
20. Miller PE, Pickett JP, Majors LJ, et al. Clinical comparison of the Mackay-Marg and Tono-Pen applanation tonometers in the dog. *Prog Vet Comp Ophthalmol* 1991;1:171-176.

21. Gelatt KN, MacKay EO. Distribution of intraocular pressure in dogs. *Vet Ophthalmol* 1998;1:109-114.
22. Priehs DR, Gum GG, Whitley RD, et al. Evaluation of three applanation tonometers in dogs. *Am J Vet Res* 1990;51:1547-1550.
23. Görig C, Coenen RTI, Stades FC, et al. Comparison of the use of new handheld tonometers and established applanation tonometers in dogs. *Am J Vet Res* 2006;67:134-144.
24. Baudouin C, Gastaud P. Influence of topical anesthesia on tonometric values of intraocular pressure. *Ophthalmologica* 1994;208:309-313.
25. Knollinger AM, La Croix NC, Barrett PM, et al. Evaluation of a rebound tonometer for measuring intraocular pressure in dogs and horses. *J Am Vet Med Assoc* 2005;227:244-248.
26. Thompson A, Gerding P, Sissler S, et al. Comparison of intraocular pressure measurements following phacoemulsification using the Tono-Vet and Tono-Pen. *Veterinary Ophthalmology* 2007;10:398-411.
27. Smith PJ, Brooks DE, Lazarus JA, et al. Ocular hypertension following cataract surgery in dogs: 139 cases (1992-1993). *J Am Vet Med Assoc* 1996;209:105-111.
28. Broadwater JJ, Schorling JJ, Herring IP, et al. Effect of body position on intraocular pressure in dogs without glaucoma. *Am J Vet Res* 2008;69:527-530.
29. Hofmeister EH, Mosunic CB, Torres BT, et al. Effects of ketamine, diazepam, and their combination on intraocular pressures in clinically normal dogs. *Am J Vet Res* 2006;67:1136-1139.
30. May DR, Noll FG. An improved approach to aqueous paracentesis. *Ophthalmic Surg* 1988;19:821-822.
31. Carnahan MC, Platt LW. Serial paracenteses in the management of acute elevations of intraocular pressure. *Ophthalmology* 2002;109:1604-1606.
32. Lam DS, Chua JK, Tham CC, et al. Efficacy and safety of immediate anterior chamber paracentesis in the treatment of acute primary angle-closure glaucoma: a pilot study. *Ophthalmology* 2002;109:64-70.
33. John M, Soucek J, Noblitt RL, et al. Sideport incision paracentesis versus antiglaucoma medication to control postoperative pressure rises after intraocular lens surgery. *J Cataract Refract Surg* 1993;19:62-63.
34. de Boer JH, Verhagen C, Bruinenberg M, et al. Serologic and polymerase chain reaction analysis of intraocular fluids in the diagnosis of infectious uveitis. *Am J Ophthalmol* 1996;121:650-658.
35. Olin DD. Examination of the aqueous humor as a diagnostic aid in anterior uveitis. *J Am Vet Med Assoc* 1977;171:557-559.
36. Hogan MJ, Wood IS, Godfrey WA. Aqueous humor cytology in uveitis. *Arch Ophthalmol* 1973;89:217-220.
37. Michau TM, Breitschwerdt EB, Gilger BC, et al. Bartonella vinsonii subspecies berkhoffi as a possible cause of anterior uveitis and choroiditis in a dog. *Vet Ophthalmol* 2003;6:299-304.
38. Van der Lelij A, Rothova A. Diagnostic anterior chamber paracentesis in uveitis: a safe procedure? *Br J Ophthalmol* 1997;81:976-979.
39. Finger PT, Papp C, Latkany P, et al. Anterior chamber paracentesis cytology (cytopspin technique) for the diagnosis of intraocular lymphoma. *Br J Ophthalmol* 2006;90:690-692.

