PRELIMINARY ANALYSIS OF OPHTHALMIC PREDNISOLONE ACETATE AND DICLOFENAC ON DIABETES MELLITUS REGULATION IN 12 OF 40 DOGS

by

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Abstract

Objective- To evaluate the use of a topical ophthalmic steroid (1% prednisolone acetate) and non-steroidal anti-inflammatory drug (0.1% diclofenac) on blood glucose concentrations, serum fructosamine concentrations, and clinical scores in diabetic dogs with cataracts using descriptive analysis.

Animals- Twelve client-owned dogs with naturally-occurring, controlled (per history and physical examination), insulin-treated diabetes mellitus and cataract. A total of 40 dogs will be enrolled in the study, as determined by power analysis.

Procedures- This was a prospective, randomized, double-masked, experimental study with 2 phases of data collection. Dogs were enrolled from October 2011 to March 2014 and were assigned to 1 of 2 treatments (Drug Red or Drug Blue) using blocked randomization; dogs received either 1% prednisolone acetate suspension or 0.1% diclofenac solution. Patient history, physical, and ophthalmic examinations were performed and a clinical score assigned at enrollment (Phase 1 [day 0]) and upon return (Phase 2 [day 32]). At these times, a complete blood count, serum chemistry, urinalysis, and serum fructosamine concentration were performed prior to hospitalization for up to 72 hours for continuous glucose monitoring. For 4 weeks (day 3 to 31), dogs returned home, and owners administered the dispensed ophthalmic medication 4 times daily to both eyes. Descriptive analysis of data was performed; statistical analysis will follow enrollment of 40 dogs.

Results- Twelve dogs have completed the study, with 6 dogs assigned to each treatment group. Dogs received 4.44 or 0.44 mg/day of prednisolone acetate or diclofenac, respectively. Dogs assigned to Drug Red more commonly exhibited elevations in serum liver enzyme activity. Drug Red group showed a greater percent increase in fructosamine concentrations over time.

Based on glucose curves alone (22 curves analyzed), an insulin dose increase was recommended for 12 curves. An insulin dose decrease and no dose change were recommended for 5 curves each. During treatment, 1 dog reportedly developed polyuria and polydipsia.

Conclusions- Descriptive analysis revealed differences in some outcomes of interest among dogs treated with 2 different ophthalmic anti-inflammatory medications. Data collection is ongoing to determine if statistically significant differences exists for outcomes per group.

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List of Abbreviations

AA	Arachidonic acid
ALP	Alkaline phosphatase
ALT	Alkaline transaminase P5P
BG	Blood glucose
BW	Body weight
CBC	Complete blood count
CGM	Continuous glucose monitor
COX	Cyclooxygenase
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
GLUT	Glucose transporter
HAC	Hyperadrenocorticism
LIU	Lens-induced uveitis
NSAIDs	Non-steroidal anti-inflammatory drugs
OD	Oculus dexter, right eye
OS	Oculus sinister, left eye
OU	Oculus uterque, both eyes
PD	Polydipsia
PE	Physical examination
PG	Prostaglandin
PP	Polyphagia
PU	Polyuria
SD	Standard deviation
VHC	Veterinary Health Center

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Chapter 1 - Literature Review

Diabetes Mellitus

Background

Diabetes mellitus (DM) is the most common disorder of the endocrine pancreas in dogs and is characterized by an absolute or relative insulin deficiency leading to persistent hyperglycemia and glucosuria.[1] Insulin plays an integral role in the body's ability to appropriately metabolize carbohydrates, fatty acids, and amino acids. Lacking sufficient insulin leads to "cellular starvation" due to inappropriate carbohydrate absorption and utilization necessitating the formation of alternate energy sources via catabolism of stored fat and protein. Insulin deficiency places an individual at risk for electrolyte imbalance, dehydration, and at a higher risk of secondary infection facilitated by an elevated concentration of circulating glucose.[1, 2] Polyuria (PU) and polydipsia (PD) with concurrent weight loss lend suspicion to the diagnosis, which is confirmed by simultaneous elevations in blood and urine glucose concentrations. A substantial commitment is involved in the treatment of canine DM and management of concurrent disease conditions.

Incidence and Signalment

Diabetes mellitus is a common endocrinopathy in dogs, with reported prevalence ranging from 1 in 100 to 1 in 500 dogs affected.[3] The Veterinary Medical Database[a] showed the incidence of DM in dogs to increase by greater than threefold from 1970 to 1999.[4] Increase in reported incidence may be attributed to greater awareness and/or willingness of owners to have dogs evaluated at a referral institution as opposed to DM truly becoming more common. Age of onset is typically 7-9 years, and female dogs are reported to be affected approximately twice as often as male dogs.[1, 2, 5]

Some breeds reported to have an increased risk for developing DM include the Australian Terrier, Standard and Miniature Schnauzer, Bichon Frise, Finnish Spitz, Fox Terrier, Miniature Poodle, Samoyed, Cairn Terrier, Keeshond, Maltese, Siberian Husky, Toy Poodle, Lhasa Apso, and Yorkshire Terrier, listed in order of descending odds ratio according to one large

retrospective study.[4] Smaller breeds of dogs were shown to be at increased risk of developing DM.[4]

Etiology of DM

The etiology of disease development is believed to be multi-factorial, involving immune-mediated destruction of the endocrine pancreas or destruction of the pancreas secondary to inflammation.[6] Body condition and diet play a role, along with environmental factors,[7] insulin antagonistic diseases (such as other endocrinopathies, renal insufficiency, hyperlipidemia, and cardiac disease), infection, glucocorticoids, and possibly a genetic predisposition.[1, 4, 8] Breed, preexisting hyperadrenocorticism (HAC or Cushing's disease), and female gender were identified via multivariate analysis as risk factors for development of DM.[5]

Inflammation of the pancreas can cause its progressive destruction, resulting in dysfunction of the exocrine and endocrine components and subsequent disease. Chronic pancreatitis can damage the pancreatic islets (the hormone secreting cells of the pancreas), leading to the eventual development of insulin deficiency and DM.[9] Approximately 28% to 36% of dogs have been reported to develop DM secondary to chronic pancreatitis.[6, 7] Additionally, pancreatectomized dogs represent a reliable experimental model for canine DM.[10]

A genetic predisposition to canine DM has been suggested;[7, 8] however, the mode of inheritance and specific genes involved have not been identified in most breeds. Regional differences in breeds over-represented for DM may relate to popularity of particular breeds in diverse geographical locations or variations in gene pools.[8] Certain genes (human leukocyte antigen genes) have been linked to DM in humans. Canine gene analogs (dog leukocyte antigen genes) are associated with an increased risk of DM in dogs.[4, 8, 11] This tendency, along with the fact that several of these genes appear to be breed-specific, suggests that genetics are involved in development of the disease.[8] Documentation of genetic-based DM is scarce with a report of familial inheritance in five Samoyeds,[12] familial predisposition in miniature poodles,[13] and evidence of autosomal recessive inheritance in the Keeshond.[14]

Anatomy and Physiology of Glucose Metabolism

Following a meal, glucose and other nutrients are absorbed through the gastrointestinal tract into the blood stream. Insulin is required for utilization of glucose for energy. Physiologic hyperglycemia is detected via perfusion of blood through the pancreatic islets of Langerhans. Pancreatic islets are islands of endocrine tissue located within the pancreatic parenchyma and are composed of four major cell types with roles in hormone secretion: α, β, δ, and *F* cells.[1] Insulin is secreted into the blood stream by the centrally located β cells. In order to accommodate cellular energy demands, insulin facilitates glucose transport into cells of organs and muscles following activation of a cell membrane receptor protein. The four subunit receptor requires that insulin binds to the two external α subunits to cause autophosphorylation of the two internal β subunits leading to activation of tyrosine kinase. An intracellular cascade is activated that allows cellular uptake of nutrients and subsequent metabolism.[15]

The primary function of insulin is to initiate anabolic reactions involving carbohydrates, nucleic acids, and lipids. Insulin enhances glucose oxidation and formation of glycogen, while also stimulating lipogenesis.[2] Glucose is utilized via cellular aerobic and anaerobic metabolism to ultimately form utilizable energy in the form of adenosine-5' triphosphate (ATP). When glucose is transported into the cell via mobilized transporter proteins (GLUT proteins), it is phosphorylated for use either through glycolysis or the citric acid cycle, stored as glycogen in the liver and muscles, or converted to fat and stored in adipose tissue.[15]

Insulin and glucagon act in concert to maintain glucose homeostasis but have very different roles.[2] During times of hypoglycemia, glucagon is secreted from α cells of the pancreas, which are immediately adjacent to the insulin-producing β cells. Glycogenolysis, gluconeogenesis, and lipolysis, all of which are influenced by glucagon, ensue to maintain a circulating energy source.[15]

Pathophysiology of Absolute or Relative Insulin Deficiency

Absolute insulin deficiency (Type I) is defined as an insufficient quantity of insulin produced by the body. The body's poor ability to detect insulin and respond appropriately is known as relative insulin deficiency (Type II); Types I and II both create inappropriately low tissue

glucose levels and resultant hyperglycemia.[1] These two disease conditions are also referred to as immune-mediated DM (Type 1) and insulin resistant DM (Type 2). Dogs affected by DM are considered to have Type I, immune-mediated DM.[1, 8]

Clinical Signs in Dogs

Four hallmark clinical signs of DM in dogs include PU, PD, PP, and weight loss.[1] Glucose absorption into the "satiety center" of the hypothalamus is required to suppress the adjacent "hunger center" and curb an individual's appetite. Passage into this region of the brain is mediated by insulin; in the face of relative or absolute insulin deficiency, the satiety center is not inhibited, leading to PP.[1]

Despite elevated circulating blood glucose (BG), the cells of the body are, in effect, "starved" for energy. Poor utilization of ingested glucose leads to weight loss. Insulin is a powerful inhibitor of lipolysis. In the absence of insulin, fat mobilization and muscle atrophy occur, contributing to weight loss and decline in body condition.[15]

Glucose is not present in the urine of dogs in physiologically normal conditions. Although it is freely filtered from plasma by the glomeruli, it is completely reabsorbed from the proximal renal tubules via secondary active transport.[16] When the transport maximum is exceeded, that is, when the filtered load exceeds the rate at which the tubules are capable of complete glucose reabsorption from the ultra-filtrate, glucose appears in the urine.[16] The glucose renal threshold is 180-220 mg/dL in dogs, meaning that persistent BG concentrations above this threshold can lead to glucosuria.[1] Glucose measures 180 Daltons in size compared to water's 18 Daltons.[16] Due to its relatively large molecular size, an osmotic gradient is created. Water is drawn into the tubules leading to increased urine volume, dilute urine, and the potential for dehydration due to excessive loss of water. The increased volume of urine causes diabetic dogs to become PU and secondarily PD to compensate for water loss.[1]

Diagnosis and Additional Diagnostic Test Abnormalities

Dogs are diagnosed with DM after documenting persistent, fasting hyperglycemia and glucosuria. Aside from sometimes profound hyperglycemia, additional blood work abnormalities are commonly seen in diabetic dogs. Serum biochemical analysis may reveal hepatic enzyme elevations of alkaline phosphatase (ALP), alanine transaminase P5P (ALT), or both. An elevated ALP has been reported to be a more common finding compared to an elevated ALT in insulin-treated diabetic dogs with persistent clinical signs of DM.[17] Diabetic dogs often show electrolyte derangements either related to altered renal tubule physiology with glucose retention or due to varying degrees of dehydration. Hypercholesterolemia and/or hypertriglyceridemia are common findings.[1, 17] While complete blood counts are usually unremarkable, a stress leukogram may be observed.[15]

Urinalysis may reveal dilute urine due to an osmotic gradient created by glucosuria. Proteinuria and bacteriuria are also somewhat common findings in diabetic dogs, as a glucosuric environment and lower urine specific gravity support bacterial growth.[1] Ketonuria may additionally be present. Ketones are an alternative energy source for most tissues of the body, produced under conditions of nutritional stress, such as starvation or insulin deficiency. Adipose tissue is catabolized and metabolites distributed to the blood stream. Hepatocytes are responsible for ketone production; ketone bodies (acetoacetate, beta-hydroxybutyrate, and acetone) are derived from oxidation of free fatty acids. While detection in the blood is possible, this is rarely performed in veterinary medicine; the degree of ketonemia is interpreted indirectly by documenting worsening of metabolic acidosis.[1] When ketones are formed but underutilized, ketone bodies spill into the urine (similarly to glucose) and may contribute to the osmotic gradient in urine. In dogs, identification of ketonuria is performed through the use of urine test strips.[1, 18]

Treatment of DM in Dogs

Treatment goals are directed at achieving remission of the classic clinical signs noted by owners - PP, PU, PD, weight loss and minimizing complications of the unregulated diabetic state such as secondary infection or diabetic ketoacidosis (DKA). Improvement in clinical signs is attained by lowering BG. As dogs are usually insulin dependent when DM is diagnosed,[1] exogenous

insulin is needed to improve hyperglycemia and nutrient utilization. There are several insulin options with various durations of activity, classified as either rapid-acting (Lispro, Aspart, and Glulisine, which are not commonly used in veterinary medicine), short-acting (regular), intermediate-acting (neutral protamine Hagedorn [NPH] and porcine lente [purified porcine zinc insulin suspension]), or long-acting insulin (Glargine, Detemir, and human recombinant protamine zinc insulin [PZI]). Porcine lente is the only insulin approved by the Food and Drug Administration for use in dogs.[18] The standard of initial therapy for dogs that are stable (not in DKA) and have a normal appetite is an intermediate-acting insulin administered subcutaneously twice daily. Recommended administration is at approximately 12 hour intervals, following a full meal. Empirical doses are recommended initially based on a fraction of lean body weight (BW) (typically 0.5 units per kilogram of BW) and are titrated up according to BG testing and/or persistent clinical signs of PU, PD, and PP.[18] Owners are educated on how to handle insulin and administer subcutaneous injections; it is advisable to monitor while the owner prepares and administers the first dose. Most dogs considered well-controlled receive an insulin dose within a dosing range of 0.2 to 1.0 unit/kg every 12 hours.[19] Uncontrolled dogs or dogs requiring a higher dose of insulin (> 1.5 units/kg) should be evaluated for insulin resistance.[18]

If the dog is fed a commercial diet, recommending the ideal amount of food to offer to maintain or achieve an ideal BW may be necessary; continuing the dog's original diet twice daily is preferred to prevent loss of appetite during diet transition or the need to recalculate the insulin dose based on a new diet. Once diet and insulin dose are established, feedings must follow a consistent schedule, quantity, and diet type to best maintain glycemic control.[1, 18] When an adjustment in diet is indicated, dog food low in simple carbohydrates and high in protein and soluble and insoluble fiber is reported to minimize postprandial hyperglycemia.[18] Diets designed for weight management or weight loss may also be good selections for the overweight diabetic dog when a diet change is indicated.[18]

Complications Associated with DM

Ocular Complications

Cataracts are one of the most common ophthalmic complications of DM in dogs.[1] Aqueous humor resembles an ultra-filtrate of plasma, composed of many organic (i.e., glucose, amino acids, lactate, protein, etc.) and inorganic (i.e., bicarbonate, sodium, chloride, oxygen, etc.) compounds. Passage of these compounds through the lens capsule occurs via passive diffusion or through active transport. (Appendix A) The energy source for the lens is glucose, which diffuses from the aqueous humor through the semi-permeable lens capsule or is transported via a glucose transporter (GLUT-1), found within the lens epithelial cells.[20-22] Energy is primarily derived from anaerobic glycolysis (Embden-Meyerhof pathway) with the rate of glycolysis being controlled by the amount of hexokinase enzyme and the rate of entrance of glucose through the lens capsule. In a hyperglycemic state, lens glucose metabolism is altered. The concentration of glucose-6-phosphate is increased, saturating the hexokinase enzyme and inhibiting glycolysis;[22, 23] a greater concentration of glucose is shunted to an alternate pathway where glucose is converted to sorbitol.[20, 24] The osmotic gradient formed from an excessive sorbitol concentration results in imbibition of water with subsequent swelling and rupture (hydropic degeneration) of the lens fibers, leading to cataract formation.[25, 26]

