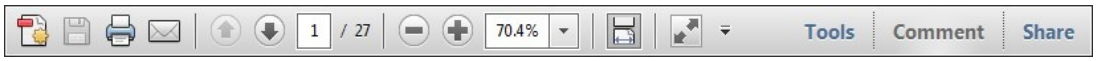
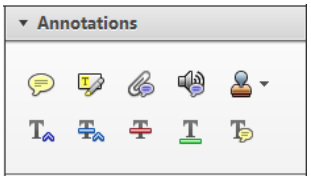
Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

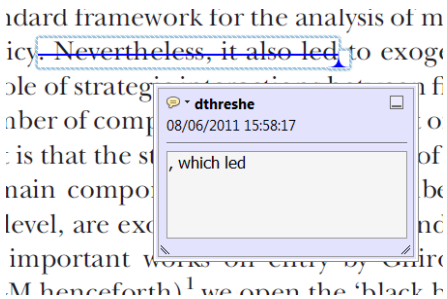


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

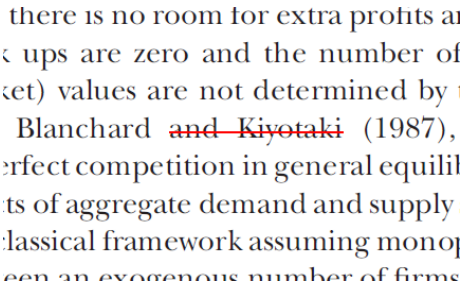


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

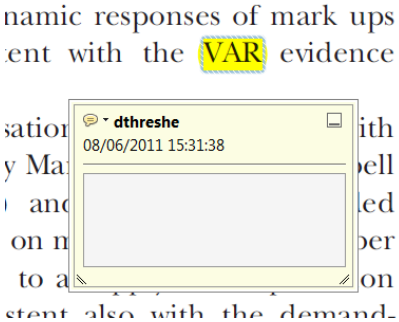


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

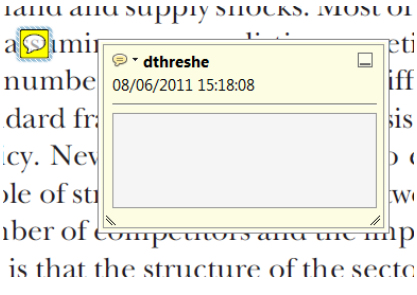


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

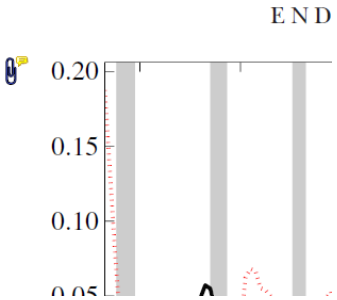


5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

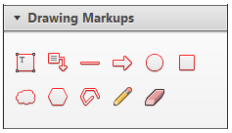
How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



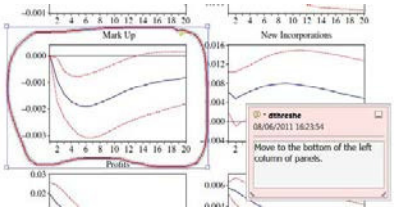
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



Preliminary findings on the effect of melatonin on the clinical outcome of cataract surgery in dogs

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Abstract

Objective Cataract is the most prevalent cause of blindness in dogs. Phacoemulsification (PE) is currently the surgical treatment of choice to remove the opaque lens; however, it is associated with varying degrees of postoperative inflammation. We assessed the effect of melatonin on postoperative complications of canine cataract surgery.

Animal studied Eleven diabetic and thirteen healthy owned dogs with cataracts.

Procedures All dogs underwent cataract surgery by PE. The anti-inflammatory effect of melatonin was compared with the reference treatments: nonsteroidal anti-inflammatory drugs (NSAIDs) for diabetic dogs, and dexamethasone for nondiabetic dogs. Eyes were examined by means of clinical evaluation and intraocular pressure (IOP).

Results In diabetic dogs, melatonin was more effective than topical and systemic NSAIDs in reducing the clinical score at 2, 7, and 20 days postsurgery, while it showed a similar efficacy to topical dexamethasone in dogs with hereditary cataracts. IOP decreased in all groups at 2 days postsurgery, but this decrease reached statistical significance only in diabetic dogs treated with NSAIDs, and persisted at 7 days postsurgery in this group. Afterward, IOP returned to normal values in all groups. Melatonin decreased the occurrence of surgical sequelae in diabetic and nondiabetic dogs.

Conclusions These results indicate that melatonin might constitute a useful tool for reducing postoperative PE complications in dogs.

Key Words: cataract, dog, melatonin, phacoemulsification, postoperative complications

INTRODUCTION

Cataract is the most common cause of treatable blindness in dogs.[1,2] Dogs of both genders can develop cataracts for different reasons, such as metabolic disorders, aging, ultraviolet irradiation, oxidative stress, nutritional deficiency, exposure to toxins, radiation, and blunt or penetrating trauma.[1,3] Most canine cataracts have a genetic component and show a higher incidence in specific breeds, such as Cockers, Poodles, Miniature Schnauzers, and Terriers.[4] Cataracts develop more frequently with increasing age, although some can be present at birth (congenital cataracts) or develop early in life (juvenile cataracts). Additionally, dogs appear to have a unique susceptibility to the development of cataracts secondary to diabetes mellitus (when compared with humans or cats, for example).[5] In fact, cataracts are one of the leading complications of canine diabetes mellitus, with a prevalence up to 75%.[5]

Cataracts are essentially caused by changes in the lens protein composition or in the arrangement of the lens fibers.[6] Depending on the cataract grade, affected dogs will display vision problems, ranging from mild visual impairment to complete blindness.[7]

