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Topical Kinostat™ Ameliorates the Clinical Development and Progression of Cataracts in Dogs with Diabetes Mellitus

Peter F. Kador, Ph.D.^{1,2}, Terah R. Webb, D.V.M., Dipl. A.C.V.O.³, I. Dineli Bras, D.V.M., M.S., Dipl. A.C.V.O.³, Kerry Ketrang, D.V.M., Dipl. A.C.V.O.⁴, and M. Wyman, D.V.M., M.S., Dipl. A.C.V.O.^{1,3,5}

¹Therapeutic Vision Inc., Omaha, NE

²College of Pharmacy University of Nebraska Medical Center, Omaha, NE

³MedVet Medical Center for Pets, Worthington, OH

⁴All Animal Eye Clinic, Cincinnati, OH

⁵College of Veterinary Medicine, The Ohio State University, Columbus OH

Abstract

Objective—To determine whether topical administration of the aldose reductase inhibitor Kinostat™ can ameliorate the onset or progression of cataracts in dogs with naturally occurring diabetes mellitus (DM).

Materials and Methods—A randomized, prospective, double-masked placebo control pilot study was conducted with 40 dogs newly diagnosed with DM with no or minimal lens changes. Twenty-eight dogs received Kinostat™ and 12 dogs received placebo.

Procedures—Owners administered the agent into both eyes three times daily for 1 year and compliance was monitored with log sheets. Complete ophthalmic examinations were performed on dilated eyes at the time of enrollment and 1, 2, 3, 6, and 12 months into treatment. Cataract severity was assessed on a scale of 0 – 3. At 12 months, full bloodwork, including HbA1C and blood Kinostat™ levels were performed.

Results—After 12 months of treatment, the cataract score in the placebo group significantly increased with 7 dogs (14 eyes) developing mature cataracts, 2 dogs (4 eyes) developing cortical opacities, and 1 dog (2 eyes) developing equatorial vacuoles with mild punctate cortical opacities. In contrast, the cataract score in the Kinostat™ treated dogs was significantly less with 7 developing anterior equatorial vacuoles, 2 developing incipient anterior cortical cataracts, and 4 developing mature cataracts. In fact, the cataract scores of the Kinostat™ group at 12 months did not significantly increase from the score at the time of enrollment. The HbA1C values between the 2 groups after 12 months of treatment were similar, and no blood levels of Kinostat™ was found in any enrolled dog.

Conclusion—The onset and/or progression of cataracts in dogs with DM can be significantly delayed by topical administration of Kinostat™.

Keywords

dogs; diabetes mellitus; cataracts; treatment; aldose reductase inhibitor

Introduction

Better nutrition and preventive veterinary care has increased the life-span of dogs and cats. However, by the age of seven, an estimated 40% of these companion animals in the United States are either overweight or obese. This excess weight is linked to the onset of diabetes mellitus (DM), a metabolic disease that is most frequently diagnosed in dogs and cats between the ages of seven and nine¹⁻⁴. DM is associated with the development of hyperglycemia-linked complications that include the onset of bilateral cataracts. Despite a similar prevalence of DM in dogs and cats, diabetes-associated cataracts primarily occur in dogs⁵ where they generally develop within 5-6 months of the onset of diabetes mellitus⁶ due to levels of the enzyme aldose reductase (AR) being much higher in the lenses of dogs compared to age-matched cats⁷.

AR is an enzyme that reduces glucose and galactose to their respective sugar alcohols sorbitol and galactitol. The accumulation of sugar alcohols in lens cells can lead to an intracellular increase in fluids that result in lens cell swelling. This swelling is associated with increased membrane permeability and a series of complex biochemical changes that are associated with cataract formation⁸⁻¹³. Known as the osmotic theory of sugar cataract formation, this theory is experimentally supported by numerous studies in diabetic, galactosemic, and transgenic animals that establish that sugar cataract formation is dependent on the levels of AR present in the lens and that the onset and reversal of the early stages of cataracts can be ameliorated with Aldose reductase inhibitors (ARIs)^{9,15,23}. More recently, osmotic stress resulting from the accumulation of sugar alcohols in lens epithelial cells which contain mitochondria, has also been shown to induce endoplasmic reticulum (ER) stress that leads to the generation of reactive oxygen species (ROS) and apoptotic signaling²⁴. This sugar alcohol linked osmotic stress can be exacerbated by fluctuations hyperglycemia. Therefore, constant inhibition of lens sorbitol formation is required in order to maintain lens clarity. The rate and severity of cataract development is directly proportional to the intracellular accumulation of sorbitol or galactitol. Experimentally, galactose-induced cataracts develop more rapidly and are more severe than cataracts in naturally occurring diabetic animals¹⁰, because sorbitol can be metabolized to fructose by the enzyme sorbitol dehydrogenase, while galactitol cannot be further metabolized.

