Effects of a topically applied 2% delta-9-tetrahydrocannabinol ophthalmic solution on intraocular pressure and aqueous humor flow rate in clinically normal dogs

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Objective—To determine the effects of topically applied 2% delta-9-tetrahydrocannabinol (THC) ophthalmic solution on aqueous humor flow rate (AHFR) and intraocular pressure (IOP) in clinically normal dogs.

Animals—21 clinically normal dogs.

Procedures—A randomized longitudinal crossover design was used. Following acquisition of baseline IOP (morning and evening) and AHFR (afternoon only) data, dogs were randomly assigned to 2 treatment groups and received 1 drop of either 2% THC solution or a control treatment (olive oil vehicle) to 1 randomly selected eye every 12 hours for 9 doses. The IOPs and AHFRs were reassessed after the final treatment. Following a washout period of ≥ 7 days, dogs were administered the alternate treatment in the same eye, and measurements were repeated.

Results—Mean ± SD IOPs in the morning were 15.86 ± 2.48 mm Hg at baseline, 12.54 ± 3.18 mm Hg after THC treatment, and 13.88 ± 3.28 mm Hg after control treatment. Mean ± SD IOPs in the evening were 13.69 ± 3.36 mm Hg at baseline, 11.69 ± 3.94 mm Hg after THC treatment, and 12.13 ± 2.99 mm Hg after control treatment. Mean IOPs were significantly decreased from baseline after administration of THC solution but not the control treatment. Changes in IOP varied substantially among individual dogs. Mean ± SD AHFRs were not significantly different from baseline for either treatment.

Conclusions and Clinical Relevance—Topical application of 2% THC ophthalmic solution resulted in moderate reduction of mean IOP in clinically normal dogs. Further research is needed to determine efficacy in dogs with glaucoma. (*Am J Vet Res* 2013;74:275–280)

Glaucoma comprises a group of ocular diseases in which progressive retinal ganglion cell death and optic neuropathy occur secondary to elevations in IOP. In 1977, the overall prevalence of glaucoma in the canine population was reported to be 0.5% on the basis of information found in the Veterinary Medical Database.[1] Investigators in a more recent retrospective study[2] of the prevalence of primary, breed-related glaucoma in North America found a gradual increase from 0.29% (1964 to 1973) to 0.89% (1994 to 2002), and glaucoma is considered a leading cause of blindness in dogs.[3]

Optic nerve head degeneration can progress despite good control of IOP via apoptotic mechanisms mediated by glutamate excitotoxicity, intraneuronal calcium accumulation, free radical formation, nitric oxide production, formation of proteases, and neurotropin deprivation.[4] Nevertheless, high IOP is the primary risk factor for progression of optic neuropathy in dogs and humans[5,6] and is currently considered the only reliably controllable variable in preventing the progression of neuropathy.[6] Therefore, the primary goal of glaucoma treatment is maintenance of the IOP in an appropriate range to preserve vision and maintain comfort.

There are currently numerous medical and surgical treatments used for control of IOP in dogs with glaucoma.[7] Medical treatments include administration of adrenergic modulators (β-adrenoceptor blockers and α-adrenoceptor agonists), carbonic anhydrase inhibitors, prostaglandin analogs, hyperosmotic agents, and parasympathomimetics. Surgical treatments generally either create an alternate pathway for aqueous humor

Abbreviations

AHFR	Aqueous humor flow rate
IOP	Intraocular pressure
THC	Delta-9-tetrahydrocannabinol

Received January 27, 2012.
Accepted May 14, 2012.
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Supported by the University of Tennessee College of Veterinary Medicine Companion Animal Fund.
Presented in abstract form at the 42nd Annual Meeting of the American College of Veterinary Ophthalmologists, Hilton Head, SC, October 2011.
The authors have no financial interest in any product used in these experiments.
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outflow (anterior chamber shunts) or focus on ciliary body destruction (cyclodestruction) to decrease aqueous humor formation. Cyclodestructive procedures include cyclocryotherapy, transscleral laser cyclophotocoagulation, or endoscopic laser cyclophotocoagulation. Because of the differences in types of glaucoma and in response to traditional glaucoma treatments, no single medical or surgical treatment is always effective in dogs. Therefore, continued research for alternative effective treatments is warranted, and cannabis and cannabis-derived products have promise in this regard.

