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Molecular, Biological, Physiological and Clinical Aspects of Melatonin

Therapeutic benefit of melatonin in experimental feline uveitis

Abstract: Uveitis is a frequent ophthalmic disorder which constitutes one of the main causes of blindness in domestic cats. The aim of this report was to analyze the effect of melatonin on experimentally induced uveitis in cats. Bacterial lipopolysaccharide (LPS) was injected intravitreally into one eye from intact cats, while the contralateral eye was injected with vehicle. Melatonin was orally administered every 24 hr to a group of ten cats, from 24 hr before until 45 days after intravitreal injections. Eyes were evaluated by means of clinical evaluation, intraocular pressure (IOP), blood–ocular barrier integrity (*via* measurement of protein concentration and cell content in samples of aqueous humor [AH]), electroretinogram (ERG), and histological examination of the retinas. In LPS-treated eyes, several clinical signs were observed until day 45 postinjection. The treatment with melatonin significantly decreased clinical signs and prevented the reduction in IOP induced by LPS. In LPS-injected eyes, melatonin significantly preserved the blood–ocular barrier integrity, as shown by a decrease in the number of infiltrating cells and protein concentration in the AH. Mean amplitudes of scotopic ERG a- and b-waves were significantly reduced in eyes injected with LPS, whereas melatonin significantly prevented the effect of LPS. At 45 days after injection, LPS induced alterations in photoreceptors and at the middle portion of the retina, whereas melatonin preserved the retinal structure. These results indicate that melatonin prevented clinical, biochemical, functional, and histological alterations induced by LPS injection. Thus, melatonin might constitute a useful tool for the treatment of feline uveitis.

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Introduction

Acute or chronic uveitis is a frequent ophthalmic disorder which constitutes one of the main causes of irreversible blindness in domestic cats. Although uveitis may be caused by corneal ulceration, penetrating wounds, blunt trauma, immune-mediated disease, lens-induced uveitis and neoplasia, common infectious systemic diseases are the most frequent causes of uveitis in cats [1]. Feline immunodeficiency virus (FIV), feline infectious peritonitis virus (FIP), feline leukemia virus (FeLV), *Toxoplasma gondii*, *Cryptococcus neoformans*, and *Histoplasma capsulatum* are the most frequent infectious agents implicated in the disease. However, *Blastomyces dermatitidis*, *Coccidioides immitis and Candida albicans* [1–4], *Bartonella henselae* [5], feline herpesvirus 1 (FHV 1) [1], *Cuterebra* spp [6], *Mycobacterium bovis*, *Mycobacterium tuberculosis and Mycobacterium avium* [7], and a *Metastrongylidae* [3] were also associated with feline uveitis.

At present, in addition to treatments for an identifiable primary cause, the symptomatic treatment for feline uveitis mainly consists in the administration of corticosteroids, directed toward reduction in pain and lesions of ocular tissues [8]. However, when used as a systemic therapy, the immunosuppressive effect of corticosteroids may contribute to the development of the systemic disease, and their chronic topical use might lead to ocular hypertension [9]. Non-steroidal anti-inflammatory drugs are also indicated for uveitis treatment in cats, but when used topically (for anterior uveitis), they may delay the corneal reparative process [10], and after a systemic use (for posterior uveitis), they may affect platelets and induce acute renal insufficiency and gastrointestinal hemorrhage or ulceration [8]. Moreover, it has been shown that only 33% of cats with systemic disease-associated uveitis respond to this treatment [11].

Uveitis has several common characteristics among species. However, many medications that are successfully used in humans or dogs for uveitis treatment are not well tolerated by cats. Therefore, the selection of appropriate drugs is limited in felines. Experimental models of feline uveitis could greatly facilitate the understanding of the cellular events involved in ocular inflammation as well as the development of new therapeutic strategies. We have recently demonstrated that an intravitreal injection of lipopolysaccharide (LPS) in cats mimics many of the clinical, biochemical, functional and histological features of feline uveitis [12].

Although uveitis is one of the main causes of eye morbidity and loss of visual functions, the complexity of

biochemical and immune mechanisms involved in its generation and development remains largely unknown. Several lines of evidence support the possibility that the disease is because of damage generated by infiltrated leukocytes which release cytokines [13, 14], and other inflammatory mediators, like arachidonic acid metabolites [15], reactive oxygen species [16, 17], and nitric oxide (NO) [18], among many others. Arachidonic acid metabolites regulate vascular permeability, chemotaxis, and contribute to uveitis amplification [19, 20]. In addition, activated phagocytes synthesize large amounts of NO through a reaction catalyzed by the inducible isoform of NO synthase (iNOS) [21, 22].