40. Faber NA, Crawford M, LeFebvre RB, et al. Detection of *Leptospira* spp. in the aqueous humor of horses with naturally acquired recurrent uveitis. *J Clin Microbiol* 2000;38:2731-2733.
41. Dadeya S, Malik KP, Guliani BP, et al. Acute lymphocytic leukemia presenting as masquerade syndrome. *Ophthalmic Surg Lasers* 2002;33:163-165.
42. Wilkerson MJ, Dolce K, DeBey BM, et al. Metastatic balloon cell melanoma in a dog. *Vet Clin Pathol* 2003;32:31-36.
43. de Boer JH, Luyendijk L, Rothova A, et al. Analysis of ocular fluids for local antibody production in uveitis. *Br J Ophthalmol* 1995;79:610-616.
44. Cheung CM, Durrani OM, Murray PI. The safety of anterior chamber paracentesis in patients with uveitis. *Br J Ophthalmol* 2004;88:582-583.
45. Arnavielle S, Creuzot-Garcher C, Bron AM. Anterior chamber paracentesis in patients with acute elevation of intraocular pressure. *Graefes Arch Clin Exp Ophthalmol* 2007;245:345-350.
46. Hardman C, Stanley RG. Diode laser transscleral cyclophotocoagulation for the treatment of primary glaucoma in 18 dogs: a retrospective study. *Vet Ophthalmol* 2001;4:209-215.
47. Ward DA, Ferguson DC, Kaswan RL, et al. Leukotrienes and sensory innervation in blood-aqueous barrier disruption in the dog. *J Ocul Pharmacol* 1992;8:69-76.
48. Krootila K, Syrjala M, Lehtosalo JI, et al. Platelets and polymorphonuclear leukocytes in experimental ocular inflammation in the rabbit eye. *Graefes Arch Clin Exp Ophthalmol* 1989;227:465-469.
49. Tjebbes GW, van Delft JL, Barthen ER, et al. d-Ibuprofen in ocular inflammation induced by paracentesis of the rabbit eye. *Prostaglandins* 1990;40:29-33.
50. Bucolo C, Spadaro A. Effect of sodium naproxen on inflammatory response induced by anterior chamber paracentesis in the rabbit. *J Pharm Pharmacol* 1995;47:708-712.
51. Bartels SP, Pederson JE, Gaasterland DE, et al. Sites of breakdown of the blood-aqueous barrier after paracentesis of the rhesus monkey eye. *Invest Ophthalmol Vis Sci* 1979;18:1050-1060.
52. Pinard C, Gauvin D, Moreau M, et al. Measurement of inflammatory mediators in aqueous humor following paracentesis of the anterior chamber in dogs. *Veterinary Ophthalmology* 2007;10:398-411.
53. Gilmour M, Lehenbauer T. Effects of tepoxalin in reducing intraocular inflammation in the dog. *Veterinary Ophthalmology* 2006;9:414-425.
54. Ward DA, Ferguson DC, Kaswan RL, et al. Fluorophotometric evaluation of experimental blood-aqueous barrier disruption in dogs. *Am J Vet Res* 1991;52:1433-1437.
55. Ward DA, Ferguson DC, Ward SL, et al. Comparison of the blood-aqueous barrier stabilizing effects of steroidal and nonsteroidal anti-inflammatory agents in the dog. *Prog Vet Comp Ophthalmol* 1992;2:117-124.
56. Ward DA. Comparative efficacy of topically applied flurbiprofen, diclofenac, tolmetin, and suprofen for the treatment of experimentally induced blood-aqueous barrier disruption in dogs. *Am J Vet Res* 1996;57:875-878.
57. van Haeringen NJ, Glasius E, Oosterhuis JA, et al. Drug prevention of blood-aqueous barrier disruption. *Ophthalmic Res* 1983;15:180-184.

58. Regnier AM, Dossin O, Cutzach EE, et al. Comparative effects of two formulations of indomethacin eyedrops on the paracentesis-induced inflammatory response of the canine eye. *Vet Comp Ophthalmol* 1995;5:242-246.