Over the past 15 years, phacoemulsification (PE), which allows ultrasonic fragmentation and aspiration of the cataractous lenses through small incisions, has supplanted extracapsular and intracapsular lens extraction as the treatment of choice for surgical correction of canine cataracts.[2,8] This surgical procedure is now minimally invasive, and the associated risks have considerably decreased due to recent advances in PE instrumentation and smaller incisions. However, different degrees of postsurgical

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inflammation of the ocular surface, the anterior chamber, and the posterior chamber remain as postsurgical complications.[9,10] Aside from ocular inflammation, ocular infection is another major concern for ophthalmologists performing cataract surgery in dogs.[11] For this reason, there is uniform agreement on the need for antibiotic prophylaxis and anti-inflammatory management after the lens extraction. In dogs with cataracts of hereditary origin (on the basis of the breed-related risk), corticosteroids are administered 3–5 days before, and up to several months after surgery. Dexamethasone, a potent synthetic corticosteroid, is one of the most widely used ophthalmic corticosteroids in canine cataract surgery.[12,13] However, hypothalamic–hypophysis–adrenal axis suppression was reported in five small dogs as a result of ophthalmic instillation of 1% prednisolone acetate for 2 weeks,[14] and the chronic systemic use of topical corticosteroids might lead to adrenal suppression and histopathologic changes in the liver.[15] Nonsteroidal anti-inflammatory drugs (NSAIDs) therapy is also used to treat postoperative ocular inflammation and is the treatment of choice when diseases such as diabetes mellitus or systemic infection preclude the use of systemic corticosteroids.[16] Moreover, according to current medical standards in Argentina, diabetic dogs are not treated with topical corticosteroids. However, topical application of NSAIDs may delay the corneal reparative process and increase intraocular pressure,[16] and after systemic use, even for short periods, NSAIDs may affect platelets and induce renal insufficiency and gastrointestinal hemorrhage or ulceration.[17,18] Although the rate and degree of complications associated with the use of these anti-inflammatory medications is quite low, the development of an additional anti-inflammatory approach would be useful for dogs that develop postoperative uveitis that responds poorly to traditional approaches.

Melatonin is biosynthesized in several anatomic locations of the eye, including the retina,[19] ciliary body,[20] lacrimal gland,[21] and lens.[22] Melatonin acts as a widespread free radical scavenger and antioxidant in different tissues including ocular structures, and it may provide neuroprotection in several ocular diseases. In that context, it has been demonstrated that melatonin may act as a protective agent in photokeratitis,[23] retinal ischemia/reperfusion injury,[24] and retinopathy of prematurity.[25] In addition, we have previously shown the beneficial effect of melatonin against retinal glaucomatous,[26] and diabetic[27] damage. Furthermore, we have demonstrated the anti-inflammatory effect of melatonin in experimental uveitis in hamsters[28] and cats.[29]

It has been shown that melatonin reduces cataract development in several experimental models,[30,31] probably by scavenging free radicals, and preserving antioxidant enzyme activities of the lens, which result in lower levels of oxidatively damaged products and lower accumulation of mineral ions in the lens under stress.[32] However, the effect of melatonin as a treatment for postoperative cataract surgery complications has not been previously examined. In that context, the purpose of this study was to analyze the effect of melatonin on the clinical outcome of cataract surgery in dogs.

MATERIALS AND METHODS

Animals

All animal procedures were in strict accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The ethics committee of the School of Medicine, University of Buenos Aires (Institutional Committee for the Care and Use of Laboratory Animals (CICUAL)) approved this study. Informed consent was obtained from all owners prior to the commencement of the study.

Forty-three eyes from 24 owned dogs (11 diabetic dogs and 13 healthy dogs with cataracts) were included in the study. Dogs were considered diabetic if blood and urine tests revealed persistent hyperglycemia (>200 mg/dL) and glucosuria, respectively, and if the dog had a history of clinical signs consistent with diabetes (i.e., polyuria, polydipsia, weight loss, and/or polyphagia). Only diabetic dogs with bilateral cataracts were included in this study. Diabetes was controlled by a certified endocrinologist, and all diabetic dogs included in this study had a stable state of the disease. Dogs were included in the nondiabetic cataractous group if they had no clinical signs indicative of diabetes, blood glucose concentrations were within the normal reference range (84–124 mg/dL), and no glucose was detected on urinalysis. One dog with a pharmacologically compensated cardiopathy (chronic mitral insufficiency) was included in this group, whereas no evidence of other systemic disease was found on physical examination, or blood and urine test results in the other dogs from this group. Dogs with any other ophthalmic disease except cataracts were excluded from the study.

Animals were examined in a Veterinary Ophthalmic Clinic ('La Estación', Pico 1701, (1430), Buenos Aires, Argentina). Preoperatively, a complete ophthalmic examination including slit-lamp biomicroscopy, applanation tonometry, indirect ophthalmoscopy, Schirmer tear test, and fluorescein staining test was performed in all animals. Moreover, prior to surgery, electroretinography (Akonic BIO-PC, Buenos Aires, Argentina) and ocular ultrasonography (Lindscan; OcuScience, Henderson, NV, USA) were performed in all eyes to rule out vitreal or retinal disease. In the presurgical examination, none of the dogs showed any sign of uveitis. According to the ocular and systemic diagnosis, animals were classified into two groups: diabetic dogs with cataracts and nondiabetic dogs with cataracts; each of these groups was randomly subdivided into two groups: one receiving the anti-inflammatory reference treatment (i.e., NSAIDs for diabetic dogs, and corticoids for healthy dogs), and the other receiving melatonin. The randomizing protocol was performed by giving a consecutive number

to each dog included in this study, and then assigning them to each treatment, using a random number generator (http://www.randomizer.org/form.htm).