Cannabis (*Cannabis sativa*; also known as marijuana) has been used for medical purposes for centuries, with the earliest written record dating to 2737 BCE by the Chinese emperor Shen Nung and subsequent widespread use reported in other ancient cultures.[8–11] Cannabinoids are a group of compounds that are constituents of marijuana, with THC mediating most of the physiologic and psychoactive effects.[12] Marijuana is classified as a schedule I controlled substance in the United States because of a high potential for abuse. Medical use of orally administered cannabinoids in the United States is increasing, with drugs such as dronabinol and nabilone commonly prescribed as antiemetics, appetite stimulants, and analgesics for patients with AIDS, cancer, and some neurodegenerative diseases.[8,13,14]

The IOP-lowering effects of marijuana in humans have been known for several decades.[15] Numerous studies in humans have subsequently been performed to evaluate the benefit of cannabinoids in glaucoma treatment via inhalation,[15–17] injectable,[16,18–20] oral,[16,21] and topical[20,22–31] delivery. However, the IOP-lowering potential of cannabinoids in dogs has received scant attention, with only 2 extant studies,[25,32] neither of which accounted for the influence of diurnal variation. The purpose of the study reported here was to evaluate the effect of twice-daily administration of a topically applied 2% THC ophthalmic solution on AHFR and IOP in clinically normal dogs. We hypothesized that IOP would decrease in association with reduction in AHFR following administration of 2% THC solution.

Materials and Methods

Animals—Twenty-one adult client-owned dogs of various breeds were included in the study. Five were used in preliminary experiments, and 16 (11 males and 5 females; mean ± SD age, 4.9 ± 3.4 years [range, 1 to 12 years]; body weight, 11.2 ± 3.6 kg [range, 6.3 to 17.5 kg]) were included in the main study. All dogs were evaluated and determined to be free of ocular disease via slit-lamp biomicroscopy,[a] applanation tonometry,[b] gonioscopy, indirect ophthalmoscopy, Schirmer tear test evaluation, and fluorescein staining. Dogs were receiving no other medications apart from routine heartworm and ectoparasite control products. No abnormalities were detected during physical examination of any dog. The study was conducted in accordance with Animal Care and Use Committee guidelines at the University of Tennessee. Owner consent was obtained prior to enrollment of dogs in the study.

Formulation of THC solution—The 2% THC ophthalmic solution was formulated in an olive oil vehicle by a licensed compounding pharmacist. Extra virgin olive oil was heated to 150°C and then cooled. The contents of 10-mg dronabinol[c] capsules were extracted aseptically and mixed in olive oil with an autostirrer to achieve a 2% THC solution prior to transferring the medication to a sterile 5-mL dropper bottle.

Selection of THC concentration—In preliminary experiments, 2% THC ophthalmic solution was applied to 1 eye in each of 5 dogs (1 drop/eye; approximate volume, 50 µL/drop), and IOPs were measured 30 minutes and 1, 2, 4, 6, and 8 hours after THC administration. The 2% concentration was chosen for use in the main study on the basis of previous studies[23,26,27] evaluating the effects of topically applied THC in rabbits and humans and our observation of apparent decreases in IOP in the preliminary investigation (mean ± SD change, 5.0 ± 2.8 mm Hg at 2 hours after THC administration; data not shown).

Treatment administration and IOP measurement—In the main study, 16 dogs received twice-daily treatment with 2% THC ophthalmic solution or a control treatment (the sterilized olive oil vehicle used in preparation of the THC solution) in a longitudinal randomized crossover design. Baseline IOPs (determined via applanation tonometry) were measured in both eyes at 10:00 AM (morning) and 5:00 PM (evening) on the first study day. On a subsequent day, 1 drop (approximate volume, 50 µL) of either 2% THC solution or the control treatment (selected at random from a table of random numbers) was administered to 1 randomly selected eye (ie, the treated eye) of each dog. Drops were placed in identical dropper bottles and identified by code. The investigators and owners were blinded as to the contents of each bottle. Administration was repeated every 12 hours for a total of 9 doses, with drops administered at home by the dogs' owners between 7:00 AM and 9:00 AM for the morning dose and 7:00 PM and 9:00 PM for the evening dose. Measurements of IOP were repeated at 10:00 AM and 5:00 PM on the day of the final dose. After a minimum 7-day washout period, the process was repeated with the alternate treatment administered in the same eye.