59. van Delft JL, van Haeringen NJ, Glasius E, et al. Comparison of the effects of corticosteroids and indomethacin on the response of the blood-aqueous barrier to injury. *Curr Eye Res* 1987;6:419-425.
60. Kennard G, Gilmour M. Effects of ketoprofen, meloxicam and flunixin meglumine in reducing intraocular inflammation. *Veterinary Ophthalmology* 2004;7:437-453.
61. Gilmour M, Kennard G. Effects of oral meloxicam, deracoxib, tepoxalin and carprofen in reducing intraocular inflammation in the dog. *Veterinary Ophthalmology* 2004;7:437-453.
62. Pinard C, Moreau M. Effect of carprofen on aqueous humor levels of PGE₂, NO_x and TNF- α in an experimental canine uveitis model. *Veterinary Ophthalmology* 2006;9:414-425.
63. Laus J, Ribeiro A, Escobar A, et al. Effects of carprofen administered by different routes to control experimental uveitis in dogs. *Veterinary Ophthalmology* 2007;10:398-411.
64. Gilmour M, Lehenbauer T. Comparison of tepoxalin, carprofen and meloxicam in reducing intraocular inflammation in the dog. *Veterinary Ophthalmology* 2008;11:413-429.
65. Eakins KE. Prostaglandin and non-prostaglandin mediated breakdown of the blood-aqueous barrier. *Exp Eye Res* 1977;25 Suppl:483-498.
66. Jampol LM, Neufeld AH, Sears ML. Pathways for the response of the eye to injury. *Invest Ophthalmol* 1975;14:184-189.
67. Al-Ghadyan A, Mead A, Sears M. Increased pressure after paracentesis of the rabbit eye is completely accounted for by prostaglandin synthesis and release plus pupillary block. *Invest Ophthalmol Vis Sci* 1979;18:361-365.
68. Latour E, Driot JY, Coquelet C, et al. The role of platelets in blood-aqueous barrier breakdown induced by anterior chamber paracentesis in the rabbit. *Curr Eye Res* 1989;8:1105-1110.
69. Okisaka S. Effects of paracentesis on the blood-aqueous barrier: a light and electron microscopic study on cynomolgus monkey. *Invest Ophthalmol* 1976;15:824-834.
70. Jampel HD, Brown A, Roberts A, et al. Effect of paracentesis upon the blood-aqueous barrier of cynomolgus monkeys. *Invest Ophthalmol Vis Sci* 1992;33:165-171.
71. Unger WG, Cole DF, Hammond B. Disruption of the blood-aqueous barrier following paracentesis in the rabbit. *Exp Eye Res* 1975;20:255-270.
72. Neufeld AH, Jampol LM, Sears ML. Aspirin prevents the disruption of the blood-aqueous barrier in the rabbit eye. *Nature* 1972;238:158-159.
73. Oosterhuis JA, van Haeringen NJ, Glasius E, et al. The effect of indomethacin on the anterior segment of the eye after paracentesis. *Doc Ophthalmol* 1981;50:303-313.
74. Salminen L. Effect of paracentesis on ocular antibiotic concentration. *Acta Ophthalmol Suppl* 1974;123:75-76.
75. Regnier A, Whitley RD, Benard P, et al. Effect of flunixin meglumine on the breakdown of the blood-aqueous barrier following paracentesis in the canine eye. *J Ocul Pharmacol* 1986;2:165-170.
76. Regnier A, Bonnefoi M, Lescure F. Effect of lysine-acetylsalicylate and phenylbutazone premedication on the protein content of secondary aqueous humour in the dog. *Res Vet Sci* 1984;37:26-29.

77. Kubota T, Ohnishi Y, Ishibashi T, et al. [Morphological study of the disruption of the blood-aqueous barrier following paracentesis]. *Nippon Ganka Gakkai Zasshi* 1990;94:243-249.