Surgical procedure

Surgery was performed by the same veterinarian (PHS) using a phacoemulsification system (AMO Phaco plus; Allergan Laboratories, Irvine, CA, USA), under a surgical microscope (OSM 75; Topcon Laboratories, Tokyo, Japan). General anesthesia was induced using routine procedures and maintained with isoflurane inhalation. One-handed PE with a modification of the 'crater divide and conquer' method was performed through a 2.7-mm corneal incision.[36,37] The corneal incision was closed with Nylon 10-0 sutures (Alcon Laboratories, Fort Worth, TX, USA). All eyes underwent uncomplicated ultrasonic lens PE and aspiration. After the successful completion of surgery, the recovery from anesthesia was recorded by observation of vocalization, exhibition of struggling during recovery, and presence of head righting reflex. All owners were advised to restrict dog activity for at least 1 month, and dogs wore an Elizabethan collar for at least 2 weeks postoperatively. No intraocular lenses were implanted because of the volition of the owners, and all the patients were visual postsurgery.

Pre- and postsurgery medication

Table 1 summarizes the pre- and postsurgery medication for all the experimental groups. One drop of 1% atropine sulfate (Holliday Laboratories, Buenos Aires, Argentina), BID, was administered to all animals for 5 days after surgery. One drop, TID, of antibiotic tobramycin sulfate (0.3%, Tobra Oftal; Love Sudamericana Laboratories, Buenos Aires, Argentina) was administered to all animals from day 5 before up to day 21 after surgery. The postoperative anti-inflammatory reference treatment for diabetic dogs was 1 drop every 6 h of 0.1% sodium diclofenac (Diclovet, Paul Laboratories, Buenos Aires, Argentina) for 1 week, 1 drop, TID, in the second week, and afterward, 1 drop BID, until day 180. In addition, diabetic dogs from the reference treatment group received 1.5 mg/kg, BID, oral carprofen (Rimadyl; Pfizer Laboratories, Buenos Aires, Argentina) for 10 days. For nondiabetic dogs, the anti-inflammatory reference treatment was 1 drop every 6 h of 0.1% dexamethasone (Holliday Laboratories, Buenos Aires, Argentina) for 1 week, 1 drop, TID, in the second week, and afterward, 1 drop, BID, until day 180. The reference treatments for diabetic and nondiabetic dogs were according to current medical standards in Argentina.

Melatonin was orally administered to five diabetic and five nondiabetic dogs at a dose of 0.3 mg/kg, BID, for 180 days. The dose of melatonin was selected on the basis of a previous report addressing the use of melatonin in cats.[29] All the anti-inflammatory treatments, except carprofen, started at 7 days before surgery, as shown in Table 1.

Postsurgical clinical examination

All dogs were examined by direct and indirect ophthalmoscopy and slit-lamp biomicroscopy by the same veterinarian (JA), who was masked for the treatment applied to each dog. At 2, 7, and 20 days after surgery, dogs were evaluated for inflammatory signs such as blepharospasm, episcleral congestion, corneal edema, aqueous flare, and miosis. Clinical severity of these inflammatory signs was assessed; each individual item (blepharospasm, episcleral congestion, corneal edema, aqueous flare, and miosis) was graded from 0 to 3 (0 = absent; 1 = mild; 2 = moderate; and 3 = severe), and then, the numbers for each category tallied for a number out of 15. Miosis was quantified in

Table 1. Pre- and postsurgery medication for each experimental group

Group	Presurgery medication	Postsurgery medication
Diabetic Reference treatment	# One drop, TID, of 0.3% tobramycin sulfate (starting 5 days before). # One drop every 12 h of 0.1% sodium diclofenac (starting 7 days before).	# One drop, TID, of 0.3% tobramycin sulfate, for 21 days. # One drop of 1% atropine sulfate, BID, for 5 days. # One drop every 6 h of 0.1% sodium diclofenac for 1 week, 1 drop, TID, in the second week, and afterwards, 1 drop BID, until day 180. # 1.5 mg/kg, BID, oral carprofen for 10 days.
Diabetic Melatonin	# One drop, TID, of 0.3% tobramycin sulfate (starting 5 day before). # 0.3 mg/kg melatonin orally, BID (starting 7 days before).	# One drop, TID, of 0.3% tobramycin sulfate, for 21 days. # One drop of 1% atropine sulfate, BID, for 5 days. # 0.3 mg/kg melatonin, orally, BID, for 180 days.
Hereditary Reference treatment	# One drop, TID, of 0.3% tobramycin sulfate (starting 5 day before). # One drop every 12 h of 0.1% dexamethasone (starting 7 days before).	# One drop, TID, of 0.3% tobramycin sulfate, for 21 days. # One drop of 1% atropine sulfate, BID, for 5 days. # One drop every 6 h of 0.1% dexamethasone for 1 week, 1 drop, TID, in the second week, and afterwards, 1 drop, BID, until day 180.
Hereditary Melatonin	# One drop, TID, of 0.3% tobramycin sulfate (starting 5 day before). # 0.3 mg/kg melatonin orally, BID (starting 7 days before).	# One drop, TID, of 0.3% tobramycin sulfate, for 21 days. # One drop of 1% atropine sulfate, BID, for 5 days. # 0.3 mg/kg melatonin, orally, BID, for 180 days.