The critical role of the accumulation of sugar alcohols in cataract formation has been confirmed through the administration of ARIs. In numerous animal studies, AR inhibition at the onset or early stages of diabetes or galactose-feeding has been shown to arrest the onset and progression of cataract formation^{10,14-19}. Since surgery is the only treatment for restoring vision loss from cataracts, a medical treatment that preserves vision and prevents the need for surgery in diabetic dogs would be beneficial. In galactose-fed dogs, cataract formation has been delayed or prevented either by oral administration of the ARI 2-methyl sorbinil, or topical administration of the formulation KinostatTM^{18-19,22}. In order to determine the effectiveness of topical KinostatTM on naturally occurring diabetics, the following study was designed.

Materials and Methods

A randomized, prospective, double-masked placebo control clinical study was conducted in 40 dogs newly diagnosed with DM. The study protocol was reviewed and approved by the animal care and use committee of the Ohio State University College of Veterinary Medicine, and conducted at MedVet Medical Center for Pets, Worthington, OH and the All Animal Eye Clinic, Cincinnati, OH. Dogs were considered candidates for the study with an initial or recent diagnosis of DM by a primary care practitioner or veterinary internist. A complete physical examination was performed and only animals without clinical or laboratory data

incriminating other systemic diseases that could complicate the development of a cataract or the diabetes control, were considered candidates. All patients were required to be on insulin therapy and diabetes management under the guidance of the diagnosing veterinarian. Breed, sex, and age were not considered when enrolling dogs in the study.

A complete ophthalmic examination was required to be performed by one of the veterinary ophthalmologists (DB, KK, TW) at the time of enrollment and at 1, 2, 3, 6, and 12 months after the initiation of therapy. After one year, owners could elect to keep their dogs in the study and have evaluations done every 6 months. The complete ophthalmic examination included a Schirmer Tear Test (STT), determination of intraocular pressure (IOP), biomicroscopy and indirect ophthalmoscopy following mydriasis. Lens changes were subjectively graded on a scale of 0 – 3 as follows: 0- no cataract formation; 1- equatorial vacuoles only/punctate cortical opacities; 2- equatorial and cortical vacuoles/diffuse cortical opacities; 3- late immature or mature cataract. Only dogs with normal STT, normal IOP, and no lens opacities or only equatorial vacuoles were considered candidates for the study.

Preliminary data in galactose-fed dogs treated with ARI indicated that 33% of animals on placebo was an acceptable positive control group, so it was decided that 33% of dogs would receive placebo and 67% would receive Kinostat™ topically. At the time of initial examination, owners were informed that there was a 33% chance that the agent they would be using would be a placebo (vehicle) that would have no affect on delaying cataract formation. Owners then signed a consent form for participation in the study that clearly stated the mandatory examination schedule, specific procedures to be performed, and the potential risks involved in the study. Owners were instructed on how to properly administer the topical agent OU TID. To ensure compliance, owners recorded each time of administration on a log sheet that was then given to the examiners at the time of each examination. As compensation for the study, owners who wished to continue treatment at the termination of the study (either drug or vehicle treated group) were given complimentary Kinostat™ for the remaining life of their dog. Each dog was randomly assigned a coded vial containing either Kinostat™ or vehicle. The coded vials were masked from all study participants and examiners. At each examination time point, dogs were removed from the study if owners reported sensitivity to the drug, or progression to an advanced cataract was noted.