Melatonin is an endogenous neuromodulator in the retina of vertebrates [23–25]. It was demonstrated that melatonin has an immunomodulatory role, and it may provide neuroprotection in different systems [26–28]. Moreover, melatonin possesses widespread free radical scavenging and antioxidant activities in different tissues [29, 30], including the retina [31, 32]. We have shown that melatonin may directly react with NO yielding at least one stable product, N-nitrosomelatonin [33]. In addition to a direct scavenging of NO, low concentrations of melatonin significantly decrease hamster retinal NOS activity and L-arginine uptake, indicating that melatonin may be a potent inhibitor of the retinal nitridergic pathway [34]. In fact, it was demonstrated that melatonin is protective against oxidative damage in situations where NO is known to account for molecular destruction [35]. Moreover, it was demonstrated that melatonin inhibits cyclooxygenase activity [36]. Several lines of evidence support the possibility that melatonin may act as a protective agent in ocular conditions such as photokeratitis, cataract, retinopathy of prematurity, ischemia/reperfusion injury, and glaucoma [31, 32]. In this context, the aim of the present work was to analyze the therapeutic effect of melatonin in feline LPS-induced uveitis.

Materials and methods

Animals

All animal procedures were conducted in strict accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Cats were obtained from the research colony of the Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires. Twenty intact young European short-hair male cats (2.5 ± 0.5 kg) that were seronegative for *T. gondii*, FIV, and FeLV were housed individually in a temperature- and light-controlled environment (fluorescent lights were automatically turned on and off every 12 hr). Cats were fed a balanced diet, and water was available ad libitum. Cats were adapted to human contact for 4–6 wk. Prior to inclusion in the study, each cat's physical and ophthalmologic health conditions were determined based on the results of general and ocular examinations. Ocular examination included Schirmer tear test measurements (Schirmer tear test strips; Schering-Plough Animal Health Corp., Union, NJ, USA), fluorescein staining (Love Sudamericana Laboratory, Buenos Aires, Argentina), applanation tonometry (Tono-Pen XL; Mentor, Norwell, MA, USA), biomicroscopy (Slit lamp HLS 150; Heine Optotechnik, Herrsching, Germany), and direct ophthalmoscopy (Heine Beta 200; Heine Optotechnik).

Intravitreal injections

Cats were anesthetized via intramuscular administration of ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (1.5 mg/kg). By use of a disposable 1-mL syringe with a 25-gauge needle, 20 μg of LPS from *Salmonella typhimurium* (Sigma, St Louis, MO, USA) in 100 μL of sterile saline solution (0.9% NaCl) was injected intravitreally into the right eye, and the left eye was injected intravitreally with 100 μL of sterile vehicle. A drop of topical anesthetic (0.5% sterile proparacaine hydrochloride ophthalmic solution, Anestalcon; Alcon Laboratories, Buenos Aires, Argentina) was instilled before injection of vehicle or LPS. The single intravitreal injection of vehicle or LPS was given at a location 5 mm posterior to the limbus and was directed toward the optic nerve to avoid trauma to the lens. Before and after injections, the conjunctival sac was thoroughly rinsed with sterile saline solution.

Melatonin administration

A group of ten cats received 3 mg of melatonin (Eliseum Laboratory, Buenos Aires, Argentina) orally at 9:00 hr, 1 day before and every day after vehicle and LPS injection, up to 45 days postinjections.

Clinical score

During a period of 45 days after intravitreal injections, cats were evaluated for signs of ocular pain, conjunctival hyperemia, chemosis, keratic precipitates, aqueous flare, hypopyon, hyphema, miosis, iridial swelling, iris heterochromia, synechiae, and cataracts using a slit lamp, as well as for vitreal opacity, retinal detachment, chorioretinitis, and optic neuritis, by direct and indirect ophthalmoscopy. Clinical severity of the inflammatory signs was assessed at several time points after injection of vehicle or LPS and was graded on a scale from 0 to 50 (Table 1). Miosis was quantified in reference to the pupil size. Severity of other clinical signs was graded by assignment of the following scores: 0 (absent) or 1 (present), 0–2 (0 = absent, 1 = moderate, and 2 = severe), or 0–3 (0 = absent, 1 = mild, 2 = moderate, and 3 = severe). For synechiae, the criterion was based on the number of synechiae (0 = absent, 1 = one, 2 = two, and 3 = 3 or more) and aqueous flare was quantified in accordance with the scale described by Hogan [37, 38]. Despite changes in the anterior portions of the eyes, it was possible to observe alterations induced by LPS in the posterior portions because in all examined eyes, cataracts were mainly immature (not complete) and miosis was severe but not complete even 24 hr after the injection. Each eye was examined by two observers, who were unaware of the treatment applied. Clinical signs were graded, and a cumulative score was assigned to each eye by each observer.