AHFR determination—Evaluation of AHFR was conducted concurrently with IOP determinations in the same dogs during the main study. Baseline AHFRs were measured prior to the first treatment, and measurement was repeated after the final administration of 2% THC ophthalmic solution or control treatment.

A protocol validated for dogs was followed for determination of AHFR via ocular fluorophotometry.[33] Briefly, 1 drop (50 µL) of 10% fluorescein sodium solution[d] was applied to the treated eye every 5 minutes for a total of 3 drops. The first drop was always administered at 8:00 AM. Five minutes after the final drop, the eyes and periocular areas of each dog were irrigated with eye wash, and dogs were then bathed thoroughly to remove any residual fluorescein from the face and body. At 1:00 PM, fluorescein concentrations in the cornea and midcentral anterior chamber were measured noninvasively with a computerized scanning ocular fluorophotometer[e] fitted with an anterior chamber adapter. No sedation was used, and dogs were scanned with minimal

restraint. Fluorophotometry readings were repeated 3 more times at 1-hour intervals until 4:00 PM. Slit-lamp biomicroscopy, applanation tonometry, and fluorescein staining were repeated after the final scan for each dog.

Changes in fluorescein concentrations over time were used in calculations to assess the rate of aqueous humor production as described by Jones and Maurice[34] and modified by Yablonski et al[35] and Ward et al[33]:

$$\text{Flow} = K_0 V_a$$

where V_a is the anterior chamber volume (assumed to be 400 µL for all dogs[33]) and K_0 is the anterior chamber loss coefficient. The K_0 was calculated according to the following formula:

$$K_0 = -A(1 + [1.53V_cC_c/1.2V_aC_a])$$

where A is the average slope of the lines of diminishing corneal and aqueous humor fluorescence, V_c is the corneal volume (assumed to be 100 µL for all dogs[33]), C_c is the corneal fluorescein concentration at the midpoint of the fluorescein decay curve, and C_a is the anterior chamber fluorescein concentration at the midpoint of the fluorescein decay curve.

All fluorophotometric scans were reviewed for evidence of motion artifact. Scans without narrow, gently arching corneal peaks and broad, flat aqueous humor plateaus were rejected (**Figure 1**). As a further assurance of accuracy, scans were rejected if the ratios of the slopes of the corneal versus aqueous humor decay curves were < 0.5 or > 1.5 (because a ratio of unity is assumed in the mathematical derivation of flow) or if the correlation coefficients of either corneal or aqueous humor fluorescein concentrations were < 0.75 (indicating lack of semilogarithmic fluorescein decay, another assumption in the mathematical argumentation).[33] If a dog did not have acceptable scans for all 3 assessments (ie, baseline, after THC administration, and after control treatment), fluorophotometry data for that animal were not used in the analysis.

Statistical analysis—All data were normally distributed (as assessed with Kolmogorov-Smirnov analysis) and are expressed as mean ± SD. The IOPs were assessed in treated and untreated eyes, and AHFRs were assessed in treated eyes only. Baseline IOPs for treated versus untreated eyes were compared via a paired *t* test. The IOPs and AHFRs measured at baseline, after THC administration, and after control treatment were compared via repeated-measures ANOVA, with Holm-Sidak post hoc analysis where appropriate. Values of $P < 0.05$ were considered significant. Power analyses were conducted on normally distributed data when *P* values were between 0.051 and 0.100. Analyses were performed with statistical software.[f]

Results

Treated eyes included 8 right eyes and 8 left eyes of 16 dogs. The 2% THC ophthalmic solution was selected as the first treatment administered in 8 eyes, and the control treatment was selected as the first treatment administered in the remaining 8 eyes. Although a grading scale was not used to assess ocular surface inflammation, slit-lamp examination at the conclusion of each treatment regimen did not reveal substantial irritation following administration of THC solution or the control treatment. Additionally, no owners reported any adverse effects during treatment. In general, dogs were cooperative for IOP measurement and for fluorophotometry, although movement of the head in some instances prevented accurate placement of the focal diamond (ie, the diamond-shaped intersection of the excitation and emission beams, from which fluorescence is measured[33]) of the fluorophotometer within the corneal stroma and therefore resulted in rejected scans. Initially, we began monitoring IOP only in the evening because of concerns regarding study logistics; however, we later determined that it would be possible to obtain morning readings. Thus, morning IOP data were obtained for only 7 dogs.