Table 2. Characteristics of the dogs included in the study

Dog	Breed	Age (year)	Sex	Systemic Disease	Stage of cataract	Time PE (min)	Eye	Treatment	Sequelae at 180 days postsurgery		
									PCO	CE	S
1	Mixed	5	SF	Diabetes	Immature	3.0	RE	NSAIDs	0	0	0
					Immature	2.9	LE	NSAIDs	0	0	0
2	Breton	5.5	SF	Diabetes	Immature	2.6	RE	NSAIDs	1	1	0
					Mature	3.2	LE	NSAIDs	0	0	0
3	Siberian Husky	5	M	Diabetes	Mature	3.6	RE	NSAIDs	0	0	0
					Mature	2.9	LE	NSAIDs	0	1	1
4	Mixed	6	SF	Diabetes	Immature	3.0	RE	NSAIDs	0	0	0
					Mature	3.6	LE	NSAIDs	0	0	0
5	Rottweiler	7	M	Diabetes	Mature	3.8	RE	NSAIDs	0	0	0
					Mature	3.5	LE	NSAIDs	0	0	0
6	Poodle	6	SF	Diabetes	Mature	2.1	RE	NSAIDs	2	1	2
					Mature	2.6	LE	NSAIDs	1	1	1
7	Mixed	7	SF	Diabetes	Mature	2.8	RE	Mel	0	0	0
					Mature	2.5	LE	Mel	0	0	0
8	Siberian Husky	6.5	SF	Diabetes	Mature	2.2	RE	Mel	0	0	0
					Mature	3.0	LE	Mel	0	0	0
9	Dachshund	5	M	Diabetes	Hypermature	3.8	RE	Mel	0	0	0
					Mature	3.5	LE	Mel	1	0	1
10	Rottweiler	5.5	SF	Diabetes	Immature	2.2	RE	Mel	0	0	0
					Immature	2.4	LE	Mel	0	0	0
11	Mixed	7	SF	Diabetes	Mature	3.0	RE	Mel	0	0	0
					Mature	3.5	LE	Mel	0	0	0
12	Poodle	7	M	–	Mature	1.8	RE	Dexa	0	0	0
					Mature	3.6	LE	Dexa	0	0	0
13	Beagle	7.5	F	–	Immature	2.0	RE	Dexa	1	0	1
					Mature	3.0	LE	Dexa	0	0	0
14	Fox Terrier	6.5	M	–	Mature	4.0	LE	Dexa	1	0	1
15	Poodle	7	SF	–	Immature	2.5	RE	Dexa	0	0	0
16	Maltese	7.5	F	–	Immature	0.6	RE	Dexa	0	0	0
17	Poodle	8	SF	Cardiopathy	Immature	1.1	RE	Dexa	0	0	0
					Mature	4.5	LE	Dexa	0	0	0
18	Poodle	7	M	–	Mature	4.4	RE	Dexa	0	2	0
					Mature	3.6	LE	Dexa	0	1	0
19	Mixed	7.5	M	–	Mature	3.4	LE	Dexa	0	2	0
20	Golden Retriever	7	M	–	Immature	3.0	RE	Mel	0	0	0
					Immature	2.9	LE	Mel	0	0	0
21	Breton	7.5	M	–	Immature	2.6	LE	Mel	0	0	0
22	Poodle	6.5	SF	–	Mature	2.0	RE	Mel	0	0	0
					Mature	2.5	LE	Mel	0	0	0
23	Siberian Husky	8	F	–	Mature	3.5	RE	Mel	0	0	0
					Mature	3.1	LE	Mel	0	0	0
24	Poodle	7.5	M	–	Mature	0.6	RE	Mel	1	0	1
					Mature	2.0	LE	Mel	0	0	0

Diabetic dogs treated with NSAIDs: 1–6 (12 eyes); diabetic dogs treated with melatonin: 7–11 (10 eyes); dogs with hereditary cataract treated with dexamethasone: 12–19 (12 eyes); and dogs with hereditary cataract treated with melatonin: 20–24 (9 eyes). Time PE = time of phacoemulsification; M = male; F = female; SF = spayed female; RE = right eye; LE = left eye; PCO = posterior capsule opacification; CE = corneal edema; S = synechiae.

reference to the pupil area. At 180 days postsurgery, eyes were characterized by the presence of sequelae (corneal edema, synechiae, and posterior capsule opacification (PCO)), and the incidence of sequelae in each group was expressed as number of sequelae/number of eyes.

IOP assessment

IOP was assessed 1 week before and on days 2, 7, 20, 90, and 180 postsurgery. Tonometric measurements were performed by a single investigator (JA) using a Tono-Pen XL applanation tonometer (Mentor®, Norwell, MA, USA). Dogs were manually restrained, and a drop of topical anesthetic (0.5% sterile proparacaine hydrochloride ophthalmic solution, Anestalcon®; Alcon Laboratories, Buenos Aires, Argentina) was applied to the cornea immediately before tonometry. Five independent IOP readings (SE < 5%) were obtained from each eye, and IOP was determined as the mean of these readings. IOP measurements were

performed during the morning (from 9 AM to noon) to correct for diurnal variations of this parameter.[33]

Statistical analysis

For the clinical score, in the case of bilateral cataracts, both eyes from the same dog were averaged. Comparison of clinical scores between treatment groups among diabetic and nondiabetic patients was performed separately for each day, while IOP values on each day postsurgery were compared back to the baseline value. Statistical analysis of the data was performed by a nonparametric Mann–Whitney test (for age, PE time, and clinical score) and by Kruskal–Wallis test (for IOP). Statistical significance was set at $P < 0.05$.

RESULTS

Table 2 summarizes the characteristics (breed, age, gender, stage, and origin of cataract) of dogs included in this study, and the PE time spent for each surgery. The diabetic cataractous group consisted of three males and eight spayed female dogs, while the nondiabetic cataractous group consisted of seven male dogs, three female dogs, and three spayed females. The age of all dogs ranged from 5 to 8 years. The mean age for all diabetic dogs was 5.9 ± 0.2 (diabetic dog with NSAIDs: 5.75 ± 0.3 years; diabetic dogs with melatonin: 6.2 ± 0.41 years), while for all nondiabetic dogs, the mean age was 7.3 ± 0.1 years (dogs with dexamethasone: 7.25 ± 0.2 years; and dogs with melatonin: 7.3 ± 0.3 years), which resulted in a significant difference in age between the diabetic and nondiabetic groups ($P < 0.01$, Mann–Whitney test), but not between the reference treatment and melatonin within each group. Many breeds were included in this study, with a predominance of Poodle in the hereditary cataract group (~50%). Twenty-nine of the cataracts removed were classified as mature, one was classified as hypermature, and thirteen were classified as immature. Nineteen of the dogs underwent bilateral surgery, while five dogs had unilateral surgery. The PE time did not significantly differ among groups (i.e., diabetic dogs with NSAIDs: 3.0 ± 0.1; diabetic + melatonin: 2.9 ± 0.2; hereditary cataract + dexamethasone: 2.9 ± 0.3; hereditary cataract + melatonin: 2.5 ± 0.4 min, Mann–Whitney test).