For all dogs, a complete blood count (CBC), serum biochemical profile, Glycosylated hemoglobin concentrations (HbA1C), and Kinostat™ blood levels were performed after 12 months of the study. The CBC and serum biochemical profile were performed to ascertain any possible systemic effects of the drug. These included measurement of serum glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, albumin, globulin, and total bilirubin concentrations and serum aspartate transaminase, alanine transaminase, and alkaline phosphatase activities. CBC and serum biochemical profile were performed by IDEXX Laboratories (Worthington, Ohio). Glycosylated hemoglobin concentrations (HbA1C) were determined with commercially available A1CNow+ meters (Bayer HealthCare, LLC Headquarters, Tarrytown, NY), and blood Kinostat™ levels were performed at The College of Pharmacy, University of Nebraska Medical Center Laboratory in Omaha, Nebraska.

Blood Kinostat™ levels were determined by adding 0.5 mL of whole blood to corex® centrifuge tubes containing 1.5 mL of double distilled water containing 50 µL of a 1.0-mM internal standard solution of sorbinil. After sonicating the mixture for 30 seconds, the solution was deproteinized with 2 mL of 48 mM NaF and then acidified with 70 µL of 4 M HCl. This solution was then mixed with 5 mL diethyl ether for 1 minute in a vortex and then separating the ether layer by centrifugation at approx $10,000 \times g$ for 25 minutes. The upper

ether layer was then transferred to another corex® tube, washed with an equal amount of 0.25 M phosphate buffer, pH 7.0 and again separated by centrifugation. The ether layer was then evaporated with a stream of nitrogen gas, and the residue was dissolved in 200 µL of methanol. The methanol residue was analyzed by reverse-phase high pressure liquid chromatography (HPLC) (Waters 600 Series; Milford, MA) by injecting 20 µL aliquots onto a (Phenomenex Inc.; Torrance, CA) 5 µm C18 column (250 × 4 mm). The samples were eluted with isocratic 55% aqueous methanol at a flow rate of 1.2 mL/min, and the eluent was monitored with a variable wavelength detector set at 220 nm. All analyses were conducted in triplicate. Using sorbinil as the internal standard, linear standard curves were generated for 2-MS at concentrations between 0 and 70 µg. Both compounds were readily separated by HPLC, with 2-MS displaying a 1.5-min slower retention time than its structurally similar parent compound, sorbinil.

Statistics

Analysis of variance between groups (ANOVA) Statistical analyses were conducted using the ProStat version 5.01 software (Poly Software International, Pearl River, NY 10965). Values of $p < 0.05$ were considered significant.

Results

The study population consisted of 20 female spayed and 20 male neutered dogs with a mean age of 9 years (range 4-14 years), recruited over an 18 month period. Twenty-eight dogs received Kinostat™ in both eyes TID and 12 received only the vehicle in both eyes TID.

Lens changes were primarily bilateral. There were only 2 dogs in the Kinostat™ group and two dogs in the placebo group initially with asymmetry between lens opacities. Analyses were conducted on the total eyes in the study rather than on the average opacity grade for each dog with the Kinostat™ group consisting of 56 eyes and the placebo group of 24 eyes. At the initiation of the study, there was no significant difference in the presence of lens opacities (cataract score, mean ± SEM) between the placebo (0.73 ± 0.06) and Kinostat™ (0.83 ± 0.08) treated groups ($p = 0.297$).

Placebo Group

Cataract formation, assessed by changes in the cataract score, was not significantly ($p = 0.13$) inhibited in the placebo group (Fig. 2). In the dogs receiving only vehicle, lens changes were observed in 20 of 24 eyes within 12 months. Seven dogs (14 eyes) developed mature cataracts, 2 dogs (4 eyes) developed cortical opacities, and 1 dog (2 eyes) developed equatorial vacuoles. All changes were bilateral. No evidence of cataract formation was present in 2 of 12 dogs (4 of 24 eyes) (17%). The population variance of the placebo group was not significantly different ($p < 0.13$). Fig. 3 illustrates the lens changes commonly observed in the vehicle-treated group.