Diabetic retinopathy occurs in both canine and human diabetic patients.[27-30] Persistent hyperglycemia leads to capillary basement membrane thickening, collagen and fibronectin deposition in the extracellular matrix, and loss of pericytes.[27] Retinal blood vessels of diabetic patients display fragility, which may be manifested funduscopically as intraretinal or preretinal hemorrhages or microaneurysms, termed diabetic retinopathy. This finding is commonly recognized in humans[27] but was documented to occur in 20 and 21% of dogs in two reports.[29, 31] In a galactosemic canine model of DM, 10 juvenile Beagles that were unilaterally aphakic developed microaneurysms, retinal hemorrhages, and other vascular pathology by 28 months after starting a 30% galactose diet; progression of retinal pathology was also documented with fluorescein angiography and monitored for 41 months while on this diet.[32] Retinal thinning, either focally or diffusely, may also be noted as a component of diabetic retinopathy.[29]

Other ocular complications reported to occur with DM in dogs include: keratoconjunctivitis sicca,[33, 34] reduced corneal sensitivity (independent of glycemic control or duration of DM),[34, 35] conjunctival epithelial dysplasia, reduced goblet cell densities, significantly reduced tear film break up times,[34] and endothelial degeneration manifested by polymegethism and pleomorphism.[36] The etiopathogeneses for the development of each of these conditions have not been confirmed. Some anatomical changes show direct relationship with degree of diabetic control in both dogs and humans.[25, 27, 30, 36]

Two of the leading causes of blindness in humans in the United States are consequences of ocular disease associated with DM. These include proliferative diabetic retinopathy and diabetic macular degeneration, which often occur concurrently. Diabetic retinopathy in humans is subdivided into proliferative and non-proliferative forms,[27, 30] and together, they account for 12% of new cases of blindness each year.[27] Microaneurysms, intraretinal hemorrhages, venous looping, and intraretinal microvascular abnormalities typify the non-proliferative form. Proliferative diabetic retinopathy, which is the more severe form, is characterized by neovascularization on or adjacent to the optic disc or in the vitreous or presence of vitreal/preretinal hemorrhage. New vessel growth occurs as a result of retinal ischemia, but these vessels are often fragile and lead to vitreal hemorrhage. Diabetic macular degeneration can occur with any level of severity of diabetic retinopathy; it involves vascular leakage and edema causing thickening of the retina with deposition of hard exudates (accumulations of lipid and serum), adjacent to or involving in the cone-rich macula, which obscures retinal function and visual acuity.[27] Diagnosis may require fluorescein angiography, optical coherence tomography, or ultrasonography in cases where the fundus is poorly visualized. Proliferative or fragile vasculature may require laser photocoagulation (panretinal or grid/focal) in order to reduce the risk of vitreal hemorrhage and retinal detachment, and vitreal hemorrhage can be removed by pars plana vitrectomy. Glycemic and blood pressure control, along with eating a low fat diet and eliminating smoking help delay development of or prevent exacerbation of diabetic retinopathy.[27, 30] Severe complications of diabetic retinopathy include traction retinal detachment, vitreous hemorrhage, and neovascular glaucoma.[27]

Heightened Susceptibility to Infection

Diabetic dogs often experience chronic or recurrent infections due to a diminished resistance to infection.[2] The innate inflammatory response to bacterial pathogen-associated molecular patterns in these dogs is altered, as pro-inflammatory cytokine (TNF, IL-6, and IL-10) production is increased without a corresponding and equivalent anti-inflammatory cytokine production.[37] This potentially explains a diabetic dog's increased susceptibility to inflammation and infection. Affected dogs also experience impaired chemotactic, phagocytic, and microbiocidal functions of leukocytes.[2] Urinary tract infections are a common complication seen in unregulated diabetic dogs.[38] The urine can be more dilute and saturated with glucose, favoring presence and growth of glucose-fermenting bacteria such as *Escherichia coli, Proteus* species, and *Aerobacter aerogenes*.[2] Retrospective studies report, 21-24% of diabetic dogs evaluated had an occult urinary tract infection with bacteriuria that was not always accompanied by pyuria. *Escherichia coli* was the most common bacteria isolated from the urine. [17, 38] Bacterial infections can contribute to insulin resistance.[17, 19]

Other Complications

Hepatomegaly occurs in diabetic dogs due to chronic, inappropriate fat deposition and hepatic parenchymal remodeling.[2] Hepatomegaly was documented in 61% of diabetic dogs, in a retrospective study.[17] Systemic hypertension and urine protein loss are relatively common findings in diabetic dogs.[39] In a prospective, observational study of 11 diabetic dogs with evaluation over 2 years, 55% were diagnosed with systolic hypertension (defined as blood pressure >150mmHg) and 64% were diagnosed with diastolic hypertension (defined as blood pressure>95mmHg).[31] Also, microalbuminuria and elevated urine protein:creatinine ratio were documented in up to 73% and 55% of dogs, respectively. No significant association between these vascular complications and time since diagnosis of DM or degree of BG control was found.[31] Diabetic ketoacidosis is a possible complication of chronic insulin deficiency, as ketones are produced as an alternate energy source. Excessive circulating levels of ketones alter acid-base balance (leading to decreased total carbon dioxide and a high anion gap metabolic acidosis), contribute to alterations in electrolytes, and encourage dehydration, making this condition a medical emergency depending on the severity of clinical signs.[1, 18] Peripheral neuropathy[1, 40] and the presence of a concurrent endocrinopathy, primarily HAC,[1] are

additional possible sequela associated with DM. The clinical signs and physical exam (PE) findings of both DM and HAC have considerable overlap, sometimes making diagnosis of concurrent HAC a challenge in known diabetic dogs.[41, 42] In a retrospective study of 221 diabetic dogs, HAC was diagnosed in 23%.[17]

Glycemic Control

Evaluation of Glycemic Control

Evaluation of glycemic control in diabetic dogs is challenging, with no consistent recommendations in the literature outlining a specific diagnostic testing protocol, nor an available test able to yield a definitive yes (well regulated) or no (poorly regulated) answer.[1] Evaluation of the degree of BG regulation is based on interpretation of a compilation of tests, which may include any or several of the following: a glucose curve (currently the gold standard of glycemic testing), urine glucose and ketone concentration monitoring, serum fructosamine and/or glycated hemoglobin concentrations, and determination of interstitial glucose concentrations using a continuous glucose monitoring system. History and PE findings are highly valuable in determining of degree of DM control.[43]

Despite being considered the gold standard of testing, glucose curves are affected by stress, the amount and timing of food consumed, inherent error when measuring insulin doses in a syringe, the variability in the rate and amount of insulin absorbed from the injection site,[44] and the inaccuracy of handheld glucometers.[45] A study comparing whole BG using a handheld glucometer to serum glucose using the laboratory plasma hexokinase method reported glucometers to slightly underestimate circulating blood glucose with the concentration typically measuring 2 mmol/L lower with glucometers; this is not a clinically important discrepancy for most scenarios.[45]

A recent study demonstrated that there is a large day-to-day variation in BG concentrations causing a single diabetic dog to have very different BG curves over subsequent days with no changes in therapy.[44] Although serial BG curves have limitations as a clinical tool in diabetic dogs, they remain a useful test for evaluating glycemic control in this species.[44]

Fructosamines are stable ketoamine compounds formed by the irreversible, insulin-independent binding of glucose to an amino group on serum proteins. The concentration of fructosamine reflects the average BG concentration over the preceding one to three weeks.[46] Fructosamine concentrations are not affected by day-to-day variations that affect serial BG concentrations. The test has a high sensitivity and specificity (93% and 95%, respectively, according to one study)[47] and may be chosen as a screening test for initial diagnosis, as well as a method of assessing glycemic control, in diabetic dogs.[46, 47] Correction of the fructosamine concentration for various serum proteins is not recommended, as there was no difference between corrected and uncorrected concentrations in discriminating between dogs with and without DM.[47] Well-controlled diabetic dogs show significantly lower fructosamine concentrations on average compared to poorly controlled diabetic dogs.[43] However, hypoalbuminemia, hyperlipidemia, and azotemia can affect serum fructosamine concentrations in healthy dogs,[48] and presumably in diabetic dogs, as well. Glycated hemoglobin concentration measures glucose bound to hemoglobin and is therefore representative of the average BG concentration over the previous six to eight weeks. Fructosamine estimation is more frequently performed in veterinary medicine compared to glycated hemoglobin.[46]

The Guardian® continuous glucose monitoring system[b] estimates glucose concentrations by converting interstitial glucose into gluconic acid and hydrogen peroxide through a reaction that creates an electric current. The Guardian unit uses proprietary algorithms to convert the signal into a numeric value representative of glucose concentration. The use of a continuous glucose monitoring system to measure glucose concentration alleviates the need for repeated venipunctures, minimizes stress associated with repetitive glucose testing,[49] and provides considerably more information than that obtained with a standard BG curve.[50] The use of the Guardian monitoring system has been validated for use in veterinary medicine; it was found to correlate well with whole BG concentrations in clinically normal dogs.[49] Limitations of the system include the need to calibrate the system multiple times in a 24 hour period and a limited glucose concentration recording range (40 to 400 mg/dL).[49]

Insulin Resistance

When hyperglycemia is controlled, clinical signs of PU, PD, weight loss, and PP improve or subside. Return of these clinical signs indicates a loss of glycemic control, which may be explained by insulin resistance. Insulin resistance occurs when there is a subnormal biologic response and decreased tissue responsiveness to endogenously produced or exogenously administered insulin. It is suspected in diabetic dogs when a dog is given >1.5 units/kg per dose of insulin.[19] Conditions that most commonly cause insulin resistance in dogs include the following: treatment with diabetogenic drugs (glucocorticoids), oral infections or urinary tract infections, severe obesity, HAC, diestrus, renal insufficiency, chronic pancreatitis, hyperlipidemia, neoplasia, and the development of insulin antibodies.[1, 7, 17, 38, 42]

Glucocorticoids, whether endogenously released (stress-induced, HAC) or exogenously administered, exacerbate hyperglycemia by stimulating gluconeogenesis in the liver. Not only is glucose production increased, but relative insulin resistance also ensues.[1] Insulin antagonism occurs as a result of a decreased quantity of cell membrane insulin receptors, altered insulin receptor binding affinity, or impairment in a post-receptor step involving activation of the glucose transport system.[1] Pituitary-dependent HAC leads to decreased function of pancreatic β cells and causes insulin resistance and hyperglycemia. Having HAC places dogs at a 13 times greater risk of developing DM. Approximately 10% of dogs with HAC develop DM.[41] Similarly, systemic administration of steroids to diabetic dogs increases the insulin requirement. Systemic glucocorticoid administration is, therefore, considered "relatively contraindicated," [1] depending on the individual dog and the concurrent diseases.

Cataracts

Definition

A cataract is an opacity within the lens that forms due to alterations in the intralenticular metabolic environment. Decreased activity of lens epithelial Na-K ATPase pumps causes alterations in electrolyte concentrations. Oxygen consumption and ATP production decrease, and antioxidant activity is diminished;[20, 22] free radicals and reactive oxygen species are elaborated from damaged intracellular organelles, perpetuating damage to lens fibers and lysis of

fibers.[25] The opacity develops due to a shift in concentration of lens proteins from soluble (crystalline) to insoluble, high-molecular weight proteins (albuminoids), which normally account for only approximately 15% of the lens protein.[20]

Classification of Cataracts

Cataracts are classified into 4 stages by the amount of the lens that is affected. Incipient cataracts occupy from 1-15% of the lens volume. Immature cataracts account for all opacities with 15-99% affected lens volume. In mature cataracts, 100% of the lens is affected, and the animal is blind. Hypermature cataracts are characterized by loss of lens volume, as degradation of lens proteins into amino acids and polypeptides creates small byproducts that diffuse through the intact lens capsule along with water.[20, 25] Some dogs merely develop focal lenticular opacities that never progress to a more advanced stage; however, dogs may experience all stages of cataract with inconsistent rate of progression to each stage. Lenses of diabetic dogs, due to the osmotic force created by sorbitol within the lens capsule, imbibe water and can quickly loose transparency secondary to hydropic degeneration. Lenses may develop a "swollen" appearance, termed intumescence. This type of cataract is diagnosed by the presence of a subjectively shallow anterior chamber caused by enlargement of the lens and anterior displacement of the iris diaphragm. A potential side effect of an intumescent cataract is ocular hypertension. The pathway of aqueous humor flow from the posterior chamber through the pupil can be compromised by the swollen lens causing buildup of fluid in the posterior chamber and elevation of the intraocular pressure. Lens capsule rupture may also occur with rapid lens intumescence.[20, 33] Spontaneous lens capsule rupture in non-diabetic patients is poorly documented in the literature but has been observed.[20] Hypermature cataracts, rapidly progressing cataracts, and lenses with capsular ruptures and have been shown to more frequently lead to intraocular inflammation,[33, 51, 52] although all stages of cataracts can incite inflammation.[53, 54]

Etiology

Cataract development in the dog can occur secondary to advanced age, ocular disease or inflammation, embryologic developmental issues leading to congenital cataracts, direct trauma,

toxic ocular environments (i.e., progressive retinal atrophy or other types of retinal degeneration), various medications (i.e., chronic ketaconazole administration[55]), electrocution,[56] radiation, inappropriate nutrition (amino acid imbalance) during early life, or systemic disease processes (i.e., DM, hypoparathyroidism), however, an inherited etiology is the most common cause in this species.[20] The severity of visual compromise depends on the location and degree of the lenticular opacity. Cataracts are a common complication of DM in dogs. Cataracts in diabetic dogs typically begin at the equator of the lens as vacuoles within the cortex[20, 24, 26] that may progress to 100% involvement of the lens material, resulting in blindness. The rate of cataract formation depends on the degree and duration of hyperglycemia, lenticular aldose reductase activity, degree of oxidative stress and free radical formation, and sorbitol concentration within the lens.[25, 57-60] A study evaluating 200 insulin-treated, diabetic dogs (degree of regulation not specified) showed 50% of dogs developed cataracts by 170 days following the diagnosis of DM, 75% by 370 days post diagnosis, and 80% by 470 days post diagnosis.[23]

Pathophysiology of Cataract Development Secondary to Hyperglycemia

Glucose diffuses passively through the semi-permeable lens capsule or via facilitated transport.[20, 22] Two glucose transporters have been identified in the lenses of rats; GLUT-1 is primarily present in the lens epithelial cells, while GLUT-3 was the predominant isoform in the lens fibers.[21] Energy in the lens is primarily derived from anaerobic metabolism through the glycolytic or Embden-Meyerhof pathway with approximately 5% of glucose being processed through the sorbitol (also known as polyol) pathway.(Appendix A) In a hyperglycemic state, lens glucose metabolism is altered in effort to prevent excessive lactic acid production and due to saturation of the hexokinase enzyme;[22, 23] a greater concentration of glucose (10-33%) is shunted to the sorbitol pathway for metabolism.[20, 24] Glucose is converted to sorbitol (or in the induced galactosemic model of cataract development, galactose to dulcitol) in a reaction catalyzed by aldose reductase. Sorbitol is subsequently converted by sorbitol dehydrogenase to fructose,[2] which is able to slowly diffuse through the lens capsule or reenter the glycolytic pathway for further metabolism.[25] Dulcitol is not further metabolized, causing more rapid cataract formation compared to that caused by sorbitol.[61] PubChem Compound Database[c] documents sorbitol and dulcitol to have a similar molecular weight of approximately 182

Daltons, which is only slightly larger than that of glucose (180 Daltons).[15] The polar character of sorbitol prevents efficient diffusion back through the lens capsule. This, along with the fact that sorbitol is produced faster than it can be converted to fructose, causes accumulation of sorbitol.[25] An osmotic gradient is formed from a high sorbitol concentration resulting in imbibition of water with subsequent swelling and rupture (hydropic degeneration) of the lens fibers.[25, 26] Disruption of lenticular fibers, formation of interfibrillar clefts, changes in cell membrane permeability, oxidative damage, nonenzymatic glycosolation of lens crystallins, and generation of free radicals and reactive oxygen species all contribute to cataract formation.[2, 20, 25, 57]