There were no differences in baseline IOPs between treated and untreated eyes in the morning ($P = 0.689$) or evening ($P = 0.791$), indicating normal aqueous humor dynamics prior to initiation of drug treatment. There were no differences among IOPs measured in untreated eyes at baseline, after THC treatment, or after control treatment in the morning ($P = 0.329$) or evening ($P = 0.061$; power to detect a 25% change, 0.813), indicating

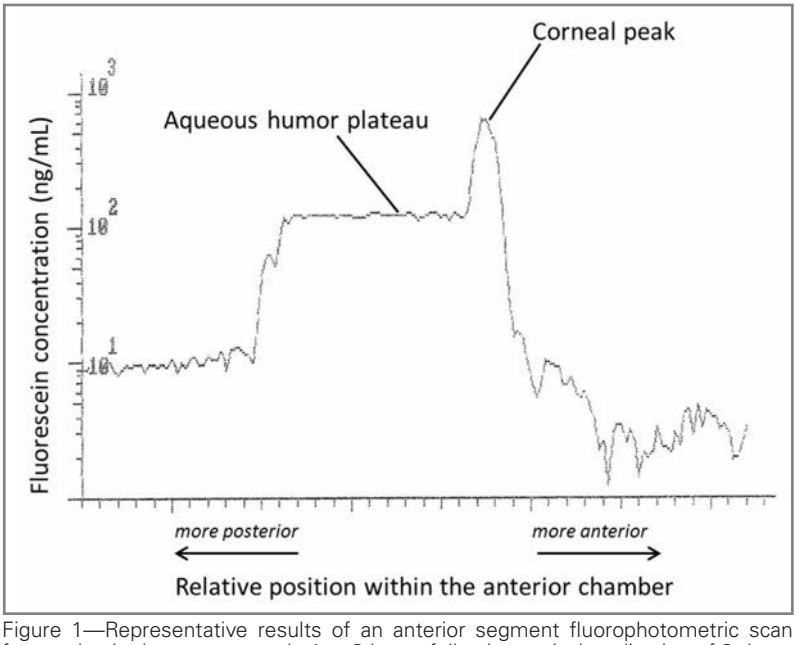

Figure 1—Representative results of an anterior segment fluorophotometric scan from a dog in the present study 4 to 8 hours following topical application of 3 drops of 10% sodium fluorescein solution. The far left side of the scan represents the anterior aspect of the lens, and the far right side represents the tear film. The aqueous humor plateau and corneal peak are positioned as indicated. The plateau of the aqueous humor indicates stable positioning within the midcentral anterior chamber. The rapid rise, smooth arch, and rapid decline of the corneal peak indicate that the corneal stroma was well positioned within the focal diamond of the fluorophotometer. Failure to achieve this scan pattern is typically caused by patient motion and can lead to erroneous AHFR calculations.

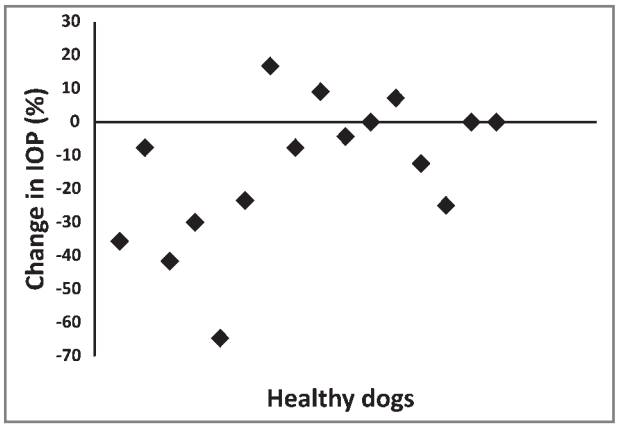

Figure 2—Scatterplot showing the percentage change from baseline (pretreatment) values in evening (5:00 PM) IOPs following topical administration of a 2% THC ophthalmic solution in 16 clinically normal adult dogs. Each diamond represents the value for 1 animal. One drop was administered every 12 hours (for a total of 9 doses) to 1 randomly selected eye in each dog. Intraocular pressures were measured via applanation tonometry. Most dogs had decreased IOP in the treated eye, but responses varied substantially among individual dogs, suggesting that some were more responsive to THC treatment than others.

that application of the THC solution in one eye did not alter IOP in the other eye.