Table 1. Score system used to grade the clinical severity of lipopolysaccharide-induced uveitis in cats

Clinical sign	Score
Pain	
Photophobia	0 or 1
Blepharospasm	0 or 1
Epiphora	0 or 1
Conjunctival hyperemia	0 or 1
Chemosis	0 or 1
Keratic precipitates	0 or 1
Corneal edema	0 or 1
Corneal vascularization	0 or 1
Tyndall effect	0–4
Hyphema	
None	0
≤ 33% of the anterior chamber	1
>33% of the anterior chamber	2
Hypopyon	
None	0
≤ 33% of the anterior chamber	1
>33% of the anterior chamber	2
Miosis	
None	0
Mild	1
Moderate	2
Severe	3
Maximal	4
Iridial swelling	0–2
Iridial hyperemia or rubeosis	0–2
Synechiae	0–3
Iris heterochromia	0–2
Cataract	0–3
Lens subluxation or luxation	0–2 or 3
Vitritis	0–3
Chorioretinitis (active or inactive)	0–3
Optic neuritis	0–3
Negative menace response	0–3
Pupillary light reflex	
Fast	0
Slow	1
Absent	3
Maximum cumulative clinical score	50

Severity of clinical signs was graded by assignment of scores of 0 (absent) or 1 (present), scores of 0–2 (0 = absent; 1 = moderate; and 2 = severe), or scores of 0–3 (0 = absent; 1 = mild; 2 = moderate; and 3 = severe). Miosis was quantified in reference to the pupil size. For synechiae, the criterion was based on the number of synechiae (0 = absent; 1 = one; 2 = two; and 3 = three or more).

The mean of the cumulative clinical scores assigned by the two observers was recorded as the clinical score for a given eye at a given time point. At each time point, the mean values from vehicle- or LPS-injected eyes in animals untreated or treated with melatonin were averaged to compute the group mean ± S.E. clinical score.

Intraocular pressure assessment

Intraocular pressure (IOP) was assessed on days 3 and 10 after intravitreal injection, as previously described [11]. Tonometric measurements were performed by a single investigator using a Tono-Pen XL applanation tonometer (Mentor®). Cats were manually restrained, and a drop of topical anesthetic (0.5% sterile proparacaine hydrochloride ophthalmic solution, Anestalcon®; Alcon Laboratories) was applied to the cornea immediately before tonometry. Five independent IOP readings (standard error [S.E.] $< 5\%$) were obtained from each eye, and IOP was determined as the mean of these readings. IOP measurements were performed at the same time each day (between 11:00 hr and noon) to correct for diurnal variations [39].

Inflammatory cells and protein concentration in aqueous humor

On days 2, 7, 14, 21, and 28 after intravitreal injections, a sample of aqueous humor (AH) was obtained from both eyes by paracentesis. For this purpose, cats were anesthetized via intramuscular administration of ketamine hydrochloride (15 mg/kg) and xylazine hydrochloride (1.5 mg/kg). Then, the conjunctival sac was thoroughly rinsed with sterile saline solution. After fixing the eye from the superotemporal bulbar conjunctivae and by use of a disposable 1-mL syringe with a 25-gauge needle, 400 μL of AH was collected slowly and with the minimal vacuum pressure needed to obtain the samples, avoiding touching the iris, lens, and corneal endothelium. The number of infiltrating cells and protein concentration were determined in each sample. For cell counting, AH samples were centrifuged at 800 g for 5 min at 4°C. Then, 360 μL of supernatant was extracted for protein concentration measurement, and the remainder was suspended in 20 μL of sodium and potassium EDTA salts (0.342 M, pH 7.2) for cell counting. Red cells were lysed in a 1:10 acetic acid dilution. Inflammatory cells were counted using a Neubauer camera and light microscopy. The number of cells per field (equivalent of 0.1 μL) was counted, and the number of cells per microliter of AH was obtained by averaging the results of four fields and referring to the sample volume before centrifugation.

Protein content was measured by the method proposed by Lowry et al. [40] with bovine serum albumin as a standard.

Electroretinography

On day 30 after the intravitreal injections, electroretinograms (ERGs) were registered as previously described, using an Akonic BIO-PC equipment (Akonic, Buenos Aires, Argentina). [12] Briefly, after 120 min of dark adaptation, cats were anesthetized as described earlier; phenylephrine hydrochloride (2.5%) and 1% tropicamide (Alcon-Mydril; Alcon Laboratories) were applied to both eyes to dilate the pupils, and the corneas were intermittently irrigated with balanced salt solution to prevent keratopathy. Each cat was placed facing the stimulus at a distance of 20 cm. A reference electrode was placed halfway between the temporal canthus and the ear, a grounding electrode was attached subcutaneously to the occipital crest, and a contact lens electrode (ERG jet electrode; LKC Technologies, Gaithersburg, MD, USA) was placed in the central cornea. Anesthesia did not rotate the globes, and eyelids were fixed using a blepharostat. A 16-W red light was used to enable accurate electrode placement. This maneuver did not significantly affect dark adaptation and was switched