The rate of cataract development is variable and is believed to be multifactorial dependent on individual aldose reductase activity, the dog's degree of and duration of hyperglycemia, and the dog's age.[23, 33, 62] Cataracts may be reversible in young diabetic humans with improvement of diabetic control.[25] Aldose reductase activity decreases with increasing age in dogs and humans[24, 25, 63, 64] and activity levels have been shown to be species dependent. The rat possesses ≥14 times the aldose reductase activity compared to a dog and is also a species that readily develops cataracts relating to elevated blood sugar in the form of galactose.[26] Dogs and humans show similar aldose reductase activity in the lens (0.39 nm/min/mg),[26] but the prevalence of cataracts with DM is higher in dogs, giving further weight to a multifactorial pathogenesis.[23] Aldose reductase inhibitors applied topically to the ocular surface have been shown in the galactosemic dog model of DM to cause a dose-dependent reduction of cataract development but prevention of cataracts was not achieved with any concentration of the drug.[26] More recently, a study evaluating effects of a topically administered aldose reductase inhibitor, Kinostat[d], applied to the eyes of 28 newly diagnosed diabetic dogs with naturally occurring DM showed delay the onset and progression of diabetic cataracts. After 12 months, the cataract score in the treatment group was significantly lower than the control group, and cataract scores of the treatment group did not significantly increase from the time of enrollment.[65, 66]

One study reported humans with Type 2 diabetes to have a 3.3% incidence of cataract formation over a 3.6 year observation period.[67] A retrospective study evaluating information from two

major medical databases over 35 years reported that diabetic humans had a high risk (mean risk ratios ranging from 2.30-2.95) for developing cataracts, when compared to a reference cohort.[68] The 10-year cumulative incidence of cataract surgery in diabetic humans was 8.3% and 24.9% in patients suffering from Type 1 and Type 2 diabetes, respectively.[69] Humans have higher sorbitol dehydrogenase activity compared to dogs, which converts sorbitol to fructose.[64] Fructose is able to slowly diffuse through the lens capsule or it may reenter the glycolytic pathway for further metabolism.[25] This may be an explanation for the lower prevalence of diabetic cataracts in humans compared to dogs and other animals.[64]

The prevalence of DM in cats is 1 in 50 to 1 in 400 depending on the population studied[7, 70] and generally occurs in cats greater than 7 years of age.[24] Cataracts are not a common complication of DM in cats, as aldose reductase activity decreases with age and is significantly lower in adult cats.[24] Lenses of dogs and cats that were incubated in a high glucose medium (30 mmol/L) for 14 days showed glucose-related lens changes (vacuole development and lens fiber swelling) in both species; glucose-induced lens alterations between dogs and cats differed in location and extent. Dogs developed large vacuoles at the equator of the lens. Lenses from cats ≤4 years old developed prominent suture lines from lens fiber swelling and extensive posterior cortical opacities with associated small vacuoles. All lenses from young cats (≤ 4 years old) and no lenses from aged cats (10-18 years old) developed cataracts.[24]

Anatomic and Immunologic Considerations with Anterior Uveitis

Blood Aqueous Barrier

Anatomically, the blood aqueous barrier (BAB) is composed of the posterior pigmented epithelium of the iris, the vascular endothelial cells of the iris, and the tight junctions between the non-pigmented ciliary body epithelial cells.[71] The following inflammatory mediators are known to play a role in destabilization of the BAB: prostaglandins (PG), leukotrienes, platelet-activating factor, neuropeptides like calcitonin gene-related peptide and substance P, nitrous oxide, bradykinin, and interleukins.[72] Vascular permeability is increased, allowing larger, intravascular molecules (namely proteins) to pass between endothelial cells and enter the anterior chamber. Prostaglandins are the main substances that result in the clinical manifestation of

intraocular inflammation.[73] Leukotrienes were shown to not be an important mediator of BAB disruption; leukotriene inhibitors may actually exacerbate disruption by shunting arachadonic acid (AA) toward the cyclooxygenase (COX) pathway.[74]

Phacolytic Uveitis or LIU

As the osmotic pressure within the aqueous humor equilibrates across the lens capsule of a cataractous lens, soluble proteins (α, β-heavy, β-light, and γ-crystalline), which are antigenic, migrate from the lens. Lens proteins released in small amounts are phagocytized by antigen presenting cells, which then exit the eye via the trabecular meshwork and travel to the spleen where afferent (CD4+) and efferent (CD8+) regulatory T cells are formed. A scenario of anergy or immune tolerance in the eye is created by preventing activation and differentiation of naïve T cells (afferent phase) and suppression of Th1 immunity (the effector phase) of cell-mediated immune response. This mechanism is accomplished through anterior chamber associated immune deviation.[75] Subsequent lens protein release follows an altered antigen presentation; CD8+ cells are activated, which suppress Th1-mediated, delayed-type hypersensitivity in the eye. During excessive exposure of lens proteins in the anterior chamber, T-cell tolerance homeostasis is overwhelmed, and Th1 immunity is activated.[75] Release of inflammatory cytokines results in compromised BAB integrity. Protein leakage and leukocyte (monocyte) chemotaxis ensue.[76] This exaggerated, non-specific inflammatory response to translocated lens protein causing breakdown of the BAB is known as phacolytic or LIU.[33, 76, 77] The prevalence of LIU with any stage of cataract is reported to be 71%,[53] but subclinical uveitis may account for an even greater number affected.[78, 79] Histologically, phacolytic uveitis typically manifests as a mild lymphoplasmacytic uveitis, but a severe granulomatous anterior uveitis (macrophages, lymphocytes, and neutrophils) is possible associated with hypermature cataracts.[33, 76, 77]

Phacoclastic Uveitis

Phacoclastic uveitis is an exaggerated inflammatory response of the eye following exposure to large volumes of lens proteins released with rupture of the lens capsule.[76, 77] Lens capsule rupture may occur spontaneously, secondary to rapid progression of diabetic cataracts in dogs or

by ocular trauma with direct damage to the lens capsule.[33, 77] Wilkie et al documented lens capsule ruptures in 9% (20/215) of diabetic dogs that received cataract surgery in a 6 year period. Cataracts in these dogs were noted to have been present for a significantly shorter duration (39 days) than diabetic dogs without lens capsule rupture (mean 63 days).[33] Most ruptures occurred in the weaker, equatorial aspect of the capsule, approximately half of the dogs experienced this complication bilaterally, and the Labrador Retriever was over-represented (6/20 dogs) for this finding.[33] Light microscopy of globes diagnosed with phacoclastic uveitis shows intralenticular polymorphonuclear cells; it is characterized by zonal inflammation with a more chronic change being perilenticular fibroplasia and granulomatous uveitis.[77]

Clinical Signs of Anterior Uveitis

A hallmark clinical sign of uveitis is aqueous flare, in which proteins in the aqueous humor are visualized with slit-lamp biomicroscopy.[80] A phenomenon known as the Tyndall effect occurs when light from the slit-lamp is scattered as it contacts the proteins in the anterior chamber, creating a characteristic "fog" within the normally transparent aqueous humor. The degree of aqueous flare appreciated is directly proportional to the severity of breakdown of the BAB.[72] Aqueous flare is quantified on a 0 to 4+ scale, and although the assignment is subjective, a common grading scheme is followed. If aqueous flare is barely detectable, it is considered 1+, whereas 2+ is moderate, 3+ is typified by hazy detail of intraocular structures such as the iris and lens. Intense flare with fibrin is classified as 4+, and would require the most aggressive treatment. Intraocular structures are barely visible to totally obscured with this degree of inflammation.[81]

Other consistent findings with uveitis are hypotony (decreased intraocular pressure), a miotic pupil, resistance to pharmacologic dilation, rubeosis iridis (a meshwork of small vessels on the iris surface), suspended cells in the anterior chamber, change in iris color, and keratic precipitates (accumulations of inflammatory cells that adhere to the corneal endothelium). Preiridal fibrovascular membranes may be identified histologically, which are termed rubiosis iridis clinically.[80] The pathogenesis of its development is suspected to be due to hypoxia, angiogenic, and fibroblastic stimulatory factors associated with chronic inflammation.[82] Hyperemia of the conjunctiva or episcleral injection, ciliary flush (hyperemia of the

circumcorneal anterior ciliary vessels), photophobia, or other signs of ocular pain (blepharospasm, elevated third eyelid, pawing at the affected eye, or epiphora) can be observed concurrently but are not findings specific to uveitis.[80]

Treatment of Anterior Uveitis

Routes of Anti-inflammatory Drug Administration

Topical anti-inflammatory medications are indicated for treatment of anterior segment inflammation, with the drug type and frequency based on severity of clinical disease. Medications applied topically penetrate to the level of the posterior lens capsule, making other routes of administration necessary for the treatment of posterior uveitis.[83, 84] Ease of administration and the ability to minimize systemic side effects are two benefits of topical therapy.[85] One of the main disadvantages of topical administration involves the difficulty in penetrating the lipophilic corneal epithelium, which represents a major barrier to absorption of many medications.[84] The corneal epithelium and Descemet's membrane are lipophilic, whereas the corneal stroma is hydrophilic. Drugs with an intermediate solubility profile are better able to penetrate intact cornea than are drugs that are strictly lipophilic or hydrophilic.[83] Acetate and alcohol corticosteroid formulations, which are more lipophilic, show superior corneal penetration compared to phosphate formulations.[86]

Subconjunctival administration of anti-inflammatory medications can achieve high concentrations in the anterior chamber and may be used concurrently with other therapeutic routes.[84] Administration in the bulbar subconjunctival space allows for use of hydrophilic drugs and affords improved efficiency, as the drugs do not have to pass through the conjunctival epithelium, a major rate-limiting step. Drugs given by this route are absorbed directly through the cornea and sclera and into the anterior chamber, ciliary body, and vitreous.[87] Medications may also ooze from the injection site and bathe the cornea, from where they then penetrates, or they may be absorbed into the conjunctival blood vessels and return hematogenously to the uveal tract.[83] Long-term therapy (8 hours to 2-3 weeks depending on formulation) via slow release of drug can be achieved,[83, 84] which is advantageous for the aggressive or feral dog or the poorly compliant owner. Corticosteroids are commonly used anti-inflammatory medications

administered by subconjunctival injection. Phosphate formulations are water soluble and therefore quickly absorbed from the injection site, but acetate and diacetate esters have a longer duration of action, as they are poorly water soluble. Betamethasone, dexamethasone, methylprednisolone acetate, and triamcinolone acetonide are commercially available corticosteroids that may be administered subconjunctivally.[84] A disadvantage of a subconjunctival injection is that the medication cannot be discontinued if complications arise; topical anti-inflammatory medications should not be administered in the face of a corneal ulcer or corneal infection, as they reduce migration of neutrophils and macrophages, impair host defenses and increase the risk of or exacerbating infection.[84] Additionally, subconjunctival granulomas may form at the injection site that can serve as a chronic source of inflammation and may require surgical excision.[88] Subconjunctival administration of NSAIDs is not recommended, as it has been shown to cause severe inflammation at the injection site.[89]

Systemic anti-inflammatory therapy is effective for treating anterior segment inflammation and may be selected for combined treatment with topical or subconjunctival corticosteroid therapy.[84] When selecting this route and determining a dose, judicious use must be employed. Consideration should be given to underlying diseases that preclude treatment with systemic steroids (i.e., generalized infection, diabetes mellitus, pancreatitis, etc.) and adverse systemic side effects that occur with administration.[84]

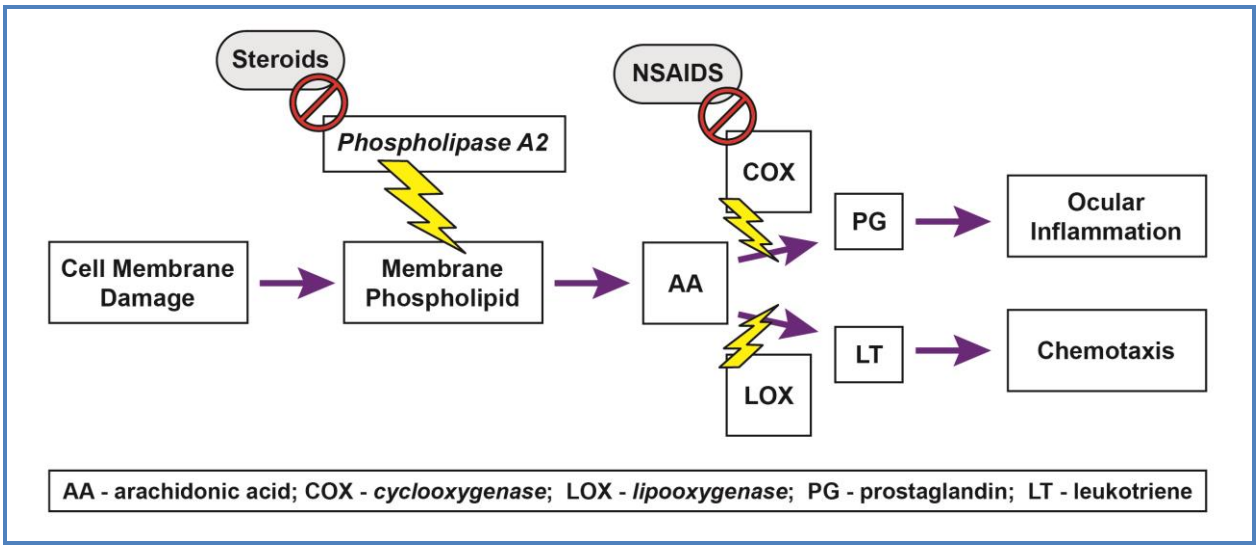
Intravitreal injections and sustained release dexamethasone intravitreal implants are rarely used in veterinary medicine but can treat posterior segment inflammation.[83, 84] Lens-induced uveitis causes inflammation primarily of the anterior segment, making these routes of administration less indicated.

Drug Properties and Available Ophthalmic Medications

Treatment of uveitis is accomplished through the use of topical and systemic anti-inflammatory drugs, either steroidal or non-steroidal.[51, 73, 76-78, 83, 84, 89-94] (Figure 1.1) Corticosteroids bind intracytoplasmic receptors present in most tissues, which then translocate to the nucleus and modify gene transcription.[1, 95] The synthesis of lipocortins is increased, blocking phospholipase A_2 and preventing conversion of phospholipids to arachidonic acid

(AA).[95] Suppression of AA inhibits subsequent formation of PG, prostacyclin I_2, thromboxane A_2, and leukotrienes (LTD_4, LTE_4).[73, 84, 91] Corticosteroids may induce expression of somatostatin, which has been shown to elicit anti-inflammatory effects.[96] Glucocorticoids inhibit prostaglandin synthesis at the cyclooxygenase pathway; they also have been proposed to have lysosomal membrane stabilization properties, which would protect organelle membranes from lysis and leakage of cell contents.[84]

Figure 1.1: Inflammatory Cascade and the Therapeutic Mechanism of Action



Lightning bolts represent points of activation and red, slashed circles represent points at which steroids and NSAIDs inhibit the inflammatory cascade. Figure created by Jane Ashley Stuckey and Mal Rooks Hoover, 2014.