Kinostat™ Group

In dogs receiving topical Kinostat™ for 12 months, lens changes were observed in 26 of 56 eyes (46%). After 12 months of treatment, anterior equatorial vacuoles were present in 14 eyes, cortical opacities were present in 4 eyes, and mature cataracts were present in 8 eyes, with all lens changes bilateral. In the dogs with mature cataracts, these developed within 6 months in 3 dogs and by 12 months in 1 dog. Cataract development was absent in 30 of 56 eyes (54%; 15 of 28 dogs) and the mean cataract severity score of the Kinostat™ treated group after 12 months of treatment was significantly less ($p = 0.0016$) compared to the mean cataract score of the 12 month placebo treated group Fig. 2).

Several dogs treated with Kinostat™ showed reversal of lens changes as vacuoles formed. This wax and waning of cataract formation is illustrated in Fig. 4 where anterior and posterior cortical opacities were present in this dog at the onset of the study and the presence of equatorial vacuoles was observed at 3 months. By nine months, these equatorial vacuoles appeared to recede and at 12 months these vacuoles were absent. Over this 12 month period the appearance of the anterior and posterior cortical cataracts did not change.

Of the 28 dogs initially in the Kinostat™ group, 20 remain in the study at the time of publication with treatment periods ranging from 14 to 29 months (mean 18 months). To date, the average cataract score in these 20 dogs (40 eyes) was significantly less ($p = 0.0043$) than the 12 month cataract score of the entire group of 28 dogs (56 eyes, Fig. 5), due primarily to the departure of dogs with mature cataracts from the study. Of the remaining dogs in this group, 13 dogs (65%) have no lens changes, 6 (30%) have cortical vacuoles and 1 (5%) has a cortical opacity. After 12 months of only vehicle treatment, all dogs in the placebo group were switched to Kinostat™ treatment. Because of the high prevalence of mature cataract in these dogs, only 7 remaining dogs from the original 12 are receiving Kinostat™. The mean cataract score of these remaining 7 dogs is significantly higher ($p < 0.001$) than the average score of the remaining 20 dogs of the initial Kinostat™ treated group (Fig. 5). To date, a diabetic Cocker Spaniel is the longest Kinostat™ treated dog (29 months). On initial examination, the presence of equatorial vacuoles were noted at 360 degrees in this dog; however, after 29 months of Kinostat™ treatment, the lens is clear and the vacuoles are no longer present (Fig. 6).

HbA1C values obtained after 12 months of treatment were not significantly different ($p = 0.369$) between the Placebo (6.7 ± 0.95) and Kinostat™ (7.7 ± 1.8) groups. CBCs and serum biochemical profiles from each dog performed after 12 months of the study, showed no differences between the placebo and Kinostat™ treated groups. No demonstrable level of Kinostat™ was found in any of the blood submitted.

Discussion

Since blood sugar levels and lens AR activity influence the onset and progression of diabetic cataracts, diabetes control and client compliance are two major factors that could influence the results of this study. The waxing and waning of lens changes observed during the study probably reflect changes in hyperglycemia and/or Kinostat™ treatment. HbA1c values were obtained from each dog at 12 months after entering the study to determine glycemic control, and although they were not significantly different ($p = 0.369$) between the vehicle and Kinostat™ groups, it is only an indicator of glycemic control over a 2-3 month period. Because results were not statistically different, it suggests that dogs in both study groups were maintained in similar glycemic control by their owners over the 2-3 months prior to level determination, but glycemic control was not closely monitored during the one year study period, which could complicate the results of this study. Client compliance, while monitored through treatment logs, appears to also be a major factor confounding the present results. This assumption is suggested by our inability to detect Kinostat™ in the blood of client treated dogs compared to our previous laboratory study in galactose fed dogs where 2 drops of Kinostat™ were administered at 5 minute intervals twice daily and it was detected²². The importance of continuous therapy is exemplified by one owner who placed his Kinostat™ treated dog in a kennel without treatment for two weeks while he went on vacation. During this period, the dog who entered with clear lenses rapidly developed bilateral mature cataracts while in the kennel. Nevertheless, the majority of owners appeared to adequately administer topical Kinostat™ since the incidence and progression of cataract formation was significantly reduced compared to vehicle.