off during the recordings. ERGs were recorded from both eyes simultaneously and ten responses to flash of white light (5 ms; 0.2 Hz) from a photic stimulator (light-emitting diodes) set at maximum brightness (9 cd s/m^2 without a filter) were amplified, filtered (1.5-Hz low-pass filter; 1000-Hz high-pass filter; notch activated) and averaged. The ERG a-wave amplitude was measured as the difference in amplitude between the recording at onset and the trough of the negative deflection and the b-wave amplitude was measured as the difference in amplitude between the trough of the a-wave and the peak of the b-wave. Electroretinographic responses were averaged for each run (ten tests). Runs were repeated three times at 5-min intervals to confirm consistency, and the mean of these runs was used for subsequent analysis.

Histological examination

Forty-five days after the intravitreal injections, both eyes from four cats (two cats untreated and two cats treated with melatonin) randomly selected were enucleated by use of a transconjunctival enucleation technique. Cats were anesthetized and immediately euthanized via intravenous administration of pentobarbital sodium (80 mg/kg) and diphenylhydantoin sodium (10 mg/kg) (Euthanyle, Brouwer, Buenos Aires, Argentina). The ocular globes were immediately placed in a fixative solution (4% formaldehyde in 0.1 M phosphate buffer, pH 7.4) for 4 hr. Then, globes were carefully incised with a razor blade in the central cornea making a small window to allow a better access of fixative. This maneuver did not affect any structure of the eye. After fixation for 48 hr, eyes were sectioned along the horizontal meridian through the optic nerve head, and photomicrographs were obtained 1.5 mm from the optic nerve head center. Retinal sections (5 μm thick) were stained with hematoxylin and eosin stain.

Statistical analysis

Statistical analysis of results was made by a two-way ANOVA followed by a Tukey's or Student's *t*-test, as stated.

Results

Clinical severity scores were assessed on 16 occasions (at days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 35, 40, and 45 after intravitreal injection) in vehicle- and LPS-injected eyes from cats untreated or treated with melatonin. Mean clinical scores for eyes in each group were averaged, and results are shown in Fig. 1. In all eyes injected with LPS from animals untreated with melatonin, signs of ocular inflammation developed, whereas no signs of uveitis were evident in vehicle-injected eyes. The treatment with melatonin significantly decreased the clinical score induced by LPS during the entire study period, except for day 1. Fig. 2 shows representative photographs of typical inflammatory signs that developed in cats injected with LPS in the absence or presence of melatonin. LPS-associated effects were most commonly signs of pain, conjunctival hyperemia, chemosis, hypopyon, hyphema, miosis, aqueous flare,

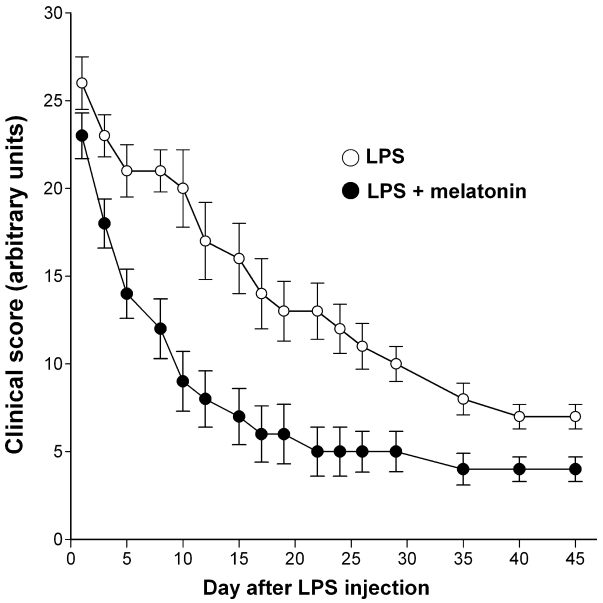

Fig. 1. Effect of melatonin on the clinical score in lipopolysaccharide (LPS)-induced uveitis. Mean ± S.E. of clinical scores assigned to each eye of 20 cats at days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26, 29, 35, 40, and 45 following a single intravitreal injection of LPS in one eye and vehicle in the contralateral eye, in the absence or presence of melatonin (n = 10 eyes/group). At all time points, the clinical score was significantly higher in the absence than in the presence of melatonin, except for day 1. $*P < 0.05$, $**P < 0.01$, by Student's *t*-test.

iridial swelling, tumefaction and rubeosis iridis, vitritis, chorioretinitis, and partial secondary blindness. The presence of melatonin significantly reduced the occurrence of clinical inflammatory signs. IOP from eyes injected with vehicle or LPS in the absence or presence of melatonin was assessed at 3 and 10 days after intravitreal injections. In the absence of melatonin, LPS induced a significant decrease in IOP as compared with vehicle-injected eyes, whereas this parameter was significantly higher in cats treated with melatonin at 10 (but not 3) days after injections (Fig. 3). In vehicle-injected eyes, melatonin did not affect IOP (data not shown).