Mean ± SD IOPs for treated eyes in the morning were 15.86 ± 2.48 mm Hg at baseline, 12.54 ± 3.18 mm Hg after THC treatment, and 13.88 ± 3.28 mm Hg after the control treatment ($P = 0.027$; n = 7). Post hoc analysis revealed a significant ($P < 0.05$) difference in mean IOP from baseline after treatment with THC solution, but not after the control treatment.

Mean ± SD IOPs for treated eyes in the evening were 13.69 ± 3.36 mm Hg at baseline, 11.69 ± 3.94 mm Hg after administration of THC solution, and 12.13 ± 2.99 mm Hg after control treatment administration ($P = 0.039$; n = 16). Post hoc analysis again revealed a significant ($P < 0.05$) difference in mean IOP from baseline after treatment with THC solution, but not after the control treatment.

The IOP changes in eyes treated with THC solution corresponded to a mean reduction in IOP of 21% in the morning and 15% in the evening, compared with baseline IOPs. Substantial variability among individual dogs was detected for changes in IOP after treatment with the THC solution, ranging from a 64% decrease to a 17% increase for measurements performed during the evening (**Figure 2**).

In 5 of 16 dogs, decreases in evening IOP of > 20% were detected following control treatment. In 4 of those dogs, the control treatment was administered following treatment with THC solution (which resulted in decreased IOP) and the 7-day washout period. There were too few dogs with morning IOP data to make meaningful statements regarding variability.

Mean ± SD AHFRs in treated eyes were 5.59 ± 2.22 µL/min at baseline, 4.63 ± 1.22 after treatment with THC solution, and 4.93 ± 2.30 after control treatment. These values were not significantly ($P = 0.667$) different (n = 11; power to detect 25% difference from baseline, 0.319). Five dogs had at least 1 scan rejected because of motion artifact resulting in uncertain corneal fluorescein peaks.

Discussion

The purpose of the present study was to investigate the effect of twice-daily administration of a topically applied 2% THC ophthalmic solution on AHFR and IOP in clinically normal dogs. Our hypothesis that this treatment would reduce IOP was supported by significant reductions in mean morning and evening IOPs (differences of 21% [$P = 0.027$] and 15% [$P = 0.039$], respectively). These IOP changes were moderate, compared with those described for other IOP-reducing agents; they were less than that reported following treatment of dogs with latanoprost,[36] bimatoprost,[37] dorzolamide,[38] and dorzolamide-timolol[39] but similar to or greater than that reported for timolol alone.[36,40] However, data in the present study did not support our hypothesis that administration of the THC solution would result in a significant reduction in AHFR.

The *C sativa* plant contains > 400 chemical compounds, and approximately 70 of these are classified as cannabinoids (ie, they exist naturally only in the cannabis plant).[41] Cannabinoids exert their physiologic effects via 2 membrane-bound G-protein–coupled receptors termed CB1 and CB2.[42] The CB1 receptor is found in a number of tissues, including ocular tissue, and CB2 is found primarily in cells of the immune system.[8,43] The most important cannabinoid is THC, which is the principal psychoactive chemical in marijuana.[12] Dronabinol, the drug used in this study, is a synthetic THC approved for treatment of anorexia, nausea, vomiting, and wasting associated with chemotherapy or AIDS in human patients. Dronabinol was the first commercial preparation of synthetic THC and is marketed as soft gelatin capsules containing the active ingredient in sesame oil.[44] Cannabinoids are additionally used in humans for treatment of glaucoma, neuromuscular disease, and pain.[14,45]

Hepler and Frank[15] provided the initial observations of the IOP-lowering effects of marijuana smoking in humans. A number of studies[15–21,23–31,46,47] have subsequently corroborated those effects in various species and with various routes of administration. Major limitations of administration via inhalation in humans include psychoactive effects that reduce a patient's ability to function in society and the toxicity of inhaled marijuana.[48] These factors have stimulated interest in topical application of THC[20,22–31,48] and, together with the impracticality of an inhalation route of administration in dogs, drove the choice to pursue topical ocular application in the study reported here. We chose an olive oil vehicle for delivery of THC on the basis of results of studies[22] that indicated superior solubility and corneal penetrance of THC in lipid solutions as well as convenience, low cost, and ease of preparation. Pharmaceutical formulation of THC for topical use can substantially influence its IOP-lowering efficacy,[22,23,28] and alternative preparations such as microemulsions or cyclodextrans[28,30] may result in improved penetration and thus potentially enhance the effects we observed.

