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Current Neuropharmacology, **2017**, *15*, **000-000**

REVIEW ARTICLE

Melatonin as a Therapeutic Resource for Inflammatory Visual Diseases

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ARTICLE HISTORY

Received: September 29, 2016
Revised: December 15, 2016
Accepted: January 06, 2017

DOI: 10.2174/1570159X15666170113122120

Abstract: ***Background*:** Uveitis and optic neuritis are prevalent ocular inflammatory diseases, and highly damaging ocular conditions. Both diseases are currently treated with corticosteroids, but they do not have adequate efficacy and are often associated with severe side effects. Thus, uveitis and optic neuritis remain a challenging field to ophthalmologists and a significant public health concern.

***Objective*:** This review summarizes findings showing the benefits of a treatment with melatonin in experimental models of these inflammatory ocular diseases.

***Results*:** Oxidative and nitrosative damage, tumor necrosis factor, and prostaglandin production have been involved in the pathogeny of uveitis and optic neuritis. Melatonin is an efficient antioxidant and antinitridergic, and has the ability to reduce prostaglandin and tumor necrosis factor levels both in the retina and optic nerve. Moreover, melatonin not only prevents functional and structural consequences of experimental uveitis and optic neuritis, but it is also capable of suppressing the actively ongoing ocular inflammatory response.

***Conclusions*:** Since melatonin protects ocular tissues against inflammation, it could be a potentially useful anti-inflammatory therapy in ophthalmology.

Keywords: Melatonin, uveitis, optic neuritis, ocular inflammation, oxidative damage, nitrosative damage.

MELATONIN IN THE EYE

Melatonin (5-methoxy-N-acetyltryptamine) was first isolated by Lerner *et al*. in the late 1950s [1]. Melatonin, a ubiquitous molecule that has been localized in plants and animals [2-4], is a key regulator of the circadian physiology, and also participates in the regulation of very diverse physiological processes, such as sleep, immune and vascular response, and reproduction, among many others [4-7].

Besides the pineal gland, the synthesis of melatonin also occurs in different ocular tissues, such as the retina [8], the ciliary body [9], and the lachrymal gland [4, 10]. The presence of melatonin has been detected in the retina of a high variety of vertebrates, from fish to mammals [4, 7, 11-13]. Even after pinealectomy, high levels of melatonin persist in the retina [4, 8], where its biosynthesis occurs through the same pathway than in the pineal gland [4, 14], *i.e*., tryptophan is converted into serotonin, which is subsequently transformed into N-acetyl serotonin in the presence of the enzyme arylalkylamine N-acetyltransferase (AA-NAT), and finally, N-acetyl serotonin is converted into melatonin in a reaction catalyzed by the enzyme hydroxyindole-O-methyltransferase (HIOMT) [4]. The earliest findings showing the biosynthetic pathway of melatonin in the rat retina were the conversion of [^{3}H]-serotonin into [^{3}H]-melatonin and the identification of HIOMT activity [4, 15, 16]. The localization of HIOMT was also demonstrated in the chick retina [17, 18]. Retinal AA-NAT which shows a circadian rhythm in chickens, rats, and monkeys with maximal levels at night, was also localized in the human eye [4, 19-22]. In the hamster retina, the photic stimulus regulates melatonin synthesis, as shown by the fact that light exposure during the night decreases melatonin levels, whereas exposure to darkness during the day significantly increases melatonin content [4, 23]. Isolated photoreceptors from *Xenopus laevis* retina rhythmically secrete melatonin, suggesting that melatonin biosynthesis is regulated by an endogenous circadian clock localized in these cells [4, 24], hypothesis that has been verified in the hamster and mouse retina [25, 26]. In the rodless mouse, regardless of a complete loss of photoreceptors, the synthesis of melatonin is not abolished; however, its circadian expression disappears [26-28], which supports a key role of rods in the rhythmicity of melatonin synthesis [4]. On the other hand, it has been shown that chick retinal ganglion

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1570-159X/17 $58.00+.00

cells (RGC), in isolated conditions are also able to synthesize melatonin with a rhythmic profile [7, 29]. Driven by a circadian clock, the photic information, or their interaction, there is an increase in the content of melatonin following darkness onset, whereas the exposure to light decreases melatonin levels in the golden hamster and rat retina [4, 7, 23, 30]. Retinal melatonin behaves as a local paracrine neuromodulator [4, 7, 31], and contributes to the regulation of several local processes [4], such as retinomotor movements [32], and dopamine release from amacrine cells [33], among others [4]. Dopamine and melatonin are key messengers, mutually inhibitory in retinal rhythmicity regulation, acting as day and night signals, respectively [4, 7]. Melatonin decreases the release of dopamine in the rabbit retina [33], whereas dopamine significantly decreases melatonin levels in the golden hamster retina [7, 34]. In humans, melatonin levels correlate with the electroretinographic response, which could suggest an association between the melatonin and electroretinogram (ERG) cycling [7, 35]. In addition, melatonin decreases retinal cAMP accumulation [4, 7, 23], and regulates the activity of the glutamate/glutamine cycle in the golden hamster retina [4, 36], and the rat retinal GABAergic system [4, 37].