At different time points after injections, the number of inflammatory cells and protein concentration was assessed in samples of AH from eyes injected with vehicle or LPS in the absence or presence of melatonin. LPS provoked a significant increase in AH cell count that lasted for 14 days. Afterward, this parameter did not differ with that observed in vehicle-injected eyes. At days 2, 7, and 14 after intravitreal injection of LPS, significantly fewer inflammatory cells were observed in the AH from melatonin-treated cats, as shown in Fig. 4. No cells were observed in the AH from vehicle-injected eyes of animals untreated or treated with melatonin throughout the study.

Aqueous humor protein concentration was significantly higher in LPS-injected eyes in the absence than in the presence of melatonin at day 14, 21, and 28 postinjection of LPS (Fig. 5). Protein concentration in vehicle-injected eyes did not change along the study or between animals untreated and treated with melatonin (i.e., 0.47 ± 0.05 mg/mL,

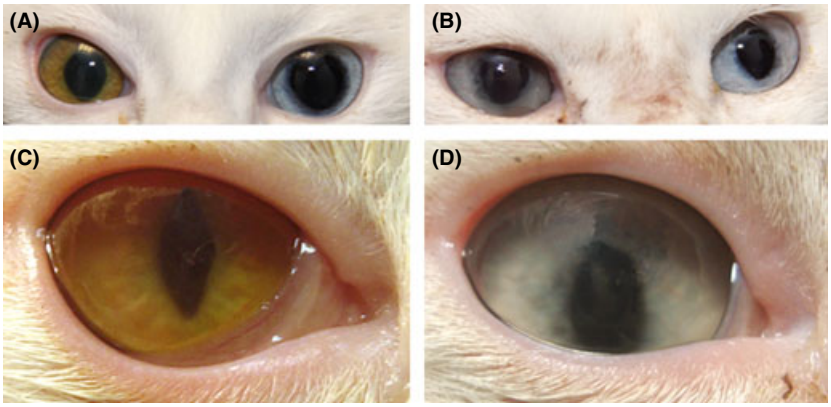

Fig. 2. Representative photographs of clinical signs observed 2 days after the injection of lipopolysaccharide (LPS) (right eye) and vehicle (left eye), in the absence (A) or in the presence (B) of melatonin. Magnification of an eye injected with LPS in the absence (C) and in the presence (D) of melatonin. (C) Note the occurrence of miosis, iridial swelling, and aqueous flare (grade 3). (D) Note a slight miosis and iridial swelling, as well as aqueous flare (grade 1). In vehicle-injected eyes, no inflammatory signs were observed in the absence or in the presence of melatonin.

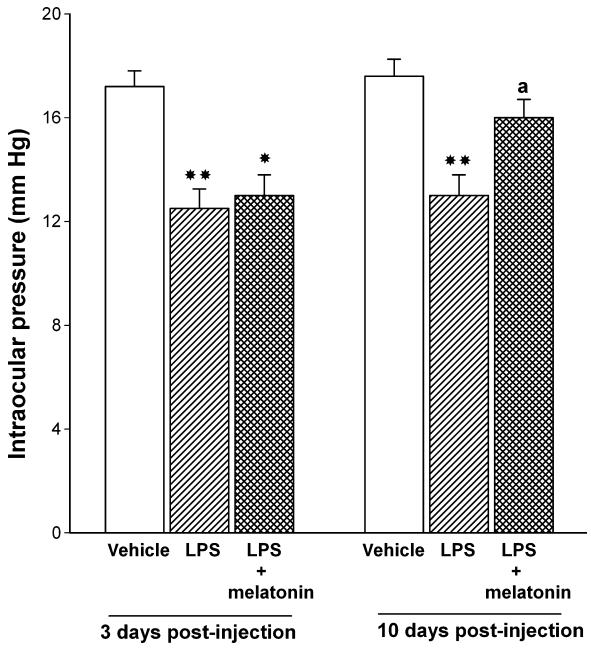

Fig. 3. Effect of lipopolysaccharide (LPS) in the absence or in the presence of melatonin on intraocular pressure, at 3 and 10 days after intravitreal injections. In the absence of melatonin, LPS induced a significant decrease in this parameter, at both time points, whereas melatonin reversed the effect of LPS at 10 days after injection. Data are mean ± S.E. (n = 6 eyes per group), $*P < 0.05$, $**P < 0.01$ versus vehicle, a: $P < 0.05$ versus LPS, by Tukey's test.