Available topical ophthalmic steroid preparations include: 0.12% and 1% prednisolone acetate, 1% prednisolone sodium phosphate (available in combination with antibiotics), 0.1% dexamethasone alcohol, 0.1% dexamethasone sodium phosphate (available in combination with antibiotics) or 0.1% dexamethasone-cyclodextrin, and hydrocortisone acetate (available in combination with antibiotics). As drugs used for local anti-inflammatory purposes do not undergo hepatic conversion to the active metabolite, prednisolone is required for topical ophthalmic use as opposed to prednisone, as the former is the metabolically active glucocorticoid.[1] The acetate and alcohol drug formulations are more lipophilic and therefore afford improved corneal penetration compared to the sodium salts of the phosphate formulation, which are water soluble.[84, 86] The cyclodextrin-based delivery system enhances the solubility

of dexamethasone in aqueous ophthalmic formulations and also improves permeability into the human eye, allowing aqueous humor drug concentrations to exceed those reported for other ophthalmic steroid medications.[97] The drug generally accepted to be the most efficacious for treating anterior segment inflammation is 1% prednisolone acetate.[84]

Additional FDA approved ophthalmic steroids less commonly used in veterinary medicine include: 0.05% difluprednate, 1% medrysone, 1% rimexolone, 0.1% and 0.25% fluorometholone alcohol, and 0.2% and 0.5% loteprednol etabonate. The latter three drugs listed are considered "soft steroids." Soft steroids are selected for use with the goal of providing local anti-inflammatory effects with a lower risk of inducing ocular hypertension in humans.[84, 95, 98] Loteprednol is a highly lipophilic, ester steroid that is metabolized to an inactive form in the aqueous humor, minimizing local side effects of ocular hypertension and cataractogenesis.[95] Humans receiving 0.5% loteprednol etabonate suspension 4 times daily to one eye showed more mild ocular hypertension (rise from 17.4 mmHg to 21.5 mmHg) after 42 days of treatment compared to those same individuals treated 4 times daily with 1% prednisolone acetate to one eye (rise from 18.1 mmHg to 27.1 mmHg) for 42 days.[85] The rise in intraocular pressure in the latter group was significant, lending merit to the study of and continued use of this drug in human medicine.[85] There are no reports on the efficacy of soft steroids or their use in veterinary medicine. Ocular hypertension from ophthalmic steroid application is not a clinical concern in dogs, although it has been induced experimentally in Beagles with primary open angle glaucoma; intraocular pressure rose by a mean of 5 mmHg after two weeks of therapy with 0.1% dexamethasone but returned to normal following cessation of the medication.[99]

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the conversion of AA to prostaglandins (PGE_2, PGD_2, $PGF_2\alpha$), prostacyclin (PGI_2), and thromboxane A_2 via inhibition of COX.[73, 84, 91] Both the constitutive form (COX-1), which is expressed on the endoplasmic reticulum of all cells in the body, and the inducible form (COX-2), which is synthesized by macrophages and is seen with inflammation, are inhibited by NSAIDs.[73] Efficacy of NSAIDs applied to the ocular surface in dogs has been thoroughly evaluated.[78, 91, 100-103] Commonly used, commercially available ophthalmic NSAIDs include: 0.1% diclofenac sodium, 0.03% flurbiprofen, 0.09% bromfenac, 0.4% and 0.5% keterolac tromethamine, and 0.1% nepafenac.[73, 84, 104] Suprofen and tolmetin in 1% formulations have been used in veterinary research.[100] Indomethacin is an

ophthalmic NSAID commercially available in Europe and Canada.[73] Ophthalmic NSAIDs are indicated in the treatment of PG-induced inflammation post cataract surgery; they are also beneficial in preventing intraoperative inflammation and miosis during cataract surgery.[73, 84]

Experimental Use of Ophthalmic Anti-inflammatory Medications

Topical anti-inflammatory medications are effective at preventing and/or controlling experimentally induced anterior uveitis in dogs. Ward et al showed that 1% solutions of ophthalmic NSAIDs were effective (diclofenac >flurbiprofen>suprofen) at preventing breakdown of the BAB following anterior chamber paracentesis.[100] Ward et al also performed a study comparing the efficacy of ophthalmic steroids and NSAIDs and their ability to inhibit paracentesis-induced anterior uveitis. Results showed that 1% prednisolone acetate and 0.03% flurbiprofen were more effective than 0.1% dexamethasone sodium phosphate at stabilizing the BAB as determined by fluorophotometry.[101] Pilocarpine-induced anterior uveitis was documented to be inhibited by 0.03% flurbiprofen, 0.1% diclofenac, and 1 % suprofen, as quantified by laser flaremetry. Non-steroidal anti-inflammatory drugs were more effective than topical 0.125 or 1% prednisolone acetate at inhibiting BAB breakdown induced by the antidromic stimulation from topical pilocarpine.[78] Millichamp and Dziezyc also showed that pretreatment with 0.03% flurbiprofen prevented signs of anterior uveitis following lens capsule disruption with neodymium:yttrium aluminum garnet laser.[102]

In cats, topical application of 1% prednisolone acetate and 0.1% diclofenac were effective at reducing aqueous flare quantified by laser flaremetry when anterior uveitis was induced by anterior chamber paracentesis; 0.1% dexamethasone and 0.03% flurbiprofen did not significantly decrease flare at any time point.[91] Topical application of 0.1% dexamethasone beloxil (AL-2512) reportedly inhibited endotoxin-induced leukocyte influx in cats by 59% compared to 1% prednisolone acetate, which inhibited influx by 37%.[105]

Side Effects of Topical Ophthalmic Anti-inflammatory Medications

Use of topical ophthalmic corticosteroids is contraindicated with corneal infection (bacterial, fungal, or viral), as therapy can exacerbate infection.[84] Corticosteroids decrease neutrophil and

macrophage migration and depress their phagocytic function.[106] Epithelialization of a corneal ulcer can be delayed by the use of these drugs, and stromal keratocyte proliferation and collagen deposition are also reduced with steroid treatment.[84, 107] Ocular hypertension occurs in normal and glaucomatous humans [85, 95] and normal cats[108] following use of topical ophthalmic steroids; this has also been shown experimentally in glaucomatous Beagles.[99] Steroid-induced cataract formation is a concern in humans.[95] This is not appreciated clinically in veterinary species,[84] yet steroid-induced cataracts have been experimentally produced in cats following topical administration of 0.1% dexamethasone sodium phosphate or 1% prednisolone acetate 2-3 times per day to 1 eye.[108] Lipid keratopathy is a clinically recognized condition noted in dogs following topical corticosteroid treatment.[84]

Reported potential side effects of topical NSAID administration in dogs include local irritation (conjunctival hyperemia and mild burning sensation) and ocular hypertension. These drugs are also contraindicated in cases of infectious keratitis.[73, 84]

Rationale for Treatment of LIU

Treatment of intraocular inflammation is important to preserve vision and minimize pain and complications associated with LIU.[109] Secondary glaucoma is a major complication of chronic uveitis that is not only a blinding condition but is also painful.[110-112] Intraocular inflammation causes alterations in the conventional aqueous humor outflow pathway. The iridocorneal angle can collapse or undergo fibrosis following chronic inflammation, or inflammatory cells and fibrin may obstruct the drainage angle. Inflammatory membrane formation (preiridal fibrovascular membrane, cyclitic membrane, retrocorneal memebrane) may develop and occlude the pupil or iridocorneal angle.[82] Posterior synechia, when the pupillary margin of the iris adheres to the anterior lens capsule, and peripheral anterior synechia, when the iris root adheres to the peripheral cornea, inhibit aqueous humor outflow.[80, 110] In a study evaluating different management plans for dogs with cataracts, failure (defined as painful, inflamed, or glaucomatous, and in the cases that received surgery, non-visual) occurred in 100% of untreated eyes (n=8 eyes [5 dogs]); the failure rate for dogs that received no treatment was 64.5 times higher than in dogs receiving medical treatment (n=35 eyes [20 dogs]) and 255 times higher than in dogs receiving surgical treatment (n=34 eyes [19 dogs]). The rate of failure for

dogs receiving medical treatment alone was 4.0 times higher than for dogs undergoing surgery. The time to failure for the no treatment group was significantly shorter than for the treatment groups, with reasons for failure being persistent uveitis and secondary glaucoma. The anti-inflammatory ophthalmic medications used in that study were not specified.[109] The population of dogs in the no treatment group was likely skewed to include dogs with noticeable ocular pathology or evidence of ocular pain, necessitating reevaluation, or potentially, to include dogs with secondary glaucoma and owners interested in a surgical procedure not typically performed in a general private practice setting (i.e., evisceration with placement of an intrascleral prosthesis).

Treatment of LIU is recommended to prepare dogs for cataract surgery, in an effort to maximize the success of the procedure while also treating dogs for iatrogenic inflammation incited by surgery.[76, 113] In a study of 151 dogs with LIU, 14% suffered complications due to uveitis;[90] eyes that underwent lens extraction and had a history of LIU had a lower long-term success rate, mainly due to glaucoma and phthisis bulbi.[90] Similar failures (glaucoma and phthisis bulbi) were documented in a retrospective study comparing the success rates of eyes with and without LIU; eyes that had a history of LIU had a reduced success rate (52%) 6 months following cataract surgery compared to eyes without a history of LIU (95%) at the same time point.[53] Miller et al also identified postoperative anterior uveitis as the main cause of failure or as causing complications in visual eyes following cataract surgery; the ophthalmic findings included glaucoma, severe anterior uveitis, retinal detachment, pupillary occlusion, posterior synechia and corneal edema.[114] However, one study suggested the presence of LIU prior to surgery was not associated with an increased risk of failure.[51] Topical treatment with steroids and NSAIDs following cataract surgery may be required for months to maintain control of post-operative uveitis.[33]

Systemic Absorption of Topically Applied Drugs

After topical application of an ophthalmic medication, a portion of the medication will be absorbed systemically through the fenestrated blood vessels of the conjunctiva and episclera and/or nasal or oral mucosa after the medication passes through the nasolacrimal system. Ingestion of the drug and subsequent absorption is also possible if the dog licks the medication

from the nose after passage through the nasolacrimal duct or if drug enters the oropharynx, as dogs may have an accessory opening of the nasolacrimal duct in the oral cavity. Some of the drug will be absorbed into the aqueous humor and enter systemic circulation following entry into the uveal vasculature or through the angular aqueous plexus.[83] Evidence of systemic absorption following ocular application of medications has been documented in rabbits and horses by identification of the drug in plasma, serum, and/or urine.[115, 116] Topically applied medications are able to bypass primary metabolism by the liver, as drugs placed on the corneal surface can be absorbed from the conjunctiva or nasolacrimal mucosa directly into the bloodstream.[83]

Suppression of the hypothalamic-hypophyseal-adrenal axis has been documented in dogs via ACTH stimulation testing following application of topical 1% prednisolone acetate suspension 4 times daily (mean dose of 4 mg/day) for two weeks[117, 118] and one study continued administration for two additional weeks at a reduced topical dose (mean dose of 2.67 mg/day). Two weeks after discontinuing steroid treatment, pre-ACTH stimulation cortisol levels returned to baseline, and ACTH stimulation responses returned to values that were within the normal response range (although they were still diminished by 26% compared to pretreatment levels).[118] Glucagon stimulation was also performed in this study, showing an exaggerated increase in BG with glucagon administration after 4 weeks of ophthalmic steroid treatment.[118] Adrenal suppression was confirmed in a group of healthy Beagles following topical application of 0.1% dexamethasone suspension four times daily to both eyes for 8 and 16 weeks (mean dose of 0.03 mg/kg of BW per day). Histopathologic changes in the liver included vacuolated hepatocytes, increased hepatic glycogen content, and ballooning degeneration of hepatocytes.[119] Subconjunctival administration of methyl-prednisolone acetate (10 mg dose repeated 21 days after initial administration) has caused similar effects in dogs.[120] Murphy et al documented iatrogenic HAC in a Boston Terrier following 5 years of ophthalmic steroid treatment; clinical signs and ACTH stimulation results returned to normal following cessation of the topical steroid therapy.[121] Topical application of ophthalmic 0.1% dexamethasone sodium phosphate has been demonstrated to significantly increase BG concentrations in diabetic humans undergoing cataract surgery.[104] Another study in diabetic humans showed a significant increase in both the fasting BG values and HbA1C levels after treatment with topical ophthalmic

steroids for 3 and 7 weeks, respectively. Patients included in the study did not receive a uniform steroid type (4 different ophthalmic medications) or dose frequency (range not listed). As HbA1C is a molecule composed by glycation of hemoglobin, its level reflects the mean BG concentration over a period of 1 to 2 months prior to collection and indicated worsening of glycemic control of patients in this study.[122]

Other studies report evidence of systemic absorption of topically applied medications in dogs. A dilation protocol for 3 dogs prior to cataract surgery included 2.5% phenylephrine drops administered 6 times in the 1.5 hours before surgery for two dogs and 11 applications of 10% phenylephrine in the 3 hours before surgery in the remaining dog. The excessive phenylephrine doses caused dogs to experience arterial hypertension, but it was medically controlled with acepromazine maleate.[123] Ophthalmic preparations of a beta-blocker, 0.5% timolol maleate, applied topically as a single dose to one eye was confirmed to decrease basal blood pressure and heart rate in dogs (mean ± standard deviation [SD] BW of 12.6±0.8 kg),[124] as well as lower the intraocular pressure (IOP) in the treated (mean of 16.1% reduction in IOP) and untreated (mean 9.0% reduction in IOP) eye of dogs; pupil size was also decreased in both eyes as a result of the drug.[125]

Exploration of alternative routes of insulin administration lead researchers to consider ophthalmic application and whether systemic absorption could achieve a desired therapeutic effect. Ophthalmic application of regular porcine insulin (administered with emulsants) in 8 euglycemic dogs was shown to reduce serum glucose and increase serum insulin concentrations.[126] Similar findings were reported for the diabetic dog and hyperglycemic rats.[127] Hypoglycemia was induced in albino rabbits following topical application of insulin.[128]

Chapter 2 - The Effects of Ophthalmic Prednisolone and Diclofenac on Diabetes Mellitus Regulation in Dogs

Introduction

An issue of debate is whether topical ophthalmic steroids exacerbate insulin antagonism and interfere with glycemic control in diabetic dogs.[1] Currently, no studies have evaluated possible systemic effects of topical ophthalmic steroids in diabetic dogs. Topical steroid and non-steroidal anti-inflammatory medications are commonly used to treat LIU, as well as to prevent and control inflammation following cataract surgery. Cataract formation is the most common ocular complication of canine DM,[1, 20] and diabetic dogs constitute a large percentage of the dog population presented to veterinary ophthalmologists for cataract surgery.[33, 51] Human diabetic patients undergoing cataract surgery who were treated post-operatively with a topical ophthalmic steroid (0.1% dexamethasone disodium phosphate) 4 times daily for one month experienced significantly increased fasting post-surgical BG concentrations compared to both pre-surgical concentrations and fasting post-surgical BG concentrations from diabetic patients that were instead treated with 0.1% diclofenac 4 times daily for one month.[104] Fasting BG concentrations for steroid-treated patients rose from baseline mean ±SD of 170±55.5 mg/dL to 229±76.8 mg/dL, which was significantly greater than the NSAID group that had a mean ±SD of 198.4±66.5 mg/dL after one month of therapy.[104] Treatment with topical ophthalmic steroids may produce similar effects in diabetic dogs.

The objective of the study was to evaluate use of a topically applied ophthalmic steroid (1% prednisolone acetate ophthalmic suspension[e]) and a non-steroidal anti-inflammatory drug (0.1% diclofenac sodium ophthalmic solution[f]) on BG concentrations, serum fructosamine concentrations, and clinical scores in diabetic dogs with cataracts using descriptive analysis. We hypothesized that 1% prednisolone acetate would negatively affect BG regulation in diabetic dogs. Results of this study are expected to influence selection of topical anti-inflammatory medications in diabetic dogs, especially in poorly controlled diabetic dogs.

Materials and Methods

Study Design

This is a prospective, randomized, double masked, experimental study involving two phases of data collection one month apart. This study was approved by the Institutional Animal Care and Use Committee of Kansas State University (approval code #3078.1).

Power Analysis Calculations for Final Enrollment Number

Power analysis calculations were conducted to determine the sample size needed to detect treatment effects on outcomes of interest, namely fructosamine concentrations, binary outcomes from the clinical score sheet, and weight loss. All calculations were designed to grant ≥80% power with a Type I error rate of 5%. Type I error is when the true null hypothesis is incorrectly rejected, resulting in a false positive determination; this would allow a conclusion to be made that does not actually exist.