78. Graff G, Brady MT, Gamache DA, et al. Transient loss of prostaglandin synthetic capacity in rabbit iris-ciliary body following anterior chamber paracentesis. *Ocul Immunol Inflamm* 1998;6:227-238.
79. Zimmerman TJ, Gravenstein N, Sugar A, et al. Aspirin stabilization of the blood-aqueous barrier in the human eye. *Am J Ophthalmol* 1975;79:817-819.
80. Floman N, Zor U. Mechanism of steroid action in ocular inflammation: Inhibition of prostaglandin production. *Invest Ophthalmol Vis Sci* 1977;16:69-73.
81. Rankin AJ, Krohne SG, Glickman NW, et al. Laser flaremetric evaluation of experimentally induced blood-aqueous barrier disruption in cats. *Am J Vet Res* 2002;63:750-756.
82. Divers TJ, Irby NL, Mohammed HO, et al. Ocular penetration of intravenously administered enrofloxacin in the horse. *Equine Vet J* 2008;40:167-170.
83. Clode AB, Davis JL, Salmon J, et al. Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. *Am J Vet Res* 2006;67:296-301.
84. Gilmour MA, Clarke CR, Macallister CG, et al. Ocular penetration of oral doxycycline in the horse. *Vet Ophthalmol* 2005;8:331-335.
85. Latimer FG, Colitz CM, Campbell NB, et al. Pharmacokinetics of fluconazole following intravenous and oral administration and body fluid concentrations of fluconazole following repeated oral dosing in horses. *Am J Vet Res* 2001;62:1606-1611.
86. Davis JL, Salmon JH, Papich MG. Pharmacokinetics and tissue distribution of itraconazole after oral and intravenous administration to horses. *Am J Vet Res* 2005;66:1694-1701.
87. Yu-Speight AW, Kern TJ, Erb HN. Ciprofloxacin and ofloxacin aqueous humor concentrations after topical administration in dogs undergoing cataract surgery. *Vet Ophthalmol* 2005;8:181-187.
88. Rowley RA, Rubin LF. Aqueous humor penetration of several antibiotics in the dog. *Am J Vet Res* 1970;31:43-49.
89. Neupert JR, Lawrence C. Protein release during aqueous withdrawal in rabbits. *Invest Ophthalmol* 1970;9:865-872.
90. Dziezyc J, Millichamp NJ, Rohde BH, et al. Comparison of prednisolone and RMI-1068 in the ocular irritative response in dogs. *Invest Ophthalmol Vis Sci* 1992;33:460-465.
91. Millichamp NJ, Dziezyc J, Rohde BH, et al. Acute effects of anti-inflammatory drugs on neodymium:yttrium aluminum garnet laser-induced uveitis in dogs. *Am J Vet Res* 1991;52:1279-1284.
92. Krohne SG, Krohne DT, Lindley DM, et al. Use of laser flaremetry to measure aqueous humor protein concentration in dogs. *J Am Vet Med Assoc* 1995;206:1167-1172.
93. Waterbury LD, Flach AJ. Comparison of ketorolac tromethamine, diclofenac sodium, and loteprednol etabonate in an animal model of ocular inflammation. *J Ocul Pharmacol Ther* 2006;22:155-159.
94. Krause U, Niemi A, Raunio V. Effect of paracentesis on protein content of aqueous humour. *Ophthalmologica* 1971;163:136-149.
95. Krohne SD, Vestre WA. Effects of flunixin meglumine and dexamethasone on aqueous protein values after intraocular surgery in the dog. *Am J Vet Res* 1987;48:420-422.

96. Chen HB, Yamabayashi S, Ou B, et al. Morphological changes in rabbit ciliary epithelium and blood-aqueous barriers after intravitreal 10^{-5} M endothelin-1. *Exp Eye Res* 1996;62:605-612.
97. Wu P, Gong H, Richman R, et al. Localization of occludin, ZO-1, and pan-cadherin in rabbit ciliary epithelium and iris vascular endothelium. *Histochem Cell Biol* 2000;114:303-310.