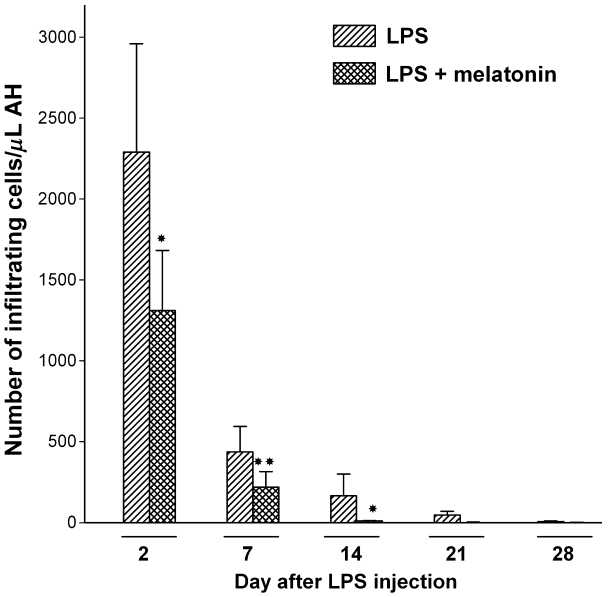

Fig. 4. Effect of lipopolysaccharide (LPS) in the absence or in the presence of melatonin on aqueous humor cell number. Cell count was significantly higher in the absence than in the presence of melatonin at days 2, 7, and 14 postinjection of LPS. Data are mean ± S.E. (n = 10 eyes per group), $*P < 0.05$, $**P < 0.01$ versus LPS-injected eyes, by Student's *t*-test.

and 0.51 ± 0.07 mg/mL, in the absence and presence of melatonin, respectively).

To analyze retinal function, scotopic ERGs were recorded before (pre-injection) and at 30 days postinjections. The average amplitudes of ERG a- and b- waves, as well as representative scotopic ERG traces from cat eyes, are shown in Fig. 6. The injection of LPS decreased scotopic ERG a- and b-wave amplitude, whereas the treatment with melatonin significantly prevented the effect of LPS on ERG a- and b-wave amplitude. No differences between pre-injection values and those obtained in vehicle-injected eyes were observed (data not shown). The ERG a- and b-wave latency did not differ among vehicle- and LPS-injected eyes from animals untreated or treated with melatonin.

Retinas from eyes submitted to different treatments were examined by light microscopy at 45 days after LPS injection (Fig. 7). No signs of inflammation were evident in the anterior segment of eyes injected with vehicle or LPS with or without melatonin (data not shown). In the retinas from eyes injected with vehicle, no signs of inflammation were evident (Fig. 7A), whereas retinal folds and loss of photoreceptors were seen in those injected with LPS (Fig. 7B). In addition, a high number of inflammatory cells (lymphocytes) mainly located in the inner retina, and disorganization of the axons from the nerve fiber layer was observed. The treatment with melatonin significantly preserved the retinal structure (Fig. 7C).

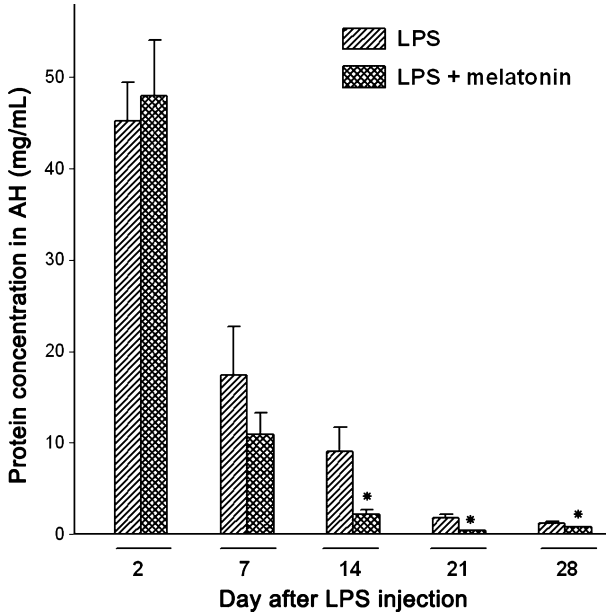

Fig. 5. Effect of lipopolysaccharide (LPS) in the absence or in the presence of melatonin on aqueous humor protein concentration. This parameter was significantly higher in the absence than in the presence of melatonin at days 14, 21, and 28 postinjection. Data are mean ± S.E. (n = 10 eyes per group), $*P < 0.05$ versus LPS-injected eyes, by Student's *t*-test.