Clinical score was assessed at days 2, 7, and 20 postsurgery in all treatment groups, as shown in Fig. 1. In the diabetic group, melatonin was significantly more effective than the reference treatment (NSAIDs) in reducing the clinical score at days 2 (Fig. 1A), 7 (Fig. 1B), and 20 (Fig. 1C) postsurgery, while in dogs with hereditary

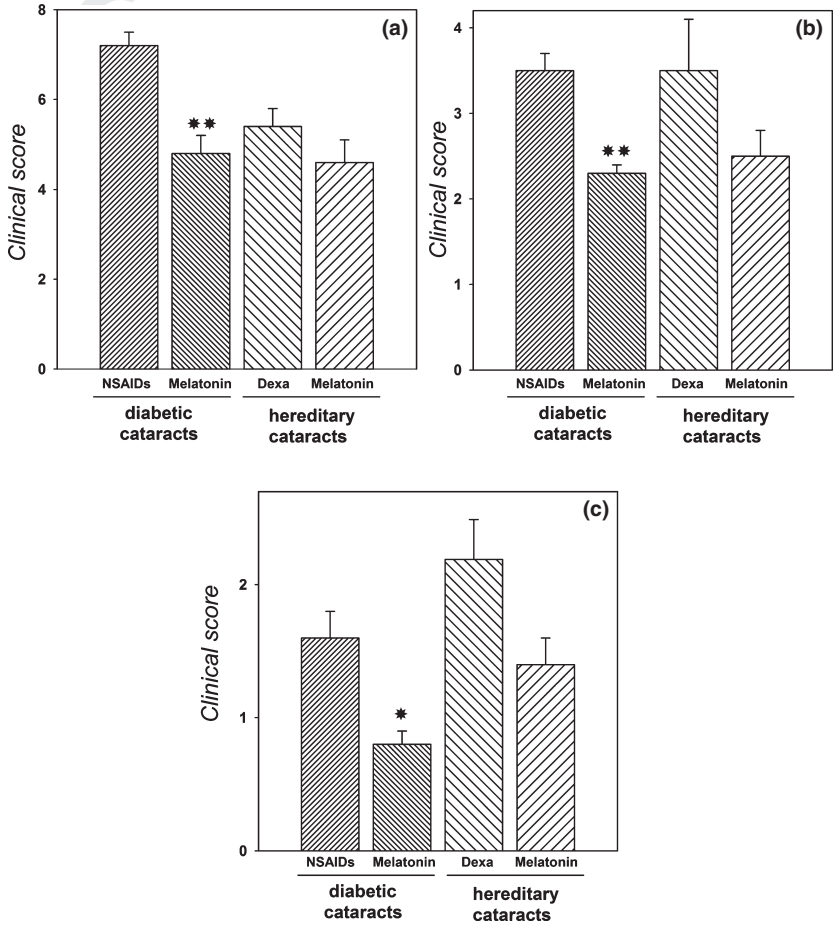

Figure 1. Median clinical scores for dogs undergoing cataract surgery *via* PE, assessed at days 2 (panel a), 7 (panel b), and 20 (panel c) postsurgery. Dogs were classified as diabetic and nondiabetic, and each group was randomly subdivided into two groups; one group received the anti-inflammatory reference treatment (NSAIDs for diabetic dogs and dexamethasone for nondiabetic dogs), and the other was treated with melatonin. In diabetic dogs, the clinical score from eyes treated with melatonin was significantly lower than that observed in dogs treated with NSAIDs at 2, 7, and 20 days postsurgery, whereas in nondiabetic dogs, melatonin was equally effective than dexamethasone. Shown are the clinical scores assigned to six diabetic dogs treated with NSAIDs, five diabetic dogs treated with melatonin, eight dogs with hereditary cataracts treated with dexamethasone, and five dogs with hereditary cataracts treated with melatonin. Dexa: dexamethasone. *$P < 0.05$, **$P < 0.01$ versus the reference treatment, by Mann–Whitney test.

cataracts, melatonin showed an efficacy similar to dexamethasone at all time points. Melatonin did not affect plasma glucose levels in diabetic dogs (data not shown).

Figure 2 shows representative photographs of typical inflammatory signs that developed in dogs submitted to cataract surgery at 2 days postsurgery. The most frequent surgical complications observed were episcleral congestion, aqueous flare, and miosis.

IOP was assessed in all experimental groups 1 week before, and at days 2, 7, 20, 90, and 180 after surgery (Fig. 3). The preoperative mean IOP was similar among groups. A decrease in IOP was observed in all groups 2 days after surgery as compared with baseline values, but this decrease reached statistical significance only in diabetic dogs treated with NSAIDs, and persisted at 7 days postsurgery in this group. Over time, IOP recovered to normal values for all groups. Table 3 summarizes individual values of clinical score and IOP for all eyes included in the study, at 2, 7, and 20 days postsurgery.

At 180 days postsurgery, the occurrence of sequelae (corneal edema, synechiae, and PCO) was assessed in all groups, as shown in Table 2. In diabetic dogs treated with NSAIDs, 4/12 eyes (33.3%) had PCO, while in diabetic dogs treated with melatonin, PCO was observed in 1/10 eyes (10%). In dogs with hereditary cataracts treated with dexamethasone, 4/12 eyes (33.3%) showed PCO, while in dogs with hereditary cataracts treated with melatonin, PCO occurred in 1/9 eyes (11.1%). The percentages of eyes with synechiae in different treatment groups were as follows: diabetic dogs + NSAIDs = 25%, diabetic dogs + melatonin = 10%, hereditary cataracts + dexamethasone = 16.6%, and hereditary cataracts + melatonin = 11.1%. In the case of corneal edema, in diabetic dogs treated with NSAIDs, the incidence was 25%, while in the hereditary cataract + dexamethasone group, corneal edema was observed in 1/12 eyes (8.3%). None of the animals treated with melatonin developed postoperative corneal edema. Figure 4 shows representative photographs of typical sequelae in dogs submitted to cataract surgery at 180 days postsurgery.