The range of fructosamine concentrations for well-controlled diabetic dogs was determined from Briggs et al[43] and divided by 4 to conduct an "empirical-rule based"[129] approximation of the residual SD. A SD of 100 µmol/L was squared to obtain the variance for power calculation. The data curves were plotted and the intersection of number of dogs (x-axis) that would grant desired power (y-axis) was recommended. Results suggested >35 dogs would be needed to ensure >80% power to detect a 100 µmol/L difference in fructosamine concentrations between dogs of each treatment group. The enrollment number was rounded up to 40 dogs, as a conservative number is more likely to reveal a clinical change in dogs when statistical significance is documented. Binary or dichotomous outcomes from the clinical score sheet (example, increase in water consumption, yes/no) were used to verify the 40 dog enrollment number by assuming a 50% and 5% difference in proportion of clinical events over time for the 1% prednisolone acetate and 0.1% diclofenac groups, respectively. The 50% change (which was used for calculation) was more stringent compared to looking for a 40, 30, or 20% change in response for the 1% prednisolone acetate group; when a smaller % change was accepted, more dogs were needed to reveal significance in findings. Enrollment number based on binary outcomes required >30 dogs to grant 80% power. Finally, 5% weight loss from a baseline of 25 kg was selected according to

an anticipated weight loss range for diabetic dogs of 0-13%[43] or 0 to 3.25kg for a 25 kg dog. Again, the approximate SD was obtained (0.8 kg) by dividing by 4,[129] and this value was squared to provide a variance for power calculation. Many more than 40 dogs would be required to find significance for a smaller % weight loss (such as 2.5%). A total sample size of 15 dogs granted 90% power when evaluating for a 5% weight loss. There was more power to detect treatment effects with weight loss than for any other variable considered; final enrollment number was selected based on the most power-constrained response of interest.

Animals

Twelve client-owned dogs diagnosed with naturally-occurring, insulin-treated DM were enrolled in the study from October 2011 to March 2014, and descriptive analysis was performed on the data collected. Diabetic dogs examined by the Kansas State University ophthalmology, internal medicine, or general pet health departments were considered for enrollment. Flyers detailing the study objective and research design were distributed to local veterinary clinics within a 20 mile radius of the Veterinary Health Center (VHC) and were posted at continuing education conferences hosted on-site. Owners of presumably controlled diabetic dogs were encouraged to contact an investigator (Dr. Jane Ashley Stuckey) to discuss history, clinical signs, possible enrollment, and details of the study.

Inclusion and Exclusion Criteria

Inclusion criteria specified that 1) all dogs enrolled were diagnosed with DM, were receiving insulin therapy, and had achieved a status where they were each deemed "controlled" per history and PE, and 2) all dogs had cataracts. The owners were questioned about clinical signs including PU, PD, PP, and weight loss (>5%) in the month prior to enrollment to help determine glycemic control. Each dog was required to have an acceptable PE (as determined by Drs. Tom Schermerhorn or Kate KuKanich) for enrollment. Owners enrolling a dog in the study were required to be willing to administer therapy to each eye for one month while the dog was home and to commit to return for repeat diagnostic testing in exactly one month in order to obtain a complete data set.

Dogs were excluded if there was a change in insulin dose or if topical or systemic steroid or non-steroidal anti-inflammatory medications had been administered within the past month. When a concurrent endocrinopathy such as HAC was present, the dog was being treated for this condition and considered controlled prior to enrollment. Dogs were not enrolled if preliminary blood work showed major abnormalities that required medical care or if bacteriuria was present, as determined by a urine sediment exam. With regard to ocular exclusion criteria (following examination by Drs. Amy Rankin or Jessica Slack), no dog could have significant uveitis defined as >1+ aqueous flare (as immediate treatment would be indicated) or a corneal ulcer (as treatment with either drug would be contraindicated and likely amplify absorption of the drug, which would potentially alter the results). A trace amount of inflammation was accepted for enrollment, as dogs would receive a topical ophthalmic anti-inflammatory agent soon after examination (3 days later) as part of the study design.

Examinations and Clinical Score Assignment

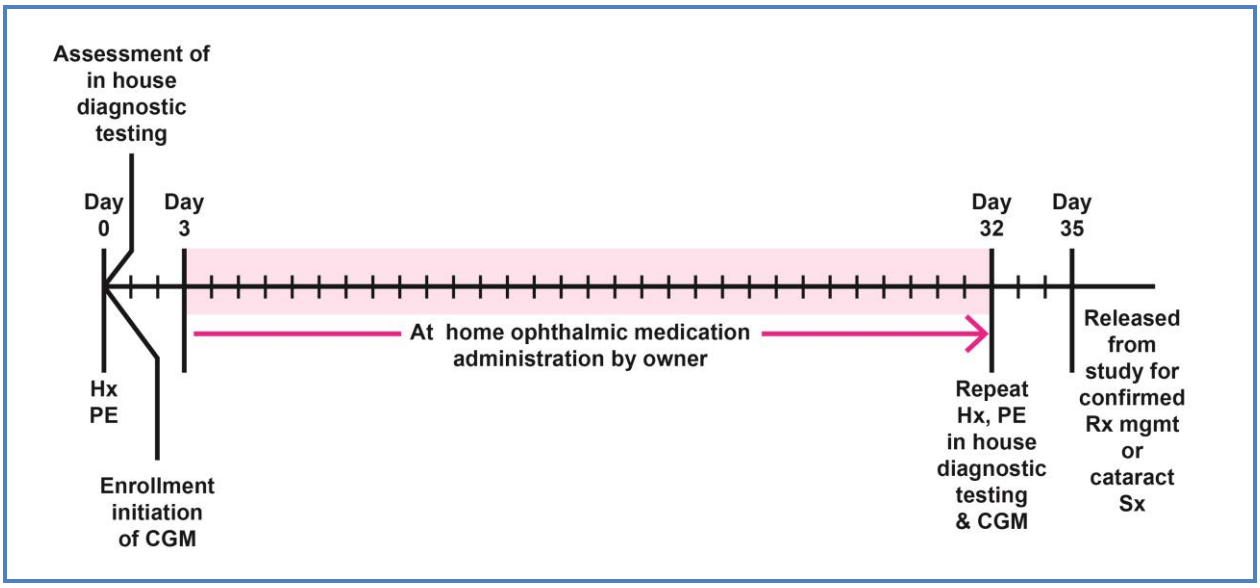
All dogs underwent an ophthalmic examination by a board certified ophthalmologist (Drs. Amy Rankin or Jessica Slack) and PE by a board certified internal medicine specialist (Drs. Tom Schermerhorn or Kate KuKanich) prior to inclusion (day 0). The complete ophthalmic examination included Schirmer tear test I,[g] fluorescein staining,[h] and intraocular pressure measurement via rebound tonometry[i] followed by slit lamp biomicroscopy[j] and indirect ophthalmoscopy[k] when cataracts did not preclude posterior segment examination.

A complete history was obtained by an internal medicine specialist who also performed the PE on the dog. Specific clinical signs and examination findings were documented on a clinical score sheet designed for this study.(Appendix B) Answers to questions on the scoring sheet were dichotomous (yes/no), allowing responses to be translated to numerical values (1/0) to obtain a cumulative score. (An example of a question, outcome, and numerical assignment would be as follows: Increase in weight gain of >5% over the past month? Answer: yes; score for that outcome= 1) Minimum and maximum cumulative clinical scores per dog were 0 and 15, respectively.

Diagnostic Testing

A complete blood count (CBC), serum chemistry panel, and urinalysis were performed on day 0, prior to enrollment. (Figure 2.1) Once examinations and preliminary diagnostics confirmed candidacy for enrollment in the study, a serum fructosamine was submitted to Antech[1] and the continuous glucose monitoring (CGM) device (Guardian®) was applied to the dog for up to 72 hours (from day 0 up to day 3). The CGM sensors were placed in the dorsal subcutaneous tissue of the thorax, 1-3 inches lateral to midline. Prior to placement, the haired skin was shaved and wiped with alcohol and allowed to air dry. Sensors were secured in place using tissue glue and/or adhesive tape. Phase 1 of the study was complete following removal of the CGM device; dogs returned home (day 3-31) to receive 4 weeks of the designated masked topical, ophthalmic anti-inflammatory medication administered by owners.

Figure 2.1: Timeline for the Research Project



CGM- continuous glucose monitoring; Hx- history; PE- physical exam; Rx mgmt- medical management; Sx- surgery. The figure illustrates the study design. There are two phases of hospitalization (day 0 up to day 3 and again from day 32 to 35). Dogs returned home between testing (day 3-31) for owners to administer the assigned ophthalmic medication as instructed. Figure created by Mal Rooks Hoover, 2014.

Following the double-masked research design, the ophthalmic medications were rebottled (in identical 10 mL amber glass vials bottles with dropper squeeze tops) and labeled by the Kansas State University dispensary staff to read "Drug Red" or "Drug Blue". This prevented both

investigators and owners from knowing the medication assigned to each color code. Owners were shown how to administer ophthalmic medications prior to each dog's discharge from the hospital. They were required to apply 1 drop of the designated topical ophthalmic anti-inflammatory medication to both eyes 4 times daily for 4 weeks (days 3-31) and were asked to record daily administration on a provided medication log. Insulin and all previously prescribed medications were continued during the 4 weeks of the study. Owners were contacted weekly by an investigator (Dr. Jane Ashley Stuckey) to ensure compliance with administration of the study drug. Dogs returned on day 32 for Phase 2, which included repeat history assessment, PE, ophthalmic examination, clinical score assignment, and all diagnostics (CBC, serum chemistry, urinalysis, serum fructosamine concentration), as well as continuous glucose monitoring for 24-72 hours (days 32-35). Ocular anti-inflammatory therapy was continued during that time (administered by a veterinary student to prevent unmasking of the investigators). The data collection was complete following day 35. Continued topical ophthalmic anti-inflammatory treatment was recommended for all dogs following completion of the study.

Treatment groups

Dogs were divided into two groups, assigned to receive either 1% prednisolone acetate ophthalmic suspension or 0.1% diclofenac ophthalmic solution. Group assignments were made by categorizing dogs using a blocked randomization design stratified by sex, age (>10 years of age), and BW (>25 kg) followed by a coin toss to determine treatment group. Blocked randomization is performed to control variability among factors not defined as outcomes of interest (i.e., age, sex, and BW). Dogs are divided into subgroups (called blocks) such that variability within is less than variability between subgroups; dogs within blocks are then randomly assigned to treatments. A single investigator (Dr. Jane Ashley Stuckey) designated the group assignments and kept the information filed with all patient examination and test results. These drugs remain masked, to date, as research is still underway. No dog received a placebo ophthalmic medication (to represent a control group) according to the construct of the study design; anti-inflammatory therapy in dogs with diabetic cataracts is standard of care and withholding this treatment was deemed unethical. Topical anti-inflammatory medications are also prescribed to prepare dogs for cataract surgery, and it would be unethical to withhold anti-inflammatory therapy prior to surgery.

Data Analysis

Descriptive analysis was performed on the data to date from 12 dogs to evaluate the following outcomes of interest: clinical scores, blood work and urinalysis findings, serum fructosamine concentrations, and recommendations for insulin dose adjustments (based solely on CGM results(.

The following method was used to assess glucose curves. Phase 1 and Phase 2 curves for 12 dogs (n= 24 curves) were analyzed. For curve analysis, identifiers for each dog were masked, group assignment per dog was not disclosed, and the curves were unpaired (i.e., 24 curves were randomly presented to evaluators). Evaluators applied previously published criteria[44] to determine whether an insulin dose adjustment was indicated and, if so, whether the dose should be increased or decreased. Decisions about insulin dose adjustments were made only by CGM curve analysis; no individual or clinical information was considered as part of curve evaluation. Each curve contained up to 72 hours of CGM data (3 consecutive 24-hr curves). Curve evaluations were carried out using the average curve generated by the CGM software from all available data for each phase. Evaluators (Drs. Tom Schermerhorn and Kate KuKanich) separately analyzed each curve. Recommendations from each evaluator were compared to determine agreement. Any disagreements were reconciled through discussion and the reconciled result recorded.

When the study is complete and data have been collected for a total of 40 dogs, statistical analysis will be performed by an investigator (Dr. Nora Bello). Generalized linear mixed models will be fitted to the responses of interest, namely serum fructosamine concentrations, glucose concentrations, and clinical scores. These models will include fixed effects for treatment, breed and age, as well as the random effect of patient to account for any repeated observations within a dog. Where appropriate, model assumptions will be evaluated. Inflation of Type I error will be prevented using Tukey-Kramer or Bonferroni adjustments.

Results

Screening and Attempted Enrollment

Although data collection for the study was from October 2011 to March 2014, coding for the Kansas State University VHC database was available through December 2013. From October 2011 to December 2013, 118 diabetic dogs were evaluated at the VHC, averaging 4.4 diabetic dogs examined per month. Of those evaluated by the investigators and deemed candidates for enrollment, participation was declined by owners of 31 dogs, either due to distance of travel (n = 3), unwillingness to be separated from the dog (n = 3), unwillingness to post-pone cataract surgery (n = 10), or were lost to follow up after initial evaluation (n = 15). Twelve dogs had significant uveitis in one or both eyes and were started on topical anti-inflammatory medications at the initial ophthalmic examination. Thirteen dogs had one or more ocular conditions that prevented enrollment (uncontrolled keratoconjunctivitis sicca, secondary glaucoma, deep corneal ulcer requiring conjunctival graft surgery, enucleation, and lens luxation), and four dogs had cataract surgery prior to October 2011. Four of the 118 dogs were newly diagnosed with DM, and insulin therapy was initiated. The remaining diabetic dogs required therapy for major systemic diseases (DKA, pancreatitis, pyelonephritis, etc.) or received soft tissue or orthopedic surgery.

Five dogs failed initial diagnostic testing. Three dogs were diagnosed with bacteriuria on the urine sediment exam. The dogs were treated with oral antibiotics according to culture susceptibility results. One dog was enrolled in the study following treatment and a negative urine sediment exam. Another dog showed persistent bacteriuria, and the owner elected to not attempt future enrollment. The final dog was sent home for systemic antibiotic treatment; following the antibiotic regimen, several unsuccessful attempts were made to contact the owner to schedule Phase 1 testing and enrollment. Two dogs required medical treatment following evaluation of initial diagnostic tests; one dog had marked hypoalbuminemia and concurrent electrolyte imbalances, and the other dog showed persistent hypoglycemia with need for an insulin dose reduction. The latter dog was enrolled approximately 6 weeks later following confirmation of glycemic control using a lower insulin dose.

Two dogs did not complete the study due to owner-admitted inability to comply with commitments of the study design. One owner requested to withdraw the dog from future testing for personal reasons unrelated to the study. The other dog was eliminated from Phase 2 testing following a weekly phone call by an investigator (Dr. Jane Ashley Stuckey), as the owner described being unable to administer the ophthalmic medication to the dog due to aggression displayed with application.

Animals Enrolled

Twelve diabetic dogs have been enrolled in the study. The study population consisted of 7 spayed females and 5 castrated males. Breeds represented include 3 Cairn Terriers and one each of the following: Beagle, Miniature Poodle, Pembroke Welch Corgi, Rat Terrier, Border Collie, Miniature Schnauzer, Chihuahua, Australian Shepherd, and a mixed breed dog. Ages ranged from 5-13 years with a mean ±SD age of 8.9±2.3 years. The mean ±SD population BW was 15.68±7.35 (range of 4.2-28.0 kg) and 15.81±7.68 (range of 4.0-28.3) for Phase 1 and Phase 2, respectively.

Hospitalization during CGM data collection was performed in 10/12 dogs. Dogs were hospitalized for a mean ±SD of 57.79±7.56 hours (range of 50 to 74 hours) for diagnostic testing. While hospitalized for Phase 1 testing, 1 dog (Drug Red group) was inadvertently under-dosed with insulin for the first 2 treatments. This was corrected for all subsequent doses administered to this dog. Two dogs were monitored as outpatients, meaning the CGM device was worn while under the owners' care and observation. Those 2 dogs were evaluated in hospital every 12 hours during Phase 1 and 2 of data collection, and the glucose monitoring system was calibrated at those intervals.