Topical application of Kinostat™ has previously been reported to arrest or reverse the development of sugar cataracts in dogs fed a diet containing 30% galactose, which suggests that this ARI formulation may be beneficial in maintaining or improving functional vision in diabetic dogs with early lens opacities²². In this randomized, prospective, double-masked placebo control pilot clinical study of 40 newly diagnosed diabetic dogs treated with Kinostat™ or a vehicle (placebo) control, both the development and severity of cataracts were significantly less in the Kinostat™ treated dogs (Fig. 2 and Fig 5). The results of this pilot study suggest that topical Kinostat™ is clinically beneficial in arresting the onset and/or progression of cataracts in dogs with DM. The use of Kinostat may be warranted in dogs with mild lens changes, but may not significantly impact more advanced lens changes. However, once ARI treatment is initiated, it must be continued indefinitely.

This is the first study to demonstrate that topical application of an ARI by owners of dogs with newly diagnosed DM can reduce the incidence of cataract formation in these dogs.

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References

1. Ionut V, Liu H, Mooradian V, et al. Novel Canine Models of Obese Pre-Diabetes and of Mild Type 2 Diabetes. *Am J Physiol Endocrinol Metab*. 2009
2. Kim SP, Catalano KJ, Hsu IR, et al. Nocturnal free fatty acids are uniquely elevated in the longitudinal development of diet-induced insulin resistance and hyperinsulinemia. *Am J Physiol Endocrinol Metab*. 2007; 292:E1590–1598. [PubMed: 17264230]
3. German AJ. The growing problem of obesity in dogs and cats. *J Nutr*. 2006; 136:1940S–1946S. [PubMed: 16772464]
4. Klinkenberg H, Sallander MH, Hedhammar A. Feeding, exercise, and weight identified as risk factors in canine diabetes mellitus. *J Nutr*. 2006; 136:1985S–1987S. [PubMed: 16772474]
5. Salgado D, Reusch C, Spiess B. Diabetic cataracts: different incidence between dogs and cats. *Schweiz Arch Tierheilkd*. 2000; 142:349–353. [PubMed: 10892302]
6. Beam S, Correa MT, Davidson MG. A retrospective-cohort study on the development of cataracts in dogs with diabetes mellitus: 200 cases. *Vet Ophthalmol*. 1999; 2:169–172. [PubMed: 11397260]
7. Richter M, Guscetti F, Spiess B. Aldose reductase activity and glucose-related opacities in incubated lenses from dogs and cats. *Am J Vet Res*. 2002; 63:1591–1597. [PubMed: 12428672]
8. Kinoshita JH. Mechanisms initiating cataract formation. Proctor Lecture. *Invest Ophthalmol*. 1974; 13:713–724. [PubMed: 4278188]
9. Kinoshita JH. Aldose reductase in the diabetic eye. XLIII Edward Jackson memorial lecture. *Am J Ophthalmol*. 1986; 102:685–692. [PubMed: 3098107]
10. Kador PF, Kinoshita JH. Diabetic and galactosaemic cataracts. *Ciba Found Symp*. 1984; 106:110–131. [PubMed: 6439498]
11. Kador PF, Lee JW, Fujisawa S, et al. Relative importance of aldose reductase versus nonenzymatic glycosylation on sugar cataract formation in diabetic rats. *J Ocul Pharmacol Ther*. 2000; 16:149–160. [PubMed: 10803425]
12. Ai Y, Zheng Z, O'Brien-Jenkins A, et al. A mouse model of galactose-induced cataracts. *Hum Mol Genet*. 2000; 9:1821–1827. [PubMed: 10915771]
13. Lee AY, Chung SK, Chung SS. Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in the lens. *Proc Natl Acad Sci U S A*. 1995; 92:2780–2784. [PubMed: 7708723]
14. Datiles MB 3rd, Fukui H. Cataract prevention in diabetic *Octodon degus* with Pfizer's sorbinil. *Curr Eye Res*. 1989; 8:233–237. [PubMed: 2523284]