DISCUSSION

Cataract extraction in dogs is an ophthalmic surgery frequently performed in veterinary medicine. Several surgical techniques to remove the opaque lens have been used, but PE is the current treatment of choice. However, PE is associated with varying degrees of postoperative inflammation. Although the number of dogs included in this study was relatively small, the present results indicate that a daily treatment with melatonin reduced postoperative complications of PE in both diabetic and nondiabetic dogs.

In this study, we used an oral administration of melatonin to prevent the side effects of corneal melts, conjunctival irritation, and dry eye that occur with the frequent use of topical corticosteroids and NSAIDs.[34] The pharmacokinetics of oral administration of different doses of melatonin ranging from 10 to 80 mg/kg in dogs was studied by Sääf *et al.*,[35] who showed that melatonin is rapidly

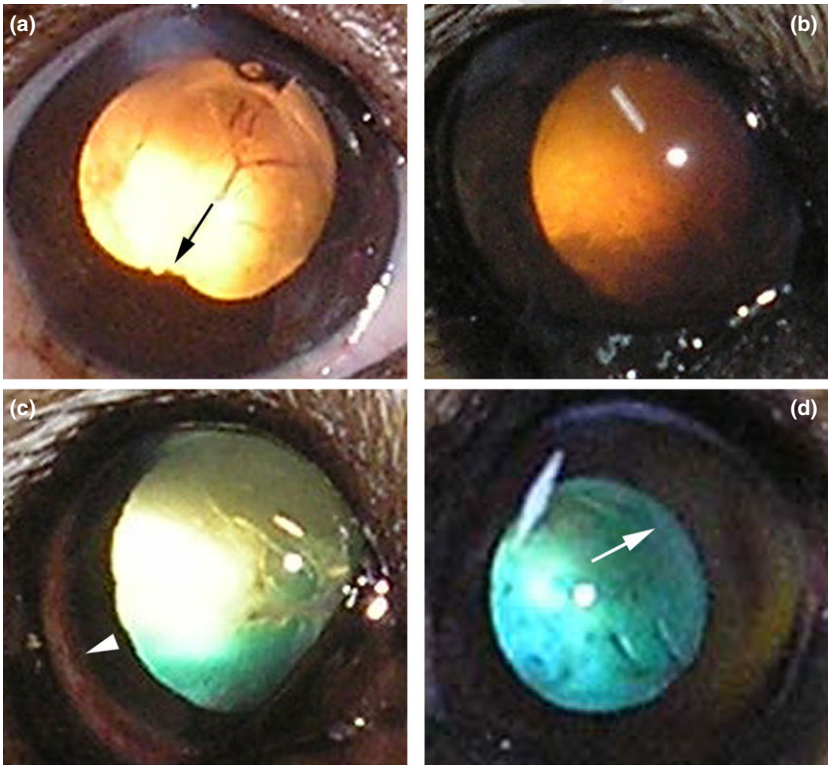

Figure 2. Representative photographs of clinical signs observed 2 days after PE in a diabetic dog treated with NSAIDs (a), a diabetic dog treated with melatonin (b), a nondiabetic dog treated with dexamethasone (c), and a nondiabetic dog treated with melatonin (d). Note the occurrence of a posterior synechia (black arrow) in a dog treated with NSAIDs, episcleral congestion in a dog treated with dexamethasone (arrow head), and a slight miosis (white arrow) in a dog treated with melatonin.

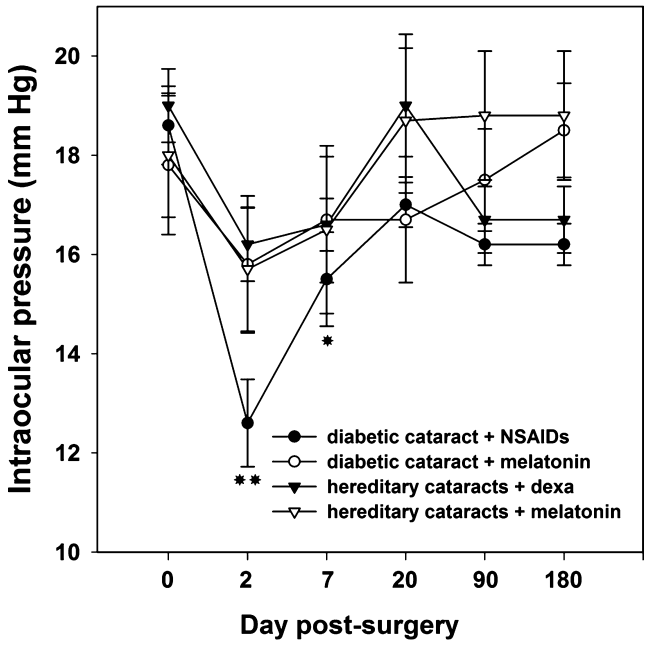

Figure 3. IOP values (determined via applanation tonometry) in diabetic and nondiabetic dogs submitted to PE and assessed before (0) and at days 2, 7, 20, 90, and 180 after cataract surgery. Data are mean ± SE (n = 12 eyes from diabetic dogs treated with NSAIDs (black circles), n = 10 eyes from diabetic dogs treated with melatonin (white circles), n = 9 eyes from six dogs with hereditary cataracts treated with dexamethasone (dexa, black triangles), and n = 12 eyes from seven dogs with hereditary cataracts treated with melatonin (white triangles)). At days 2 and 7 postsurgery, IOP significantly decreased in diabetic dogs treated with NSAIDs, as compared with preoperative values. *P < 0.05, **P < 0.01 versus preoperative values, by Kruskal–Wallis test. No differences were observed among treatments at each time point postsurgery.

absorbed and reaches a maximum serum level after 20–30 min, with a distribution phase of 3.5 h and an elimination half-life ($t_{1/2}$) of 5 h.