Dogs had the following concurrent disease conditions: HAC (n=1), neoplasia (n=1, mast cell tumor), protein-losing nephropathy (n=4), arthritis (n=3), food allergy (n=1), evidence of bacteriuria upon return for Phase 2 as diagnosed by the urine sediment exam (n=3), perivulvar dermatitis (n = 1), and severe dental disease (n=1). The dog with HAC was diagnosed and considered controlled for this condition.

Group Demographics

Drug Red group had a mean ±SD age and BW of 9.3±1.8 years (range of 6-11 years) and 15.51±9.46 kg (range of 4.1-28.15 kg), respectively. Two male/castrated and 4 female/spayed dogs were in this group. Drug Blue group had a mean ±SD age and BW of 8.5±2.9 years (range of 5-13 years) and 15.97±5.86 kg (range of 10.75-26.15 kg), respectively. Drug Blue group included 3 male/castrated and 3 female/spayed dogs. The mean ±SD insulin dose recommended for all dogs was 0.83±0.28 u/kg (range of 0.36-1.41 u/kg), with dogs from Drug Red receiving a mean ±SD dose of 0.69±0.20 u/kg (range of 0.36-0.91u/kg) and dogs from Drug Blue receiving a mean ±SD dose of 0.98±0.29 u/kg (range of 0.61-1.41 u/kg). Humulin® N[m] was administered to 10/12 dogs. One dog assigned to the Drug Blue group received Novolin® N,[n] and 1 dog assigned to the Drug Red group received Vetsulin®.[o]

History, Physical, and Ocular Examinations

Upon return for Phase 2 testing, 1 owner reported PU/PD as a newly observed clinical sign exhibited by the dog; this dog was in the Drug Red group. Two owners reported witnessing clinical signs of hypoglycemia (one from each treatment group), one of which treated for this by administering Karo syrup[p] to the dog's gingiva (Drug Red group).

Physical examination findings were fairly consistent between Phase 1 and Phase 2 for all dogs. Nine of 12 dogs were considered over-conditioned (body condition score ≥4 out of 5) according to the examiners (Drs. Tom Schermerhorn or Kate KuKanich). Weight loss was documented in 5 of 12 dogs (42%); however, no dog lost more than 5% BW from Phase 1 to Phase 2 of the study. Of the 5 dogs that lost weight, 2 dogs were in Drug Red group and 3 dogs were in Drug Blue.

Ocular examination showed a single dog to have very mild uveitis at Phase 1, manifested as trace aqueous flare in both eyes (OU); evidence of uveitis was not appreciated in this dog at Phase 2. A different dog had uveitis (manifested as keratic precipitates and resistance to pharmacologic dilation) in the left eye (OS) at the Phase 2 ocular examination. Ocular hypertension (right eye (OD): 16 mmHg, OS: 25 mmHg) was present OS in 1 dog at Phase 1 examination, and was further increased OU (OD: 32 mmHg, OS: 42 mmHg) by Phase 2. This dog was started on

Dorzolamide hydrochloride[q] OU every 8 hours prior to cataract surgery, at which time bilateral anteriorly luxated lenses were observed and intracapsular lens extractions were performed OU. These 3 dogs were assigned to the Drug Blue group. Cataract stages for all dogs (24 eyes) at Phase 1 examination were as follows: 8 immature, 14 mature, and 2 hypermature cataracts. Cataract stages for all dogs (24 eyes) at Phase 2 examination were: 8 immature, 12 mature, and 4 hypermature cataracts. Cataract progression was documented in two dogs (4 eyes) from Phase 1 to Phase 2; both dogs were assigned to the Drug Blue group. One dog had mature cataracts at Phase 1 that progressed to hypermature over the study period; this dog was the one that exhibited uveitis in the left eye at the Phase 2 examination. The other dog with cataract progression showed increased lens opacification from Phase 1 to Phase 2 examination, as the fundus was difficult to visualize only at the second examination; the cataracts were classified as immature at both time points. One dog exhibited retinal hemorrhages in the right eye at both examinations, which were attributed to diabetic retinopathy after mean blood pressure (140 mmHg) and blood work evaluation (platelet count = 332,000 K/uL). No corneal ulcers were diagnosed. Mean ±SD Schirmer tear test values for Phase 1 and 2 were 19.42±3.74 mm/min OD, 18.50±4.23 mm/min OS and 19.67±4.62 mm/min OD, 19.75±2.70 mm/min OS, respectively. Schirmer tear test values ranged from 12-28 mm/min in Phase 1 and 9-27 mm/min in Phase 2. Mean ±SD intraocular pressures for Phase 1 and 2 were 7.92±4.38 mmHg OD, 8±5.97 mmHg OS and 7.67±7.83 mmHg OD, 9.25±10.46 mmHg OS, respectively. Intraocular pressures ranged from 3-25 mmHg in Phase 1 and 3-42 mmHg in Phase 2. A total of 8 dogs had cataract surgery following Phase 2 of the study.

Clinical Score

Using the clinical score sheet (Appendix B), dichotomous outcomes of interest were documented for each dog. Clinical scores for all dogs from Phase 1 and Phase 2 had a mean of 2.1 and 2.2, respectively, which represented a 1 point total increase over time.

When comparing drug groups, the direction of change was similar. In both groups, 2 dogs showed an improvement in clinical score (reduced numerical value over time), 2 showed no change from Phase 1 to Phase 2, and 2 had a worsened clinical score. The Drug Red group showed a 17% increase in mean clinical score over time (Phase 1 mean=1.5; Phase 2 mean=1.8),

which equaled a total group score increase of 2 points. The Drug Blue group's mean score decreased by 7% (Phase 1 mean=2.7; Phase 2 mean=2.5), which equaled a total group score decrease of 1 point.

Complete Blood Count, Serum Chemistry, and Urinalysis

Lymphopenia was observed on the CBC of 9 dogs (3 from the Drug Red group, 6 from the Drug Blue group) in Phase 1 and 8 dogs (4 per drug group) in Phase 2. The mean ±SD lymphopenic counts for Phase 1 and Phase 2 measured 1.03±0.28 K/uL (normal range: 1.5-5 K/uL) and 0.84±0.33 K/uL, respectively.

An elevated ALP was documented in 11 dogs in Phase 1 (range of high values: 203-2867 U/L [normal range: 1-142 U/L]) and in 10 dogs in Phase 2 (range of high values: 309-2724 U/L). An increase in ALP (mean ±SD of increase between testing: 420.86±318.67 U/L; range of increase between testing: 154-1069 U/L) from Phase 1 to Phase 2 occurred in 7 dogs, 5 of which were in the Drug Red group and 2 of which were in the Drug Blue group. The ALT was elevated (383 and 404 U/L [normal range: 28-171 U/L]) in 2 dogs at Phase 1 testing. Phase 2 testing revealed a rise ALT in 3 dogs (1 dog previously documented with an elevated ALT and 2 additional dogs) during the treatment period, all of which were in the Drug Red group. The range of elevated ALT values for Phase 2 was 237-588 U/L with a mean ±SD increase from Phase 1 to Phase 2 of 166.67±39 U/L. Cholesterol mean ±SD values for Phase 1 were 531.75±238.01 mg/dL and 501.17±272.67 mg/dL for Phase 2. Hypercholesterolemia was documented in 10 dogs (range of elevated values from 316-1185 mg/dL [normal: 133-394 mg/dL]) and 7 dogs (range of 434-1259 mg/dL) in Phase 1 and 2, respectively; however, none of dogs were fasted prior to blood sampling. The highest degree of lipemia (denoted as mild, moderate, or marked) recorded per dog was as follows: 1 with no comment on lipemia, 5 mild, 4 moderate, and 2 dogs were reported to have marked lipemia.

Bacteriuria, diagnosed via urine sediment examination, was observed in 3 dogs in Phase 2 urinalyses, 1 from the Drug Red group and 2 from the Drug Blue group. Crystaluria (calcium oxylate [n = 1], struvite [n = 1], and amorphous [n = 4]) was noted in 6 dogs, with only one dog having crystaluria (amorphous) at the time bacteriuria was also visualized. Urine specific gravity

ranged from 1.008-1.060. Changes (an increase or decrease) in the degree of glucosuria and proteinuria (both reported on a 0 to +3 scale on the Multistix 10 SG® urine dipstick[r]) between testing intervals were similar; both were documented to change in 6 dogs. Glucosuria increased in 4 dogs (2 dogs per treatment group) and decreased in 2 dogs (1 dog per treatment group). The maximum increase and decrease of glucosuria on the urine dipstick were +3 and -3, respectively. Proteinuria increased in 2 dogs (1 dog per treatment group) and decreased in 4 dogs (2 dogs per treatment group). The maximum increase and decrease of proteinuria on the urine dipstick were +1 and -2, respectively. One dog was diagnosed with +3 ketonuria (also reported on a 0 to +3 scale on the urine dipstick) during Phase 2 testing.

Serum Fructosamine Concentrations

Serum fructosamine concentrations ranged from 293-609 µmol/L with a mean ±SD of 466±88.40 µmol/L for Phase 1. Phase 2 values ranged from 390-714 µmol/L with a mean ±SD of 529±110.0 µmol/L. Eleven of 12 dogs exhibited an increase in serum fructosamine concentrations (increased by mean ±SD of 75.82±64.03 µmol/L; range of 5-221 µmol/L) between testing intervals. When comparing serum fructosamine concentrations over time between groups (Table 2.1), the Drug Red group showed a mean ±SD increase from 433±75.20 to 516±104.18 µmol/L (mean of 16% increase), and the Drug Blue group showed a mean ±SD increase from 499± 94.60 to 543±124.30 µmol/L (mean of 8% increase).

A serum fructosamine concentration >450 µmol/L was defined as "unacceptable" on the clinical score sheet; values ≤450 µmol/L were "acceptable". Five dogs had acceptable serum fructosamine concentrations at Phase 1 (3 in the Drug Red group, 2 in the Drug Blue group), and 4 dogs had acceptable concentrations at Phase 2 (2 per treatment group). (Table 2.1, Table 2.3)

Table 2.1: Serum Fructosamine Concentration Data per Group

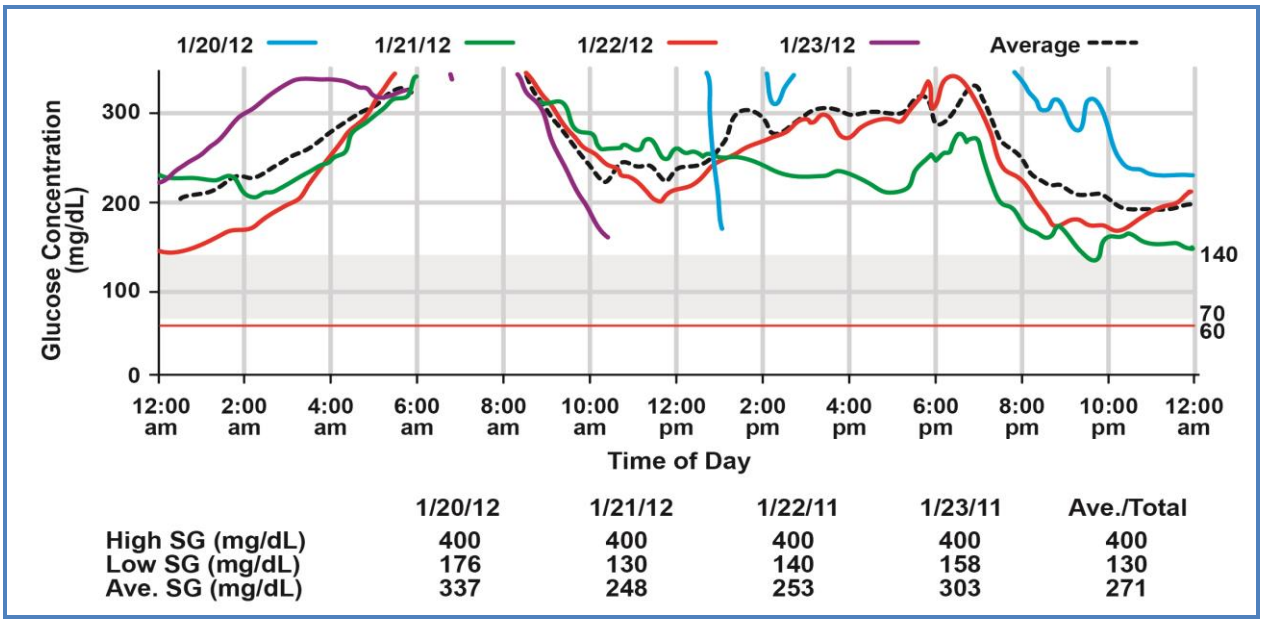
	Drug Red Group	Drug Blue Group
Mean ±SD [Fructosamine] Phase 1	433±75.20 µmol/L	499± 94.60 µmol/L
Mean ±SD [Fructosamine] Phase 2	516±104.18 µmol/L	543±124.30 µmol/L
Mean % Increase in [Fructosamine] from Phase 1 to 2	16%	8%
Acceptable Phase 1	3	2
Acceptable Phase 2	2	2

Mean ±SD serum fructosamine concentrations from Phase 1 and Phase 2, the % increase in mean fructosamine concentrations over time, and the number of "acceptable" dichotomous outcomes assigned (defined as a serum fructosamine concentration ≤450 µmol/L) per phase are compared between Drug Red and Drug Blue groups.

Continuous Glucose Monitoring

Sensors were in place a mean ±SD of 52.58±11.96 hours (Phase 1 mean ±SD: 47.92±12.6 hours; Phase 2 mean ±SD: 57.25±9.63 hours). In general, CGM sensors were well-tolerated by dogs. One dog in the Drug Blue group, however, pulled or scratched the CGM sensor out of the skin consecutively at Phase 1 despite intervention (tissue glue, tape, and an Elizabethan collar). A standard BG curve was performed in lieu of CGM, with peripheral venous blood samples obtained every two hours for the remainder of hospitalization. A single glucometer (Accu-chek Complete®S) was used for the BG curve and BG calibrations to minimize variability between values obtained. An example CGM curve is provided. (Figure 2.2)

Figure 2.2: CGM Curve (Dog 1, Phase 2)



	1/20/12	1/21/12	1/22/11	1/23/11	Ave./Total
High SG (mg/dL)	400	400	400	400	400
Low SG (mg/dL)	176	130	140	158	130
Ave. SG (mg/dL)	337	248	253	303	271

The figure represents the curves generated by the CGM device. The sensor was placed around 1:00pm on 1/20/12 (blue line). There are breaks at the peak of the curve; the CGM is unable to record values >400 mg/d. The curve peaks around 7:00pm, which is the scheduled feeding and insulin administration time for this dog. The waveform reappears around 8:00pm and declines to the nadir. The curve to represent subsequent days is demarcated by color (blue, green, red, purple). The dotted line represents the average recording from all days, used by two investigators to interpret CGM results for insulin dose adjustment recommendations.

Continuous Glucose Curve Evaluation

Of the 24 curves evaluated, 22 curves received a recommendation on insulin dosing. (Table 2.2) Two curves from Phase 1 (1 curve per drug group) could not be interpreted and were excluded. One excluded curve was from the dog (Drug Red assignment) that was initially under-dosed with insulin, as this would falsely increase all concentrations of glucose recorded by the CGM device during the inappropriate treatment time and affect the overall average tracing, which was used to determine dosing recommendations. The other excluded curve was from the dog (Drug Blue assignment) that received CGM and a standard BG curve, as an overall average tracing formed by the CGM device was not available for the standardized interpretation utilized in this study.

No dose change was recommended for 5 of 22 curves (4 assigned to Drug Red, 1 assigned to Drug Blue). Only one dog was determined to need no change in insulin dose at Phase 1; this dog

was assigned to the Drug Red group. Dose change was recommended for 17 of 22 curves. When a dose change was recommended, a dose increase was recommended for 12 curves (5 Drug Red, 7 Drug Blue), and a dose decrease was recommended for 5 curves (2 Drug Red, 3 Drug Blue). Dose increases were typically recommended because curves violated the criteria for 12-hr post insulin injection glucose concentrations (>180 mg/dl). The nadir value was outside of the desired range for most of these curves, as well. Dose decreases were typically recommended because curves showed nadirs that were unacceptably low (<90 mg/dl). When looking at change within each dog over time, the recommendations were the same for Phase 1 and 2 in 6/10 dogs; recommendations differed over time for 4/10 dogs. No dog was recommended to have no change for both phases.