15. Kador, PF. Chapter 18: Ocular Pathology of Diabetes Mellitus. In: Tasma, W.; J, EA., editors. Doude's Foundations for Clinical Ophthalmology. Vol. 84. Philadelphia: Lippincott Williams & Wilkins; 2008.
16. Hu TS, Datiles M, Kinoshita JH. Reversal of galactose cataract with Sorbinil in rats. Invest Ophthalmol Vis Sci. 1983; 24:640–644. [PubMed: 6841013]
17. Kador PF, Randazzo J, Babb T, et al. Topical aldose reductase inhibitor formulations for effective lens drug delivery in a rat model for sugar cataracts. J Ocul Pharmacol Ther. 2007; 23:116–123. [PubMed: 17444799]
18. Sato S, Mori K, Wyman M, et al. Dose-dependent prevention of sugar cataracts in galactose-fed dogs by the aldose reductase inhibitor M79175. Exp Eye Res. 1998; 66:217–222. [PubMed: 9533847]
19. Sato S, Takahashi Y, Wyman M, et al. Progression of sugar cataract in the dog. Invest Ophthalmol Vis Sci. 1991; 32:1925–1931. [PubMed: 1903366]
20. Guptill L, Glickman L, Glickman N. Time trends and risk factors for diabetes mellitus in dogs: analysis of veterinary medical data base records (1970–1999). Vet J. 2003; 165:240–247. [PubMed: 12672370]
21. Basher AW, Roberts SM. Ocular manifestations of diabetes mellitus: diabetic cataracts in dogs. Vet Clin North Am Small Anim Pract. 1995; 25:661–676. [PubMed: 7660540]
22. Kador PF, Betts D, Wyman M, et al. Effects of topical administration of an aldose reductase inhibitor on cataract formation in dogs fed a diet high in galactose. Am J Vet Res. 2006; 67:1783–1787. [PubMed: 17014334]
23. Kinoshita JH, Kador P, Datiles M. Aldose reductase in diabetic cataracts. Jama. 1981; 246:257–261. [PubMed: 6787220]
24. Mulhern ML, Madson CJ, Kador PF, et al. Cellular osmolytes reduce lens epithelial cell death and alleviate cataract formation in galactosemic rats. Mol Vis. 2007; 13:1397–1405. [PubMed: 17768385]

Abbreviations

ANOVA	ANalysis Of VAriance between groups
AR	Aldose Reductase
ARI	Aldose Reductase Inhibitor
CBC	Complete Blood Count
DM	Diabetes Mellitus
HPLC	High-Pressure Liquid Chromatography
OU	both eyes (Oculus Uterque)
TID	three times daily (Ter In Die)

Figure 1.
Illustration of lens cataract grading employed.

Figure 2.

Comparison of mean cataract scores (mean ± SEM) at the onset and at 12 months of vehicle (placebo) and Kinostat™ treatment in diabetic dogs. The vehicle treated group was composed of 24 eyes from 12 dogs and the Kinostat™ treated dog was composed of 56 eyes from 28 dogs.

Figure 3. Progression of lens changes observed in a vehicle (placebo) treated diabetic dog. While the lens was initially clear at the onset of the study, lens changes rapidly progressed in 6 months from the formation of vacuoles to the mature cataract stage.

Figure 4.

Progression of lens changes over a 12 month period in a topically Kinostat™ treated dog. Note the initial appearance of anterior and posterior cortical opacities which did not progress and the appearance and reversal of equatorial vacuoles.

Figure 5.

Comparison of mean cataract scores (mean ± SEM) obtained after 12 months of treatment and the last examination date on or before October 2009 (mean month and range) of the original vehicle (placebo) and Kinostat™ treatment groups. At 12 months, the vehicle treated group was composed of 24 eyes from 12 dogs and the Kinostat™ treated dog was composed of 56 eyes from 28 dogs. After 12 months, all dogs in the vehicle (placebo) treated group also received Kinostat™. Because of the high prevalence of cataracts in these dogs, only 7 remaining dogs (14 eyes) from the original 12-month vehicle are receiving Kinostat™. The mean treatment period of Kinostat™ in these dogs is 9 months (range 8 – 12 months). Similarly, only 20 dogs (40 eyes) remain from the original Kinostat™ treated group. This resulted in a significant ($p = 0.009$) reduction in the mean cataract score of the remaining original Kinostat™ treated group.

Figure 6. Appearance of the lens from a diabetic Cocker Spaniel on initial examination where equatorial vacuoles are noted at 360 degrees (left). The same lens after 29 months topical treatment with Kinostat™ (right). The vacuoles are no longer present and the lens appears clear.