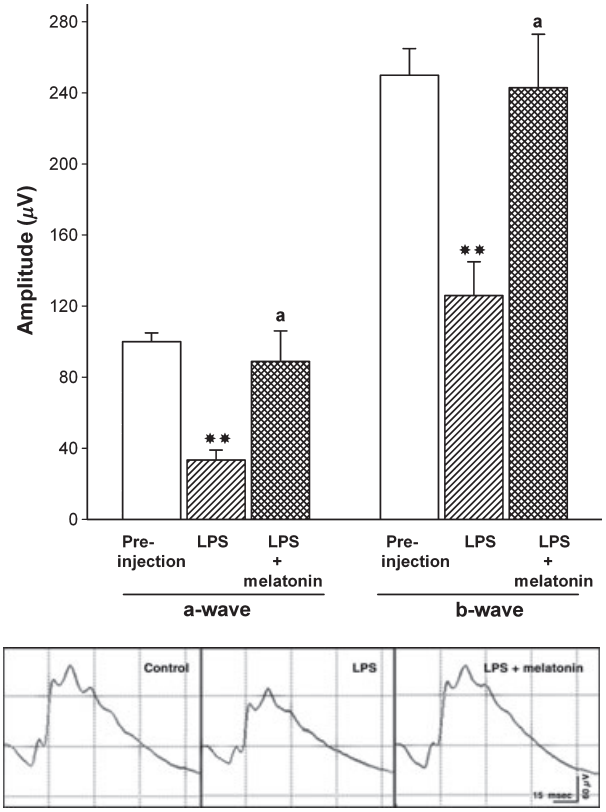

Fig. 6. Upper panel: Scotopic electroretinogram (ERG) a- and b-wave amplitude in the eyes of cats before (pre-injection) and at day 30 after an intravitreal injection of lipopolysaccharide (LPS), in the absence or in the presence of melatonin. A significant reduction in the amplitude of scotopic ERG a- and b-wave amplitude was observed in eyes injected with LPS, whereas these reductions were not seen in cats treated with melatonin. Data are mean ± S.E. (n = 10 eyes per group), $**P < 0.01$ versus control, a: $P < 0.01$ versus LPS, by Tukey's test. Lower panel: Representative scotopic ERG traces from eyes submitted to different treatments.

Discussion

Uveitis is a major cause of visual disability in cats. Although the number of cats examined in this study was relatively small, the present results indicate that the biochemical, clinical, functional, and histological alterations induced by this particular model of experimental uveitis were significantly reduced in cats daily treated with melatonin. It was previously shown that melatonin significantly reduces edematous effects of experimental uveitis in the guinea pig retina, [41] and that it prevents ocular inflammatory signs induced by LPS injection in the golden hamster [42]. The present results further support the preventive action of melatonin in experimental uveitis, by showing its beneficial effect in cat eyes.

Experimental models of uveitis were developed in several species by LPS intravenous, intraperitoneal, or footpad administration. We used an intravitreal injection of LPS, because this maneuver avoids systemic inflammation and allows the use of the contralateral eye as control, minimizing the number of animals, and decreasing their mortality. We have previously shown that a single intravitreal injection of LPS in cats mimics several features of feline

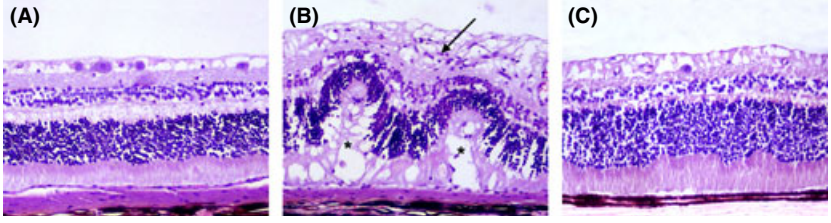

Fig. 7. Representative photomicrographs of transverse sections of retinas from one eye injected with vehicle (A), one eye injected with lipopolysaccharide (LPS) in the absence of melatonin (B) and one eye injected with LPS in the presence of melatonin (C). In the vehicle-injected eye, the retinal architecture appears normal. In the eye injected with LPS in the absence of melatonin, alterations in photoreceptors and middle portion of the retina are apparent. Note the inward foldings of the external segments of photoreceptors (asterisks) and inflammatory cell infiltration in the inner portion of the retina (arrow). The presence of melatonin preserved the retinal structure. H&E staining, 40×.

uveitis, including disruption of the blood–ocular barrier integrity, with characteristic clinical signs [12]. Endotoxin-induced uveitis is a well-validated model of uveitis in different species [15, 43–45]. However, few studies have previously addressed the temporal course of ocular alterations after injection of LPS, and largely only short-term (24 hr to 7 days) consequences were reported [46–48]. Our previous results show that at least in cats, the duration of the effect of LPS is longer than previously estimated [12]. In the presence of melatonin, a significant reduction of clinical signs induced by LPS was observed. This effect was not specific for particular signs, because a reduction of all of them was evident in the presence of melatonin. As hypotony is a cardinal sign of primary uveitis, IOP was assessed in eyes injected with vehicle or LPS in the absence or presence of melatonin. As previously shown, LPS induced a significant decrease in IOP [12], whereas melatonin reversed the effect of LPS at 10 days after the injection.