Agreement between Evaluators

Evaluators' (Drs. Tom Schermerhorn and Kate KuKanich) recommendations were in agreement for 19/22 curves. All three disagreements were the result of one evaluator recommending a need for a dose change and the other recommending no dose change. Discussion between evaluators lead to an agreement of no dose change for these three dogs, as it more closely fit evaluation by Fleeman et al.[44] For all curves for which a recommendation of dose change was given, there was complete agreement between evaluators about the nature of the change (increase or decrease).

Table 2.2: Insulin Dose Recommendations

No.	Phase 1	Phase 2	Drug
1	Increase	Increase	**Blue**
2	Decrease	Decrease	**Blue**
3	Increase	No Change	**Red**
4	Increase	Increase	**Red**
5	Decrease	No Change	**Blue**
6	Decrease	Decrease	**Red**
7	Increase	Increase	**Blue**
8	Increase	Increase	**Blue**
9	No Change	Increase	**Red**
10	Increase	No Change	**Red**
11	EXCLUDED	No Change	**Red**
12	EXCLUDED	Increase	**Blue**

Dog enrollment number is recorded in column 1. Recommendations for insulin dose adjustment from Phase 1 and Phase 2 are listed for each dog in column 2 and 3, respectively, along with group assignment in the last column.

Ophthalmic Medications and Dose Administered

Medication logs were returned by all owners at Phase 2. From day 3 to 31, owners were responsible for administering 112 treatments (4 times daily for 28 days) per eye for each dog. When treatments were missed, the medication was not administered to either eye; the number of documented missed treatments per dog ranged from 0 to 11 (mean ±SD of 4±3.98 times). Only 4 owners reported 100% adherence to the treatment regimen, and 1 owner reported a single missed treatment. All other owners had ≥4 missed treatments. One owner recorded 11 missed treatments, which would equate to 90% adherence to the requested treatment regimen.

1% Prednisolone Acetate Ophthalmic Suspension

As 1% prednisolone acetate is formulated to 10 mg/mL, 100 mg of drug are contained within each 10 mL bottle. The amber dropper bottle used in the study dispensed a total of 180 drops. The number of drops held per bottle was determined by an investigator (Dr. Jane Ashley Stuckey), who filled the 10 mL vials and counted the number of drops emitted. This was performed using 3 different bottles to verify a uniform number of drops dispensed. Considering each drop contained 0.56 mg (100 mg/180 drops) of the steroid and 8 drops were administered per day (4 times per day to both eyes), dogs received approximately 4.48 mg/day of 1% prednisolone acetate topically. The minimum and maximum BWs recorded for either phase were 4.0 kg and 28.3 kg; using these body weights, the potential maximum dose was 1.12 mg/kg/day (if the lightest dog were assigned to this treatment), and the potential minimum dose was 0.16 mg/kg/day (if the heaviest dog were assigned to this treatment) of 1% prednisolone acetate applied topically.

0.1% Diclofenac Ophthalmic Solution

As 0.1% diclofenac is formulated to 1 mg/mL, 10 mg of drug are contained within each 10 mL bottle. Considering each drop contained 0.056 mg (10 mg/180 drops) of the NSAID and 8 drops were administered per day (4 times per day to both eyes), dogs received approximately 0.45 mg/day of 0.1% diclofenac topically. Using the minimum and maximum body weights recorded from either phase, the potential maximum dose was 0.11 mg/kg/day (if the lightest dog were assigned to this treatment), and the potential minimum dose was 0.02 mg/kg/day (if the heaviest dog were assigned to this treatment) of 0.1% diclofenac applied topically.

Discussion

Results of the descriptive analysis for the study reported here suggest a difference in several outcomes of interest between data from diabetic dogs treated with a topical ophthalmic steroid compared to a topical ophthalmic NSAID. Preliminary descriptive analysis shows that dogs in the Drug Red group compared to those in Drug Blue showed a slight increase in the mean clinical score over time and more often showed a rise in ALP and ALT from Phase 1 to 2. Serum fructosamine concentrations rose in the majority of dogs (11/12) over time; the mean

serum fructosamine concentration per drug group increased by a larger percentage for the Drug Red group compared to the Drug Blue group. Insulin dose adjustments were recommended for 17/22 CGM curves from both Phase 1 and 2; insulin dose increases were recommended in 12 dogs (5 from Drug Red and 7 from Drug Blue). One owner reported observing signs of a dog assigned to the Drug Red group to become PU/PD during the treatment period; 2 dogs were reported to exhibit clinical signs of a hypoglycemic episode (one from each treatment group).

Concurrent Disease Conditions and Relevant Examination Findings

Concurrent disease conditions present in several dogs should be mentioned, as neoplasia (n=1), HAC (n=1), and concurrent bacteriuria (n=3), which may indicate an occult urinary tract infection, can all complicate diabetic regulation.[17, 38, 42] These diseases are known to cause insulin resistance. No dog in the study received an insulin dose above 1.5 U/kg, which is a dose suggested to be associated with insulin resistance.[42] The mean ±SD insulin dose was 0.83±0.28 u/kg (range of 0.35-1.42 U/kg).

One dog had mild LIU in both eyes at Phase 1 examination that was controlled at Phase 2 examination. A single dog had evidence of LIU in the left eye only at the Phase 2 examination, manifested as keratic precipitates and resistance to pharmacologic dilation. This overall good response to treatment (controlled inflammation in 23/24 eyes) indicates that the topical medications assigned were effective at treating and controlling LIU.

Clinical Score

Mean clinical score rose slightly over one month (from 2.1 to 2.2); when the means were calculated per group, this increase was a result of the Drug Red group. Although a change was seen, it was small, representing a cumulative total 2 point increase for the 6 dogs in the Drug Red group.

Diagnostic Testing

Serum Chemistry and Urinalysis

Serum chemistry analysis from dogs in this study showed elevated liver enzymes to be a common abnormal finding, which was also reported in a large retrospective study of diabetic dogs.[17] Alanine transaminase P5P is a liver specific cytosolic, leakage enzyme that indicates hepatocellular injury and necrosis. Alkaline phosphatase is a production enzyme of the liver, but elevation is not specific to pathology of this organ system. Two enzymes contribute to serum ALP in dogs: liver (L-ALP) and corticosteroid-induced isoenzyme (C-ALP).[1] Diabetic dogs can show elevations in both ALT and ALP, but these findings may be an effect of another disease process or from administration of corticosteroids or NSAIDs.[17] Cholestasis, chronic hepatitis, corticosteroid-induced hepatopathy, hepatic necrosis, and hepatic nodular regeneration (seen in older dogs) all cause elevations in ALP.[130] In this study, 11 dogs had an elevated ALP and 2 dogs had an elevated ALT at Phase 1, which is best explained by the dogs' systemic disease(s). Further elevations in these 2 enzymes over the treatment period may represent exacerbation of the underlying disease or may be due to the assigned ophthalmic medication. The fact that 5/7 dogs and 3/3 dogs that showed a rise in ALP and ALT, respectively, were in the Drug Red group lends merit to the possibility that these changes may be related to the medication administered.

Three dogs were documented with bacteriuria via urine sediment examination during Phase 2 testing. In other studies, 21-24% of diabetic dogs evaluated had an occult urinary tract infection with bacteriuria that was not always accompanied by pyuria,[17, 38] which is similar to the 25% (3/12) documented in our study.

Serum Fructosamine Concentrations and CGM Data

Serum fructosamine concentration rose between Phase 1 and Phase 2 in 11 dogs. Only 5 dogs had serum fructosamine concentrations that were defined as acceptable in Phase 1, and 4 fructosamine concentrations were defined as acceptable in Phase 2. When an increase in dose was recommended (12/22 curves) based on CGM analysis, serum fructosamine was defined as

unacceptable for 9 of those respective time points.(Table 2.3) This occurred in 3 dogs assigned to the Drug Red group and 6 dogs in the Drug Blue group. Only once was no change in insulin dose recommended in a dog that was also determined to have an acceptable serum fructosamine concentration. All dogs recommended to have an insulin dose decrease were recorded as having acceptable serum fructosamine concentrations. These findings suggest good agreement between the recommendations to increase the insulin dose based on a high (unacceptable) fructosamine concentration but the importance of further evaluating a dog with a fructosamine concentration ≤450 µmol/L (acceptable) by another testing method to better determine BG control.

Table 2.3: Comparison of Dichotomous Outcomes between Serum Fructosamine and CGM Data Interpretation for Change in Insulin Dose

No.	Phase 1	Acceptable or Unacceptable	Phase 2	Acceptable or Unacceptable	Drug
1	Increase	U	Increase	U	**Blue**
2	Decrease	A	Decrease	A	**Blue**
3	Increase	U	No Change	U	**Red**
4	Increase	A	Increase	A	**Red**
5	Decrease	A	No Change	A	**Blue**
6	Decrease	A	Decrease	A	**Red**
7	Increase	U	Increase	U	**Blue**
8	Increase	U	Increase	U	**Blue**
9	No Change	U	Increase	U	**Red**
10	Increase	A	No Change	U	**Red**
11	OMIT	U	No Change	U	**Red**
12	OMIT	U	Increase	U	**Blue**

A- acceptable; U- Unacceptable. Phase 1 and Phase 2 recommendations for insulin dose adjustment compared to dichotomous outcomes of serum fructosamine concentration for each dog. Note, dog 10 was the only dog to experience a change in the dichotomous outcome for serum fructosamine concentration.

1% Prednisolone Acetate and 0.1% Diclofenac

Compared to other ocular steroids, 1% prednisolone acetate is considered the most effective anti-inflammatory drug for treatment of inflammation in the anterior segment.[84, 101] Studies have shown that 0.1% diclofenac exhibits superior efficacy compared to flurbiprofen in preventing inflammation following experimentally induced breakdown of the BAB in dogs.[100] Both drugs are commercially available, experimentally proven to be effective methods of treatment, and are commonly prescribed by the investigators of this study with good clinical response to treatment, explaining why each was chosen to represent the ophthalmic steroid and NSAID used for comparison of outcomes.

Dogs received approximately 4.48 mg/day of 1% prednisolone acetate or 0.45 mg/day of 0.1% diclofenac topically, which equates to a potential dose range of 0.16 to 1.12 mg/kg/day for the steroid or 0.02 to 0.11 mg/kg/day for the NSAID, depending on BW and group assignment. Exogenous steroid administration (similar to the mechanism with endogenous glucocorticoid release) causes insulin resistance.[1, 15, 42] If the dog weighing the fewest kilograms were assigned to the steroid treatment, this dog would receive the high end of the steroid anti-inflammatory dosage range (0.5-1 mg/kg/day).[130] If the dog weighting the most kilograms were assigned to the steroid treatment, this dog would receive a dose less than that typically considered to represent physiologic supplementation.[130] The degree of inflammation in the eye and the efficacy of individual medications determine treatment recommendations (drug type and frequency applied).[84] It is important to remember that a low BW can lead to a larger total dose of drug administered to the patient, but it is not a factor in selection of ophthalmic medications.

Conclusions

Results of the preliminary descriptive analysis for the study reported here suggest a difference in several outcomes of interest between data from diabetic dogs treated with a topical ophthalmic steroid compared to a topical ophthalmic NSAID. Preliminary descriptive analysis shows that dogs in the Drug Red group compared to those in Drug Blue showed a slight increase in the mean clinical score over time and more often showed a rise in ALP and ALT from Phase 1 to 2.

Serum fructosamine concentrations rose in the majority of dogs (11/12) over time; the mean serum fructosamine concentration per drug group increased by a larger percentage for the Drug Red group compared to the Drug Blue group. Insulin dose changes were recommended for 17/22 curves from both Phase 1 and 2; insulin dose increases were recommended in 12 dogs (5 from Drug Red and 7 from Drug Blue). When an increase in dose was recommended, serum fructosamine was defined as unacceptable for 9 of those respective time points. This occurred in 3 dogs assigned to the Drug Red group and 6 dogs in the Drug Blue group. An insulin dose decrease was recommended for 5 curves. Interestingly, all dogs recommended to need an insulin dose decrease were evaluated to have acceptable fructosamine concentrations. One dog (Drug Red group) was reported to exhibit PU/PD during the treatment period; 2 dogs were reportedly exhibiting clinical signs of a hypoglycemic episode (one from each treatment group).

Chapter 3 - Analysis of Study Design

Introduction

A collaborative study between the ophthalmology and internal medicine services at Kansas State University was undertaken to determine whether topical ophthalmic application of a steroid can affect BG regulation in diabetic dogs. Diabetes mellitus is a common endocrinopathy in dogs,[1] and diabetic cataracts occur as the most frequent ocular complication of DM in dogs. Dogs with cataracts require ocular anti-inflammatory treatment to prevent and control LIU.[1, 20, 23] Information gleaned from this study will be a valuable contribution to the literature. Reports of systemic effects in dogs following topical application of steroids exist,[117-121] and reports from human medicine regarding systemic effects of topical steroids in diabetic patients are available.[104, 122] To the author's knowledge, no study in the veterinary literature evaluates the use of topical, ophthalmic anti-inflammatory medications in diabetic dogs and the effects of those medications on diabetic regulation.

Diabetic Dogs Eligible for Enrollment

Recruitment of Dogs

On average, 4.4 diabetic dogs are examined per month at the Kansas State University VHC. While this number of dogs should afford an adequate population for enrollment during the study period, diabetic dogs often visit a referral institution to address a current problem (for example: significant LIU, pancreatitis, DKA, etc.) and return to the primary veterinarian for routine care, making it more of a challenge to find dogs that fit enrollment criteria. Of the dogs with well-controlled DM , evidence of bacteriuria was the most common finding with initial diagnostic testing to preclude enrollment. Occult urinary tract infections are diagnosed in approximately 21-24% of diabetic dogs.[17, 38] Urinary tract infections contribute to insulin resistance and may alter glycemic control,[17, 38] potentially complicating data interpretation.

The population of diabetic dogs from surrounding areas seemed to be the most logical pool from which to draw, as distance of travel and extra visits to the hospital were deterrents to participation in the study according to some owners. Flyers were designed to detail study

objectives and benefits of enrollment. They were distributed to veterinary clinics within a 20 mile radius of the VHC and were made available at continuing education conferences hosted on-site. Discussion of the study between referring veterinarians and investigators was also performed during phone consultations.

Resistance of Owners to Permit Participation

Many dogs were presented to the ophthalmology service for the purpose of evaluation prior to scheduling phacoemulsification. Enrollment made the possible date for cataract surgery a minimum of one month following examination. Ten owners were anxious to have the procedure performed as soon as possible and declined study participation. As enrollment in the study often committed owners to a minimum of one additional transit to and from the hospital and a large portion of owners travel from >2 hours away, this occasionally negatively affected willingness to participate (3 owners). Three owners declined participation of a dog due to resistance to be separated from the dog or the belief that the dog would be stressed in a hospital environment. At home monitoring with twice daily visits for calibration of the CGM monitoring device was offered to all owners. Interestingly, the frequency of 4 times per day topical treatment was not reported to deter any owner from participation. Study diagnostics, examinations, hospitalization, and one month's treatment with an ocular anti-inflammatory medication were provided at no cost to owners, and information was relayed to primary veterinarians for continued care following conclusion of data gathering. Despite these benefits, additional incentives may have encouraged more owners to grant participation.

Details of Study Design

Clinical Score Sheet Rationale

No single test can be used to estimate the degree of glycemic control in diabetic dogs. Multiple tests exist to quantify BG control (BG curve, serum fructosamine concentration, glycolated hemoglobin, CGM, urine glucose quantification), which emphasizes this point. History, reduction in clinical signs of PU, PD, PP, and PE findings are often considered the most reliable method to judge BG control.[43] Determining control is strategic and many pieces of

information factor into assessment. The clinical score sheet was specifically designed for this study as a way to incorporate multiple evaluation points for interpretation (history, PE findings, ocular exam findings, fructosamine concentrations, and assessment of CGM data). The score sheet allowed each question to be translated to a dichotomous outcome in order to derive a cumulative numerical value. Some dogs have BG curves with a poor curve shape or a nadir higher than what the veterinarian would prefer in order to consider the dog well-controlled; however, the dog may no longer display PU, PD, PP, and has a stable BW. These dogs would be considered clinically controlled. The cumulative clinical score has the potential to represent clinical diabetic control, as it combines clinical signs with diagnostic test results. The score was used to identify change within a dog over time (Phase 1 to Phase 2) and to compare between scores of dogs assigned to each drug group.