98. Tserentsoodol N, Shin BC, Suzuki T, et al. Colocalization of tight junction proteins, occludin and ZO-1, and glucose transporter GLUT1 in cells of the blood-ocular barrier in the mouse eye. *Histochem Cell Biol* 1998;110:543-551.
99. Smelser GK, Pei YF. CYTOLOGICAL BASIS OF PROTEIN LEAKAGE INTO THE EYE FOLLOWING PARACENTESIS. AN ELECTRON MICROSCOPIC STUDY. *Invest Ophthalmol* 1965;4:249-263.
100. Freddo TF. Shifting the paradigm of the blood-aqueous barrier. *Exp Eye Res* 2001;73:581-592.
101. Pedersen OO. Electron microscopic studies on the blood-aqueous barrier of prostaglandin-treated rabbit eyes. II. Iris. *Acta Ophthalmol (Copenh)* 1975;53:699-709.
102. Smith RS. Ultrastructural studies of the blood-aqueous barrier. I: Transport of an electron-dense tracer in the iris and ciliary body of the mouse. *Am J Ophthalmol* 1971;71:1066-1077.
103. Butler JM, Unger WG, Grierson I. Recent experimental studies on the blood-aqueous barrier: the anatomical basis of the response to injury. *Eye* 1988;2 Suppl:S213-220.
104. McLaren JW, Ziai N, Brubaker RF. A simple three-compartment model of anterior segment kinetics. *Exp Eye Res* 1993;56:355-366.
105. Oshika T, Sakurai M, Araie M. A study on diurnal fluctuation of blood-aqueous barrier permeability to plasma proteins. *Exp Eye Res* 1993;56:129-133.
106. Bhattacharjee P. Prostaglandins and inflammatory reactions in the eye. *Methods Find Exp Clin Pharmacol* 1980;2:17-31.
107. Bill A, Stjernschantz J, Mandahl A, et al. Substance P: release on trigeminal nerve stimulation, effects in the eye. *Acta Physiol Scand* 1979;106:371-373.
108. Beding-Barnekow B, Brodin E, Hakanson R. Substance P, neurokinin A and neurokinin B in the ocular response to injury in the rabbit. *Br J Pharmacol* 1988;95:259-267.
109. Unger WG. Review: mediation of the ocular response to injury. *J Ocul Pharmacol* 1990;6:337-353.
110. O'Connor GR. Factors related to the initiation and recurrence of uveitis. XL Edward Jackson memorial lecture. *Am J Ophthalmol* 1983;96:577-599.
111. Holmberg BJ, Maggs DJ. The use of corticosteroids to treat ocular inflammation. *Vet Clin North Am Small Anim Pract* 2004;34:693-705.
112. Protzman CE, Woodward DF. Prostanoid-induced blood-aqueous barrier breakdown in rabbits involves the EP2 receptor subtype. *Invest Ophthalmol Vis Sci* 1990;31:2463-2466.
113. Miller JD, Eakins KE, Atwal M. The release of PGE2-like activity into aqueous humor after paracentesis and its prevention by aspirin. *Invest Ophthalmol* 1973;12:939-942.
114. Bhattacharjee P. The role of arachidonate metabolites in ocular inflammation. *Prog Clin Biol Res* 1989;312:211-227.
115. Yamauchi H, Iso T, Iwao J, et al. The role of prostaglandins in experimental ocular inflammations. *Agents Actions* 1979;9:280-283.

116. Neufeld AH, Sears ML. The site of action of prostaglandin E2 on the disruption of the blood-aqueous barrier in the rabbit eye. *Exp Eye Res* 1973;17:445-448.
117. Dueker DK, Chaudhry HA. SEM of the ciliary processes after paracentesis. *Scan Electron Microsc* 1980:441-447.
118. Vegge T, Neufeld AH, Sears ML. Morphology of the breakdown of the blood-aqueous barrier in the ciliary processes of the rabbit eye after prostaglandin E2. *Invest Ophthalmol* 1975;14:33-36.