We have previously shown that melatonin decreases inflammatory signs and prevents blood–ocular barrier disruption induced by experimental uveitis in Syrian hamsters[28] and cats,[29] while Kaur *et al.*[36] demonstrated that melatonin protects the inner blood–retinal barrier in rats. The present results support the beneficial effect of melatonin on the clinical outcome of cataract surgery in dogs. As mentioned before, the success of canine cataract surgery is mainly limited by postsurgical complications. Trauma at the surgical site induces an inflammatory response initiated by the release of membrane phospholipids and culminating in the formation of prostaglandins, leukotrienes, and other eicosanoids, as well as the recruitment of neutrophils and macrophages to the site.[37] Inflammation generally manifests as mild iritis along with corneal edema and increased cells and protein (flare) in the anterior chamber along with hyperalgesia or pain.[38] Besides the classic signs of rubor, tumor, and pain, ocular inflammation may result in additional sequelae such as PCO, culminating in suboptimal vision.[39] The use of a scoring system provides a means of achieving a quantitative measure of postoperative clinical signs and characterization of their temporal course. The clinical consequences of PE in diabetic and nondiabetic dogs were assessed at early time points (i.e., 2, 7, and 20 days) after surgery, whereas the incidence of sequelae was analyzed in the late phase (i.e., 180 days).

Canine inherited and diabetic cataracts are different from the typical senile human cataract in progression and appearance. In most affected breeds, cataracts are accepted as heritable based on the characteristic anatomic localization and appearance during the initial stages, the age of onset and progression, the absence of other causes of cataractogenesis, and bilaterality.[4] On the other hand, diabetic cataracts in dogs are rapid in progression, typically intumescent, and entirely opaque and cause more severe lens-induced uveitis.[1] Despite these differences, melatonin decreased early and late postsurgical complications in diabetic and nondiabetic dogs with a better or similar efficacy as the reference treatments (i.e., NSAIDs and corticoids, respectively). The effect of melatonin was not specific for a particular sign or sequel, as a reduction of them all was observed in melatonin-treated dogs. At early postsurgery stages, melatonin was more effective than the treatment of reference in diabetic dogs (i.e., NSAIDs), while in dogs with hereditary cataracts, it showed a similar efficacy to dexamethasone. Notwithstanding, melatonin may significantly outmatch corticoids and NSAIDs in terms of the absence of undesirable side effects, low toxicity, and high safety (for review, see Ref. [42]).

Decreased IOP is a characteristic sign of ocular inflammation in cats and dogs.[29,41] Therefore, it is important to monitor IOP during the course of postsurgical stages of dog cataract surgery, and the recovery of IOP to normal values is an important index of the surgical outcome. There were no statistically significant differences among treatments concerning IOP values along the study, with the exception of a transient ocular hypotension at 2 and 7 days postsurgery observed in diabetic dogs treated with NSAIDs, which could be associated with postoperative hypotony related to surgical ocular inflammation.

The effect of melatonin on IOP is still controversial. In our experimental setting, an oral treatment with melatonin did not affect dog IOP after cataract surgery. In contrast, it has been demonstrated that oral or topical administration of melatonin reduces IOP in monkeys,[42] rabbits,[43] and humans.[44] However, other studies did not find any effect of topical application, intravenous or intravitreal injection, and intra-arterial infusion of melatonin on rabbit IOP.[45,46] In addition, we have previously shown that the administration of melatonin as a subcutaneous pellet does not affect IOP in normal or glaucomatous rats.[26] Although we do not have any explanation for this discrepancy, differences in species, administration route, and dose, as well as the particular ocular condition (eye submitted to PE), could account for it.

Table 3. Clinical score and IOP

Dog	Breed	Systemic Disease	Eye	Tmnt	IOP mmHg 1 week before	Day 2 Clinical score/IOP mm Hg	Day 7 Clinical score/IOP mm Hg	Day 20 Clinical score /IOP mm Hg
1	Mixed	Diabetes	RE	N	19	8/16	4/15	2/15
			LE	N	18	7/14	3/14	1/14
2	Breton	Diabetes	RE	N	23	6/16	5/16	3/26
			LE	N	19	8/16	1/14	1/14
3	Siberian Husky	Diabetes	RE	N	17	8/12	4/16	0/16
			LE	N	18	5/14	3/16	2/16
4	Mixed	Diabetes	RE	N	17	10/14	6/18	2/18
			LE	N	16	7/12	2/14	1/14
5	Rottweiler	Diabetes	RE	N	19	8/16	2/16	1/16
			LE	N	21	6/25	4/25	3/25
6	Poodle	Diabetes	RE	N	17	9/12	4/12	3/12
			LE	N	19	4/13	4/14	1/14
7	Mixed	Diabetes	RE	M	19	6/14	2/18	1/18
			LE	M	16	5/12	2/17	1/16
8	Siberian Husky	Diabetes	RE	M	18	4/10	1/14	0/18
			LE	M	16	4/14	4/12	1/14
9	Dachshund	Diabetes	RE	M	17	5/10	3/18	1/18
			LE	M	18	3/16	2/17	0/18
10	Rottweiler	Diabetes	RE	M	20	6/12	2/16	1/16
			LE	M	16	6/17	2/18	0/18
11	Mixed	Diabetes	RE	M	18	6/8	4/9	1/16
			LE	M	19	3/13	1/16	0/18
12	Poodle	–	RE	D	20	4/16	1/17	1/17
			LE	D	18	7/12	3/14	2/14
13	Beagle	–	RE	D	21	3/18	3/18	2/18
			LE	D	21	6/14	4/14	2/19
14	Fox Terrier	–	LE	D	16	8/10	7/12	4/16
15	Poodle	–	RE	D	18	5/15	3/15	2/15
16	Maltese	–	RE	D	20	4/19	2/19	1/22
17	Poodle	Cardiopathy	RE	D	21	3/19	1/19	1/22
			LE	D	16	7/12	4/12	2/16
18	Poodle	–	RE	D	20	7/14	6/12	4/12
			LE	D	18	5/13	2/13	1/22
19	Mixed	–	LE	D	17	5/26	4/13	3/11
20	Golden Retriever	–	RE	M	17	4/18	2/18	1/18
			LE	M	16	5/15	2/16	1/16
21	Breton	–	LE	M	19	3/20	3/18	2/18
22	Poodle	–	RE	M	16	6/17	2/17	1/17
			LE	M	21	6/16	2/19	1/22
23	Siberian Husky	–	RE	M	16	5/15	2/16	1/16
			LE	M	19	6/12	2/14	1/29
24	Poodle	–	RE	M	18	3/17	5/15	3/15
			LE	M	21	5/16	2/16	1/20