We detected a large degree of variability in IOP changes attributable to topical administration of THC solution in the present study, with individual dogs

having either a very large decrease, little to no decrease, or a slight increase following treatment. For example, although the mean decrease in IOP following treatment with THC solution was 15% for the evening evaluations, 4 of 16 dogs had decreases > 30%, 6 had decreases between 5% and 30%, and 6 had either no decrease or a slight increase in IOP. Humans have similarly been described as either THC responders or THC nonresponders.[15,48] We also detected large decreases in IOP (> 20%) in 5 dogs following the control (olive oil vehicle) treatment, but in 4 of those dogs, the THC treatment had been administered first (followed by a 7-day washout period), and in each of these cases, the decrease was detected in an apparent THC responder. It may be possible that the duration of IOP-lowering effects in dogs that responded to THC administration was longer than we anticipated, and although it seems unlikely, the 7-day washout period may possibly have been insufficient. In addition, all dogs in the study were ophthalmologically normal. For most IOP-lowering drugs, a greater effect is detected in glaucomatous eyes than in normal eyes, and this has been demonstrated with THC as well.[28] Therefore, it is likely that a greater IOP-lowering effect would be found in dogs with glaucoma than was detected in these clinically normal dogs.

The mechanisms by which THC lowers IOP is not known with certainty.[45,49] Cannabinoid receptors are present throughout the CNS, leading to speculation that central regulation of IOP may be involved in the THC response, but most investigators believe that local ocular responses via CB1 receptors are more important.[43,45] The CB1 receptors have been found within the trabecular meshwork, ciliary muscle, and ciliary epithelium (along with other ocular tissues), suggesting that cannabinoids could affect aqueous humor production, trabecular outflow, and uveoscleral outflow.[43] There appear to be differences in distribution of these receptors among different species, suggesting that cannabinoids may act centrally to lower IOP in some mammals and locally in others.[18,49] Additionally, it may be possible that local receptor-mediated effects are responsible for IOP changes while other central effects (ie, psychotropic and systemic) are occurring. It has been shown that injection of THC into the vertebral artery in anesthetized cats causes a consistent decrease in IOP; however, this response is not centrally mediated.[18] An increase in uveoscleral aqueous humor outflow has been shown in humans,[17] an increase in outflow through the trabecular meshwork has been demonstrated in rabbits,[50,51] and reduced aqueous humor production has been shown in monkeys with glaucoma.[31]

Merritt et al[25] demonstrated a decrease in IOP in dogs without a concomitant increase in total outflow facility following topical ocular application of 0.1% THC in light mineral oil to clinically normal Beagles, suggesting the treatment may decrease aqueous humor production in dogs. We therefore hypothesized that treatment with THC solution would be associated with a decrease in AHFR in the present study. Contrary to our expectations, we did not detect a decrease in AHFR concomitant with the IOP reduction, suggesting enhancement of trabecular or uveoscleral outflow. The results of the present study appear to be at odds with those described by Merritt et al,[25] but 2 factors make it difficult to compare the Merritt et al[25] data with ours. First, they compared IOPs before and 6 hours after the THC treatment, which does not allow for the influence of diurnal variation in IOP. Thus, one cannot be sure that the IOP reduction they reported was attributable to THC rather than normal diurnal variation.[25] Second, they measured outflow tonographically, which accounts for pressure-dependent trabecular outflow but does not account for uveoscleral outflow. It is conceivable that our data in combination with that of the previous study[25] suggest that THC lowers IOP in dogs via increase in uveoscleral outflow, which has been reported after marijuana treatment in 1 human patient with glaucoma.[17] Caution is still warranted in making this assumption given that our data, which did not reveal a reduction in AHFR, carried a very low statistical power. Thus, it is possible that THC actually does reduce AHFR in dogs but that our data were too limited or too variable to detect that reduction.[52]

a. Kowa SL-15, Kowa Co Ltd, Nagoya, Japan.
b. Tono-Pen XL, Mentor O & O Inc, Norwell, Mass.
c. Marinol, 10-mg capsules, Watson Pharmaceuticals, Parsippany, NJ.
d. Fluorescite, Alcon Inc, Fort Worth, Tex.
e. FM-2 FluorotronMaster, OcuMetrics, Mountain View, Calif.
f. SigmaStat, version 3.0, SPSS Inc, Chicago, Ill.

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