At Home Care of Dogs (Day 3-31)

Client-owned dogs were enrolled in the study. Following collection of Phase 1 data, dogs returned home for continued care until return to the hospital on Day 32. Dogs in a familiar environment experience lower stress, and test results would more likely reflect the dogs' actual degree of BG control when dogs remain in the owners' care with only a single variable changed (introduction of a new topical ophthalmic medication administered 4 times daily). Conversely, the ideal research setting would be to have a single investigator administer all medications to ensure accurate and timely application of the study drug. When study participants are under the care of investigators, adherence to individual diet recommendations (not offering extra treats or table food that could affect daily glycemic control), standardization with exercise, and observance of a routine treatment schedule for administering the assigned medication are feasible. It was not possible to offer this service for the 4 week length of the study period.

Rational of the Ophthalmic Anti-inflammatory Treatment Regimen

Compared to the use of other topical ophthalmic steroids and NSAIDs for prevention and control of LIU in dogs, 1% prednisolone acetate and 0.1% diclofenac were shown to be the most effective.[100, 101] Both drugs are commercially available and are commonly prescribed by the investigators of this study with good clinical response to treatment, explaining why each

medication was chosen to represent a steroid and NSAID treatment group. The treatment frequency of 4 times per day to both eyes was practical to evaluate, as it reflects a common post-cataract surgery protocol recommended for dogs[53] or a treatment regimen that may be required during times of active LIU. Other studies evaluating systemic absorption of topically applied steroids have also employed a 4 times daily frequency.[117-119]

Limitations

Limitations of the study include the lack of a placebo or control treatment group, the lack of a single objective measure of glycemic control, challenges associated with use of the CGM in dogs, and the potential for client compliance issues. No untreated control group was assigned, as it would be unethical to administer a placebo or withhold medication in a dog that presents to the ophthalmology service with diabetic cataracts. Most dogs with cataracts will have some degree of either subclinical or mild LIU.[53] Topical anti-inflammatory medications are prescribed to prepare dogs for cataract surgery, and it would be unethical to withhold anti-inflammatory therapy prior to surgery. Prophylactic treatment with anti-inflammatory agents is the minimum accepted standard for dogs diagnosed with cataracts.[20, 84, 109]

There is no single objective measure to assess glycemic control, which is an inherent challenge of the management of DM in dogs. Blood glucose concentrations are affected by many variables including stress, inappetence, variability in the amount of insulin administered and absorbed from the injection site (particularly if different anatomic regions are used), and concurrent infections or illnesses.[1, 38, 42, 44] Only CGM curves were used to define insulin dose adjustments (increase, decrease, or no change in insulin dose). This was purposeful, however, to minimize subjectivity in this decision. In order better interpret glycemic control, aside from CGM analysis, several parameters (serum fructosamine concentrations, clinical scores, and history, PE, and ocular examination findings) were measured and evaluated.

Continuous glucose monitoring devices have inherent complications. Securing them under the skin of dogs can be challenging, as they are designed for placement in humans. Sensors were placed with the catheter in the subcutaneous tissue of the dorsolateral thorax, but most dogs enrolled in the study were over-conditioned, and subcutaneous fat in this area sometimes caused

folding of the skin, dislodging the sensor. Data could be missed for a variable length of time if displacement occurred overnight. Strategies were enforced to help ensure security of placement, including tissue glue, body tape, and having dogs wear stockingettes or Elizabethan collars. Sensors were well-tolerated among 11/12 dogs, but one dog removed multiple sensors despite intervention. Calibration times were fixed and dependent on time of sensor placement, making consideration of timing especially important for owners of dogs not hospitalized that reported to the hospital every 12 hours for calibration of the CGM device. CGM devices must also be kept within 6 feet of dogs at all times during data collection. The CGM sensor is only capable of recording glucose concentrations >40 or <400 mg/dL. While these extremes still represent significant times of hypoglycemia or hyperglycemia, respectively, the incomplete data limits interpretation.

Potential for problems relating to owner compliance with administration of topical medications is also a limitation of the study. While the maximum number of reported non-treatment times out of the 112 requested treatment times was 11, this still represents 90% adherence to the outlined treatment regimen. Only 4 owners (representing 1/3 of the dogs enrolled in the study) reported having no missed treatments during the study period. All owners of the dogs enrolled in the study were considered compliant with treatment requests, as a 90% treatment cutoff was accepted. This does assume that medications were actually applied when dosing was documented on the medication log. In order to minimize problems with compliance issues, an investigator (Dr. Jane Ashley Stuckey) taught owners how to administer the ophthalmic medication properly, giving strategies for unassisted administration. Owners were sent home with a medication log to record times of application, and they were called weekly to discuss the medication schedule and dog tolerance of topical ophthalmic treatment. Phone calls were effective in eliminating one owner with poor compliance from completing the study.

Critique of the Study Design

Revising the Clinical Score Sheet

The clinical score sheet could be refined to enable the cumulative score to be a more reliable indicator of clinical glycemic control. Some dichotomous outcomes were assigned by surveying

the dog's owner. Eight of the 15 questions on the clinical score sheet require a response from the owner; 7/15 are assigned by investigators that perform the PE and evaluate the CGM data (Drs. Tom Schermerhorn or Kate KuKanich) or perform the ocular exams (Drs. Amy Rankin or Jessica Slack). Answers from survey questions are at risk of inherent subjectivity and bias. For example, owners may be poorly educated on the signs of hypoglycemia but asked to speak on whether a dog displayed clinical signs of this metabolic state. Owners may remark that PU, PD, and PP have subsided when, in fact, the signs have improved but are still inappropriately increased. Bias may be introduced, as owners subconsciously want physical and monetary efforts (in veterinary visits, husbandry, and insulin administration) to be effective at appropriately managing the dog's DM. Some owners may attribute a reduced activity level to cataract development and associated visual compromise when the question was designed to assess a manifestation of metabolic imbalance and poor BG control. An example of how a question could be rephrased would be that instead of asking if there has been a change in water consumption, ask if the owner has had to refill the water bowl more frequently within the last month (or since Phase 1 evaluation). Or, instead of asking about a change in activity level, phrasing the question, "Have you noticed a decrease in the dog's activity, which you feel is unrelated to changes vision?"

Also, it is possible that some outcome measures on the clinical score sheet were made too stringent, namely the serum fructosamine concentration and CGM analysis (determining recommendations on insulin dose adjustment). Another study found well-controlled dogs to have a fructosamine concentration <525 µmol/L;[43] however, the definition of an unacceptable fructosamine concentration in this study was >450 µmol/L. This more strict definition of an acceptable outcome may have raised clinical scores by 1 point in dogs that were actually well-controlled. In order to standardize analysis of CGM data and recommendations on insulin dose adjustment between investigators (Drs. Tom Schermerhorn and Kate KuKanich) and between dogs, a published guideline[44] for interpretation was followed. Many clinicians would still consider a curve that was slightly elevated from that defined in the guideline to represent that of a controlled diabetic dog. It would be interesting to also evaluate curves by calling them "clinically acceptable or unacceptable." Ultimately, the decision to increase or decrease insulin will be based on a combined interpretation of PE, history, and test results, not just the CGM data.

All of the questions on the clinical score sheet relate to glycemic control with the exception of the outcome regarding owner compliance, which refers to compliance with insulin administration and application of the dispensed ophthalmic medication. While this is good information to document, assigning a point for poor compliance of the owner would increase the dog's cumulative clinical score. Poor compliance with insulin administration would absolutely contribute to loss of glycemic control. Poor compliance with application of the dispensed ophthalmic medication would mean that less of the medication was available for systemic absorption and likely have even less of an effect on glycemic control. Revising the score sheet to not assign a numerical value to this outcome (making the total equal 14 points) would allow only outcomes directly associated with the dog (not the owner) to be tallied.

Length of the Study Period

Considering a 2 weeks study period as opposed to 4 weeks, may encourage additional owners to consent to participation. Other studies[117, 118] have shown effects of topical ophthalmic steroid treatment after two weeks of 4 times daily application to both eyes, suggesting this timeline may be reasonable to assess outcome measures in our study. To counter the possibility of reduced effects, the frequency of anti-inflammatory therapy could be increased slightly, likely at the expense of owner compliance.

Conclusions

Achieving diabetic control in dogs is challenging, but assessment of control is also difficult and requires consideration of many variables and test results. For this reason, the study faces obstacles that are nearly unavoidable. The study design was carefully constructed to maximize ability to assess control, and power analysis followed a conservative approach to ensure that when statistics are performed, significant changes, when found, would more likely represent a clinical change in diabetic control, which is inherently difficult to interpret in dogs.

Footnotes

[a] Veterinary Medical Database, Savoy, IL, USA

[b] Guardian® continuous glucose monitor; Medtronic Minimed, Inc., Northridge, CA, USA

[c] PubChem Compound Database; National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD, USA

[d] Kinostat™ ; Therapeutic Vision Inc., Omaha, NE, USA

[e] 1% prednisolone acetate ophthalmic suspension; Sandoz, Alcon Laboratories, Inc., Fort Worth, TX, USA

[f] 0.1% diclofenac sodium ophthalmic solution; Akorn, Inc., Lake Forest, IL, USA

[g] Schirmer tear test®; Intervet, Summit, NJ, USA

[h] Bio-Glo,® Fluorescein sodium ophthalmic strips USP; HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA, USA

[i] TonoVet tonometer; Tiolat Oy Lumic International, Baltimore, MD

[j] Kowa Co., Ltd., Tokyo, Japan

[k] Keeler binocular indirect ophthalmoscope; Keeler Instruments Inc., Broomall, PA, USA

[l] Antech Diagnostic Laboratories, Memphis, TN, USA

[m] Humulin® N, NPH Human Insulin; Lilly USA, LLC, Indianapolis, IN, USA

[n] Novolin® N, NPH Human Insulin; Novo Nordisk A/S, Princeton, NJ, USA

[o] Vetsulin®, porcine insulin zinc suspension; Intervet/Merck Animal Health, Summit, NJ, USA

[p] Karo syrup; Ach Food Companies, Inc., Memphis, TN, USA

[q] Dorzolamide hydrochloride ophthalmic solution 2%; Hi-Tech Pharmacal Co. Inc., Amityville, NY, USA

[r] Multistix 10 SG® urine dipstick; Bayer, Leverkusen, Germany

[s] Accu-chek Complete®; Roche Diagnostics, Indianapolis, IN, USA

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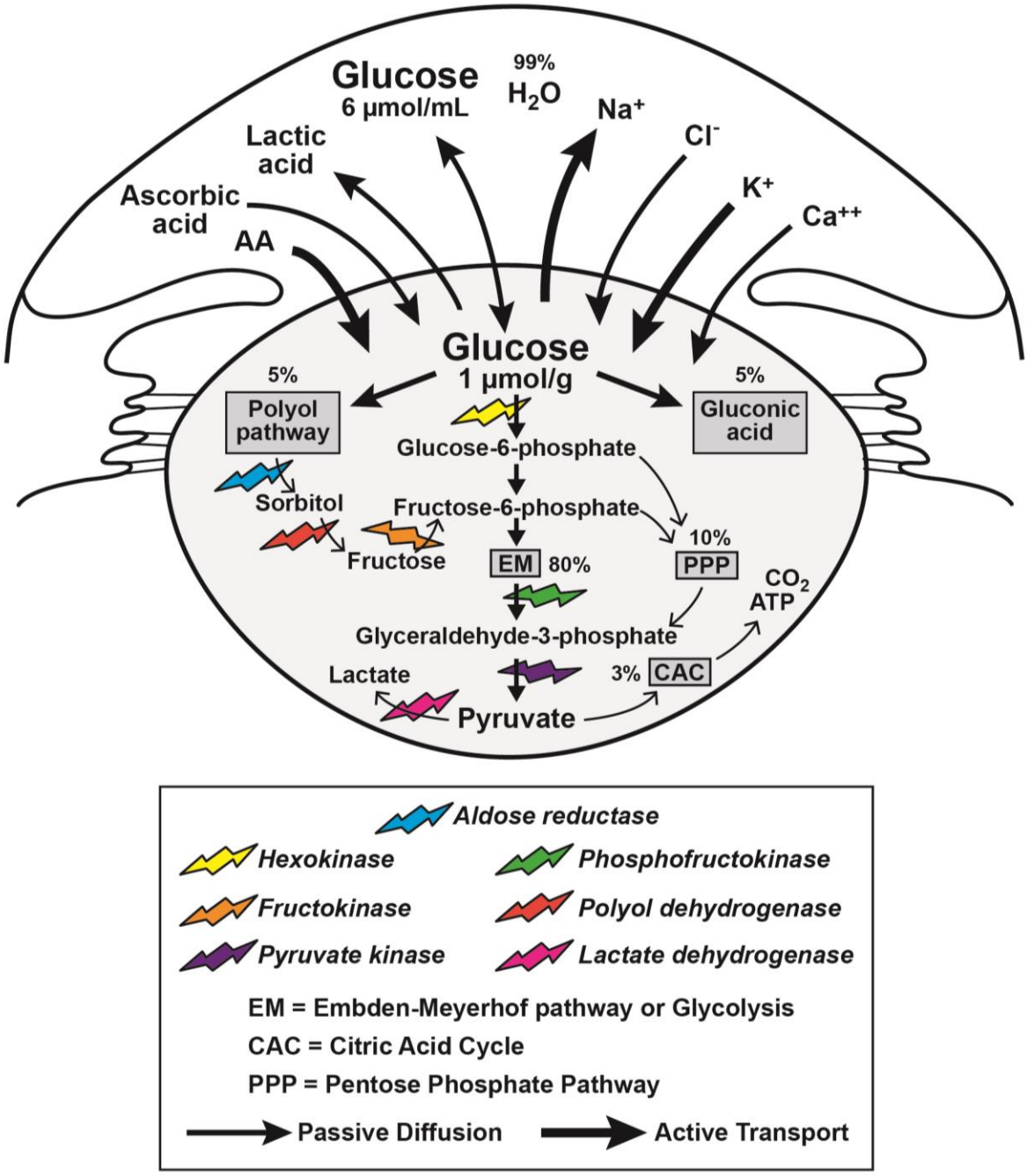
Appendix A - Normal Lens Glucose Metabolism



Aqueous humor resembles an ultra filtrate of plasma. Several of the organic and inorganic compounds normally present in the aqueous humor are displayed. Glucose undergoes passive diffusion or facilitated transport through the semi-permeable lens capsule, and under physiologic conditions (a euglycemic state), the glucose metabolism pathways are exhibited with their respective percentage of glucose processed. Figure created by Mal Rooks Hoover, 2014.

Appendix B - The Effects of Ophthalmic Prednisolone and Diclofenac on DM Regulation Evaluation of Diabetes Control

Patient History	
Need for insulin adjustment in previous 4 weeks	Yes No
Change in water consumption	Yes-increased water consumption No
Change in urine frequency/amount	Yes No
Change in appetite/food intake	Change in appetite (increased) No change in appetite
Change in activity level	Change in activity level (decreased) No change in activity level
Signs of hypoglycemia noticed	Yes No
Required treatment for hypoglycemia	Yes No
Noncompliant with insulin/ocular medications	Yes No
Physical Examination	
Change body weight ≥5%	Yes No
Body condition score	Unacceptable (poor body condition) Acceptable (good body condition)
Hydration status	Dehydrated Normal hydration
Progression of cataract	Progression No progression
Uveitis present	Yes No
Laboratory Evaluations	
Fructosamine concentration	Unacceptable (>450 µl/L) Acceptable (≤ 450 µl/L)
Glucose curve analysis: insulin adjustment	Yes No