119. van Delft JL, van Haeringen NJ, Bodelier VM, et al. Prostaglandin E2 in the vitreous body of the normal rabbit eye and after ocular trauma. *Ophthalmic Res* 1991;23:240-245.
120. Brightman AH, 2nd, Helper LC, Hoffman WE. Effect of aspirin on aqueous protein values in the dog. *J Am Vet Med Assoc* 1981;178:572-573.
121. Kronfeld PC, Lin CK, Luo TH. The Protein Content of the Reformed Aqueous Humor in Man. *Trans Am Ophthalmol Soc* 1940;38:192-213.
122. Podos SM, Becker B, Kass MA. Prostaglandin synthesis, inhibition, and intraocular pressure. *Invest Ophthalmol* 1973;12:426-433.
123. Nakano J, Chang AC, Fisher RG. Effects of prostaglandins E 1 , E 2 , A 1 , A 2 , and F 2 on canine carotid arterial blood flow, cerebrospinal fluid pressure, and intraocular pressure. *J Neurosurg* 1973;38:32-39.
124. Whitelocke RA, Eakins KE. Vascular changes in the anterior uvea of the rabbit produced by prostaglandins. *Arch Ophthalmol* 1973;89:495-499.
125. Unger WG. Mediation of the ocular response to injury and irritation: peptides versus prostaglandins. *Prog Clin Biol Res* 1989;312:293-328.
126. Millichamp NJ, Dziezyc J. Mediators of ocular inflammation. *Prog Vet Comp Ophthalmol* 1991;1:41-58.
127. Millichamp NJ, Dziezyc J. Comparison of flunixin meglumine and flurbiprofen for control of ocular irritative response in dogs. *Am J Vet Res* 1991;52:1452-1455.
128. Hendrix DVH. Diseases and surgery of the canine anterior uvea In: Gelatt KN, ed. *Veterinary Ophthalmology*. 4th ed. Ames, IA: Blackwell Publishing, 2007;812-858.
129. Krohne SG, Gionfriddo J, Morrison EA. Inhibition of pilocarpine-induced aqueous humor flare, hypotony, and miosis by topical administration of anti-inflammatory and anesthetic drugs to dogs. *Am J Vet Res* 1998;59:482-488.
130. Krohne SG, Blair MJ, Bingaman D, et al. Carprofen inhibition of flare in the dog measured by laser flare photometry. *Vet Ophthalmol* 1998;1:81-84.
131. Krohne SG. Effect of topically applied 2% pilocarpine and 0.25% demecarium bromide on blood-aqueous barrier permeability in dogs. *Am J Vet Res* 1994;55:1729-1733.
132. Sanders DR, Spigelman A, Kraff C, et al. Quantitative assessment of postsurgical breakdown of the blood-aqueous barrier. *Arch Ophthalmol* 1983;101:131-133.
133. Ino-ue M, Shirabe H, Yamamoto M. Blood-aqueous barrier disruption in experimental anterior segment ischemia in rabbit eyes. *Ophthalmic Res* 1999;31:213-219.
134. Sanders DR, Joondeph B, Hutchins R, et al. Studies on the blood-aqueous barrier after argon laser photocoagulation of the iris. *Ophthalmology* 1983;90:169-174.
135. Dziezyc J, Millichamp NJ, Smith WB. Effect of flurbiprofen and corticosteroids on the ocular irritative response in dogs. *Vet Comp Ophthalmol* 1995;5:42-45.

136. Waterbury LD, Flach AJ. Efficacy of low concentrations of ketorolac tromethamine in animal models of ocular inflammation. *J Ocul Pharmacol Ther* 2004;20:345-352.
137. Regnier A, Schneider M, Concordet D, et al. Intraocular pharmacokinetics of intravenously administered marbofloxacin in rabbits with experimentally induced acute endophthalmitis. *Am J Vet Res* 2008;69:410-415.