As previously described, the intravitreal injection of LPS in cats provoked a significant increase in cell number and protein concentration in AH, indicating a disruption of the blood–ocular barrier integrity [12]. The LPS-induced increase in AH cell number reached a maximal value at day 2 after LPS injection. Afterward, the number of cells in LPS-injected eyes progressively decreased, reaching baseline values at 21 days postinjection. A longer-lasting effect of LPS was observed on AH protein concentration. In this case, a significant effect of LPS persisted until day 28 postinjection. The fact that the concentration of protein in AH samples remained increased for a longer period than cell number is compatible with a partial repair of the disrupted blood–ocular barrier during last phases of the inflammatory process. Melatonin, which showed no effect on AH cell number in vehicle-injected eyes, induced a significant decrease in AH cell number at days 2, 7, and 14, and reduced protein concentration at days 14, 21 and 28 postinjection of LPS, indicating that melatonin may partially protect the blood–ocular barrier at early and late stages of the inflammatory process. Similarly, it was shown that melatonin attenuates the postischemic increase in blood–brain barrier permeability following ischemic stroke in mice [49], and reduces cerebral edema and decreases blood–brain barrier permeability in rats [50]. In addition, it was demonstrated that melatonin protects endothelial barrier and preserves microvascular blood perfusion after ischemia/reperfusion in the hamster cheek pouch [51]. Furthermore, the present findings are compatible with the observations by Kaur et al. [52] who demonstrate that melatonin protects the inner blood–retinal barrier in the rat hypoxic retina and from those by Sande et al. [42] which indicate that melatonin preserves the ultrastructure of blood–ocular barriers in LPS-injected hamster eyes.

Electroretinography has been successfully used to non-invasively and objectively measure retinal function following a wide range of retinal insults (e.g. toxic agents, infection, vasculopathy, and photic lesions) in several species [51–56]. To assess the effect of LPS on retinal function, scotopic flash ERGs were registered. A significant reduction in ERG a- and b-wave amplitude was observed in LPS-treated eyes, which was diminished with melatonin treatment. Several lines of evidence indicate that there is a close relationship between the degree of ocular inflammation and the depression of the ERG [54]. The a-wave of the flash ERG is classically thought to represent photoreceptor activity, whereas the b-wave reflects bipolar and Müller cell functions. Although the assessments of retinal function and histological characteristics were not performed at the same interval after injection (i.e. 30 and 45 days after the injection of vehicle and LPS, respectively), the results also support a correlation between retinal morphology and the electroretinographic activity. Inward foldings of photoreceptor external segments and infiltrating cells were observed in the inner region of retinas from eyes injected with LPS in the absence of melatonin, indicating damage to the outer, middle, and inner regions of the retina. As melatonin preserved the ERG a- and b-wave amplitude and the retinal structure, these results suggest that the protection induced by melatonin could be a panretinal phenomenon.

There remain to be established the mechanism/s involved in the protection induced by melatonin. In that sense, it has been previously shown that reducing NO levels [57, 58], manipulating intracellular redox status with antioxidants [59], and inhibiting prostaglandin synthesis [58] can reduce experimental uveitis. There is a very large body of evidence documenting melatonin as an antioxidant [31, 60–62]. In addition, we have shown that melatonin is a potent inhibitor of the retinal nitridergic pathway [34], and it has anti-inflammatory effects in the golden hamster eye [42]. These results suggest that melatonin could be a useful resource in the management of uveitis, because it exhibits antioxidant and antinitridergic properties, and may decrease the levels of prostaglandins. Feline uveitis, a disease with potentially blinding sequelae (synechiae, cataracts, secondary glaucoma, loss of vision), remains a challenging field to Veterinary Ophthalmology, as the disease causes significant morbidity and the use of traditional forms of treatment is restricted by limited effectiveness and potential side effects. Therefore, the establishment of alternative anti-inflammatory approaches is desirable to decrease the rate and degree of these complications. The present results suggest that melatonin could be a resource in the management of feline uveitis. Alone or combined with corticosteroid therapy, the anti-inflammatory effects melatonin may decrease the rate and degree of corticosteroid-induced complications. Although in the present study only the preventive effect of melatonin in one model of feline experimental uveitis was examined, these results support that even as preventive strategy, melatonin might become a future consideration for uveitis treatment in cats.

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Author contributions

María Jose Del Sole: intravitreal injections, clinical assessments, IOP measurements, electroretinography, assessment of proteins and cells in the aqueous humor, data analysis. Pablo H. Sande: clinical assessments, IOP measurements, electroretinography. Diego C. Fernandez: histological analysis. María Inés Keller Sarmiento: assessment of proteins and cells in the aqueous humor, data analysis. Marcelo A. Aba: contributions to concept/design, critical revision of the manuscript. Ruth E. Rosenstein: contributions to concept/design, data interpretation, drafting of the manuscript.

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