Diabetic dogs treated with NSAIDs: 1–6 (12 eyes); diabetic dogs treated with melatonin: 7–11 (10 eyes); dogs with hereditary cataract treated with dexamethasone: 12–19 (12 eyes); and dogs with hereditary cataract treated with melatonin: 20–24 (9 eyes). Shown are clinical score and IOP for each eye included in the study at 2, 7, and 20 days postsurgery. RE = right eye; LE = left eye. Tmnt = treatment; N = NSAIDs; M = melatonin; D = dexamethasone.

It was previously reported that surgical sequelae are more frequent in diabetic than in nondiabetic dogs.[47] In agreement, when comparing diabetic with nondiabetic dogs submitted to the reference treatment for each case, a higher incidence of sequelae was observed in diabetic than in nondiabetic dogs. However, the treatment with melatonin decreased the occurrence of sequelae in both groups. It has been demonstrated that small- and medium-sized breeds developed significantly more PCO in comparison with the large/giant breeds.[48] Consistent with these results, PCO was not observed in any of the six large breed dogs (four diabetic dogs and two dogs with hereditary cataract) included in the present study.

The mechanisms involved in the protection induced by melatonin in dog eyes remain to be established. A number of studies support that melatonin and its metabolites[49] may exert its anti-inflammatory effects through the regulation of different molecular pathways, which may be

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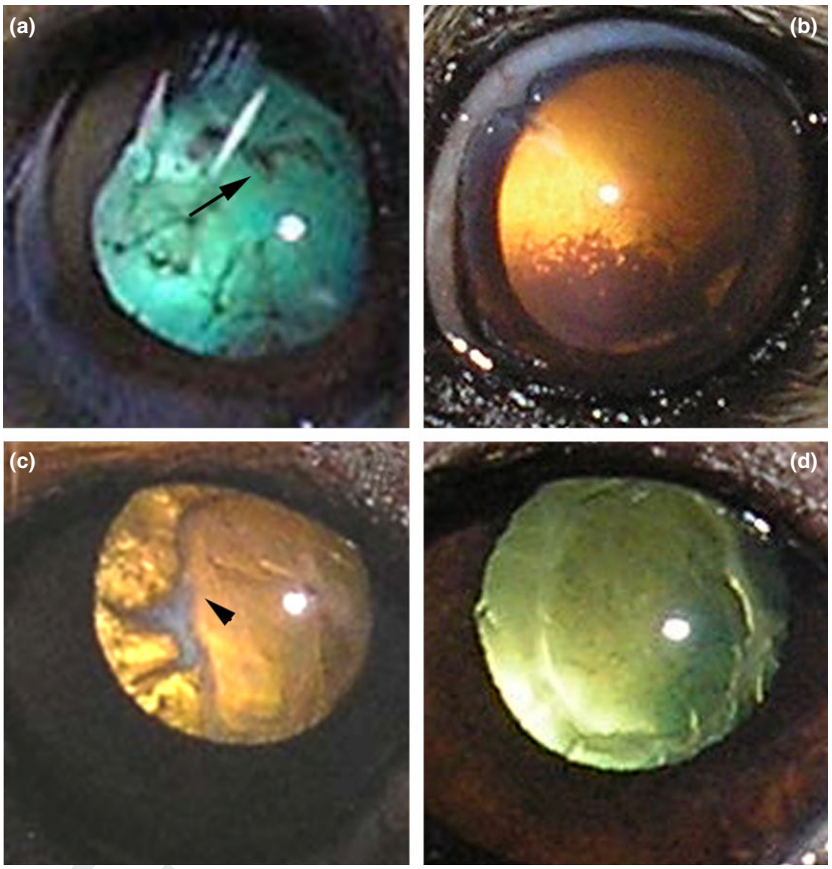

Figure 4. Representative photographs of clinical sequels observed at 180 days after surgery in a diabetic dog treated with NSAIDs (a), a diabetic dog treated with melatonin (b), a nondiabetic dog treated with dexamethasone (c), and a nondiabetic dog treated with melatonin (d). Note in A: PCO (arrow), in C: a marked rhexis capsule opacification (arrow head), and in B and D: a normal appearance.

involved in the effect of melatonin in dog eyes submitted to PE. In this sense, it has been demonstrated that melatonin inhibits the expression of cyclooxygenase-2 and of the inducible isoform nitric oxide synthase, limiting the production of excessive amounts of prostanoids, leukotrienes, and nitric oxide, as well as other mediators of the inflammatory process such as chemokines, cytokines, and adhesion molecules (for review, see Ref.[50]).

In summary, the results of this pilot study indicate that melatonin alleviated postoperative complications of PE in dogs. Although additional studies are required to determine the mechanism of action for melatonin in preventing complications in eyes treated with PE and to validate these preliminary results, melatonin may have beneficial effects. Until further studies are performed, melatonin should be used in conjunction with topical steroids or NSAIDS.

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