138. Del Sole MJ, Sande PH, Felipe AE, et al. Characterization of uveitis induced by use of a single intravitreal injection of bacterial lipopolysaccharide in cats. *Am J Vet Res* 2008;69:1487-1495.
139. Edelman JL, Lutz D, Castro MR. Corticosteroids inhibit VEGF-induced vascular leakage in a rabbit model of blood-retinal and blood-aqueous barrier breakdown. *Exp Eye Res* 2005;80:249-258.
140. Shiels IA, Sanderson SD, Taylor SM. Arterially perfused eye model of uveitis. *Aust Vet J* 1999;77:100-104.
141. Hogan MJ, Kimura SJ, Thygeson P. Signs and symptoms of uveitis. I. Anterior uveitis. *Am J Ophthalmol* 1959;47:155-170.
142. Townsend WM. Canine and feline uveitis. *Vet Clin North Am Small Anim Pract* 2008;38:323-346, vii.
143. Wilkie DA. Control of ocular inflammation. *Vet Clin North Am Small Anim Pract* 1990;20:693-713.
144. van der Woerd A. Management of intraocular inflammatory disease. *Clin Tech Small Anim Pract* 2001;16:58-61.
145. Chiang TS, Thomas RP. Consensual ocular hypertensive response to prostaglandin. *Invest Ophthalmol* 1972;11:169-176.
146. Chiang TS, Thomas RP. Consensual ocular hypertensive response to prostaglandin E 2. *Invest Ophthalmol* 1972;11:845-849.
147. Kottow MH, Seligman LJ. Consensual reactions to anterior chamber paracentesis in the rabbit. *Am J Ophthalmol* 1978;85:392-399.
148. Miyake K, Asakura M, Maekubo K. Consensual reactions of human blood-aqueous barrier to implant operations. *Arch Ophthalmol* 1984;102:558-561.
149. Raviola G. Effects of paracentesis on the blood-aqueous barrier: an electron microscope study on *Macaca mulatta* using horseradish peroxidase as a tracer. *Invest Ophthalmol* 1974;13:828-858.
150. Bito LZ. Species differences in the responses of the eye to irritation and trauma: a hypothesis of divergence in ocular defense mechanisms, and the choice of experimental animals for eye research. *Exp Eye Res* 1984;39:807-829.
151. Oshika T, Nishi M, Mochizuki M, et al. Quantitative assessment of aqueous flare and cells in uveitis. *Jpn J Ophthalmol* 1989;33:279-287.
152. Laurell CG, Zetterstrom C, Philipson B, et al. Randomized study of the blood-aqueous barrier reaction after phacoemulsification and extracapsular cataract extraction. *Acta Ophthalmol Scand* 1998;76:573-578.
153. Hess JB, Pacurariu RI. Acute pulmonary edema following intravenous fluorescein angiography. *Am J Ophthalmol* 1976;82:567-570.
154. Pacurariu RI. Low incidence of side effects following intravenous fluorescein angiography. *Ann Ophthalmol* 1982;14:32-36.

155. Fearnley IR, Spalton DJ, Smith SE. Anterior segment fluorophotometry in acute anterior uveitis. *Arch Ophthalmol* 1987;105:1550-1555.
156. Shah SM, Spalton DJ, Allen RJ, et al. A comparison of the laser flare cell meter and fluorophotometry in assessment of the blood-aqueous barrier. *Invest Ophthalmol Vis Sci* 1993;34:3124-3130.
157. Ferguson VM, Spalton DJ. Recovery of the blood-aqueous barrier after cataract surgery. *Br J Ophthalmol* 1991;75:106-110.
158. Ferguson VM, Spalton DJ. Quantification of the ocular response to treatment in posterior uveitis. *Acta Ophthalmol (Copenh)* 1992;70:824-831.
159. Dziezyc J, Millichamp NJ, Keller CB, et al. Effects of prostaglandin F2alpha and leukotriene D4 on pupil size, intraocular pressure, and blood-aqueous barrier in dogs. *Am J Vet Res* 1992;53:1302-1304.
160. Sanders DR, Kraff M. Steroidal and nonsteroidal anti-inflammatory agents. Effect on postsurgical inflammation and blood-aqueous humor barrier breakdown. *Arch Ophthalmol* 1984;102:1453-1456.
161. Kraff MC, Sanders DR, McGuigan L, et al. Inhibition of blood-aqueous humor barrier breakdown with diclofenac. A fluorophotometric study. *Arch Ophthalmol* 1990;108:380-383.
162. Johnstone McLean NS, Ward DA, Hendrix DV. The effect of a single dose of topical 0.005% latanoprost and 2% dorzolamide/0.5% timolol combination on the blood-aqueous barrier in dogs: a pilot study. *Vet Ophthalmol* 2008;11:158-161.
163. van Best J, del Castillo JB, Diestelhorst M, et al. Diffusion coefficient through the blood-aqueous barrier using a standard protocol. *Br J Ophthalmol* 1996;80:356-362.
164. Schalnus R, Ohrloff C. Quantification of blood-aqueous barrier function using laser flare measurement and fluorophotometry--a comparative study. *Lens Eye Toxic Res* 1992;9:309-320.
165. Sawa M, Tsurimaki Y, Tsuru T, et al. New quantitative method to determine protein concentration and cell number in aqueous in vivo. *Jpn J Ophthalmol* 1988;32:132-142.
166. Saari KM, Guillen-Monterrubio OM, Hartikainen J, et al. Measurement of protein concentration of aqueous humour in vivo: correlation between laser flare measurements and chemical protein determination. *Acta Ophthalmol Scand* 1997;75:63-66.
167. Shah SM, Spalton DJ, Taylor JC. Correlations between laser flare measurements and anterior chamber protein concentrations. *Invest Ophthalmol Vis Sci* 1992;33:2878-2884.
168. Krohne SG, Reagan WJ, Welch PM. Use of laser flare-cell photometry to count anterior chamber canine leukocytes and latex beads in vitro. *Am J Vet Res* 1998;59:1221-1226.
169. Nicolas T, Benitez del Castillo JM, Diaz D, et al. Effects of subconjunctival methylprednisolone on the blood aqueous barrier following cataract surgery. *Int Ophthalmol* 1995;19:235-238.
170. Kadoi C, Hiraki S, Hayasaka S, et al. Inhibitory effect of nilvadipine on disruption of blood-aqueous barrier induced by prostaglandin E2 application in pigmented rabbits: A morphologic study. *Ophthalmic Res* 1999;31:236-242.
171. Rodriguez-Peralta L. The blood-aqueous barrier in five species. *Am J Ophthalmol* 1975;80:713-725.
172. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-254.

173. Leiva M, Naranjo C, Pena MT. Comparison of the rebound tonometer (ICare®) to the applanation tonometer (Tonopen XL®) in normotensive dogs. *Vet Ophthalmol* 2006;9:17-21.
174. Jampol LM. Consensual reactions to anterior chamber paracentesis. *Am J Ophthalmol* 1978;86:286-288.
175. Wilkie DA, Latimer CA. Effects of topical administration of timolol maleate on intraocular pressure and pupil size in cats. *Am J Vet Res* 1991;52:436-440.
176. Wilkie DA, Latimer CA. Effects of topical administration of timolol maleate on intraocular pressure and pupil size in dogs. *Am J Vet Res* 1991;52:432-435.
177. Stadtbäumer K, Köstlin RG, Zahn KJ. Effects of topical 0.5% tropicamide on intraocular pressure in normal cats. *Vet Ophthalmol* 2002;5:107-112.
178. Wilkie DA, Latimer CA. Effects of topical administration of 2.0% pilocarpine on intraocular pressure and pupil size in cats. *Am J Vet Res* 1991;52:441-444.