MELATONIN IN THE RETINA REDUCES FREE OXYGEN AND NITROGEN RADICALS

The effect of melatonin on reactive oxygen (ROS) and reactive nitrogen species (RNS) can be mediated both by high affinity membrane receptors and by a direct donation of electrons [38-39]. Ianas *et al*. were pioneers in demonstrating melatonin capacity to detoxify ROS [40]. Afterwards, the ability of melatonin to directly scavenge the reactive hydroxyl radical was demonstrated [41, 42]. Since then, melatonin efficiency as a free radical scavenger and antioxidant was confirmed by many reports [4, 43-46]. Besides melatonin, several of its metabolites generated as a product of free radical scavenging in the kynurenic pathway also behave as antioxidants [4, 28, 47-50], strongly increasing the efficacy of melatonin as an antioxidant. In addition to its effect as a direct free radical scavenger, melatonin may protect against oxidative stress by stimulating antioxidant enzymes, improving mitochondrial oxidative phosphorylation efficiency, and by increasing the effectiveness of other antioxidants [4, 50-53].

The retina is highly sensitive to oxidative damage because of its direct exposure to light, its high oxygen consumption, and content of polyunsaturated fatty acids [4]. In this line, it was shown that in rod outer segment membranes, melatonin inhibits peroxidation of polyunsaturated fatty acids [4, 54], prevents oxidative stress after retinal ischemia/reperfusion [55], and glaucomatous damage [37]. Furthermore, picomolar and low nanomolar concentrations of melatonin are more potent than vitamin E in inhibiting light-induced oxidative injury in frog photoreceptors [56]. Melatonin has a protective effect on retinal pigment epithelium (RPE) cells against H_2O_2-induced damage, across a wide range of concentrations (100 pM- 0.1 mM), acting through the activation of melatonin membrane receptors at low concentrations, and through both direct antioxidant and indirect receptor activation effects at high concentrations [57]. Melatonin increases reduced retinal glutathione levels and superoxide dismutase activity, and decreases lipid peroxidation [4, 37], strongly supporting the effectiveness of melatonin as a retinal antioxidant [4]. Recent studies using melatonin receptors knock-out mice have shown that melatonin signaling *via* melatonin receptor type 1 (MT1) and type 2 (MT2) can positively modulate photoreceptors and RGC viability [58-60], supporting that the retinal protective effect of melatonin can involve receptor-mediated and non-receptor-mediated mechanisms (Fig. **1**). By X-ray spectroscopy and NMR, we have characterized a product of the direct reaction between melatonin and nitric oxide (NO), N-nitrosomelatonin [4, 61]. In addition, melatonin significantly decreases retinal nitric oxide synthase (NOS) activity [62], in agreement with its effect on NOS activity from other neural tissues [63-65]. However, while the effective concentration on NOS activity in crude homogenates from striatum, cerebellum, or hypothalamus is 1 nM, in the retina, melatonin decreases the synthesis of NO at pM concentrations, and only when the whole tissue is preincubated in its presence. These discrepancies could indicate that in the retina, unlike the other central structures, melatonin which is locally synthesized, regulates NO biosynthesis acting through a receptor-mediated pathway, but not through its direct interaction with calmodulin, and could be effective only in cell types that express both NOS and melatonin receptors [4].

Another key step in NO biosynthesis is the availability of L-arginine, which depends on its uptake through a specific transporter [62]. In this line, we have shown that in the hamster retina, low concentrations of melatonin decrease L-arginine uptake [4, 62], as well as the positive effect of L-arginine on cGMP accumulation [62], supporting that melatonin is an effective anti-nitridergic compound at retinal level. Moreover, it has been shown that melatonin significantly reduces the effect of NO on the thickness and nuclei count of inner retinal layer, RGC apoptosis, and lipid peroxidation in rats [4, 66, 67]. Melatonin is also able to scavenge peroxynitrite, a deleterious pro-oxidant formed through the reaction of NO and superoxide [68]. Furthermore, melatonin protects from oxidative stress in situations in which NO is deleterious [69].

UVEITIS

The term uveitis describes different forms of ocular inflammation affecting the uveal tract (iris, ciliary body, and choroid), and adjacent ocular structures (retina, vitreous, and optic nerve) [4]. Being the fourth most frequent cause of blindness in the active population from developed countries, uveitis is a major cause of severe visual diminishment [70-72]. Uveitis comprises a wide range of clinical presentations, with different patterns, extent, etiologies, and tissue involved. Panuveitis, which is associated with the worst clinical prognosis, is a generalized inflammation affecting not only the whole uveal tract, but also the vitreous humor and the retina [4, 72]. Uveal inflammation can appear as an acute disease, with a sudden onset, as a chronic long lasting inflammatory process, or a recurrent relapsing ailment. The most important uveitic complications are cataract, vitreous opacities, cystoid macular edema, secondary glaucoma, band keratopathy, dragged disk vessels, retinal detachment,

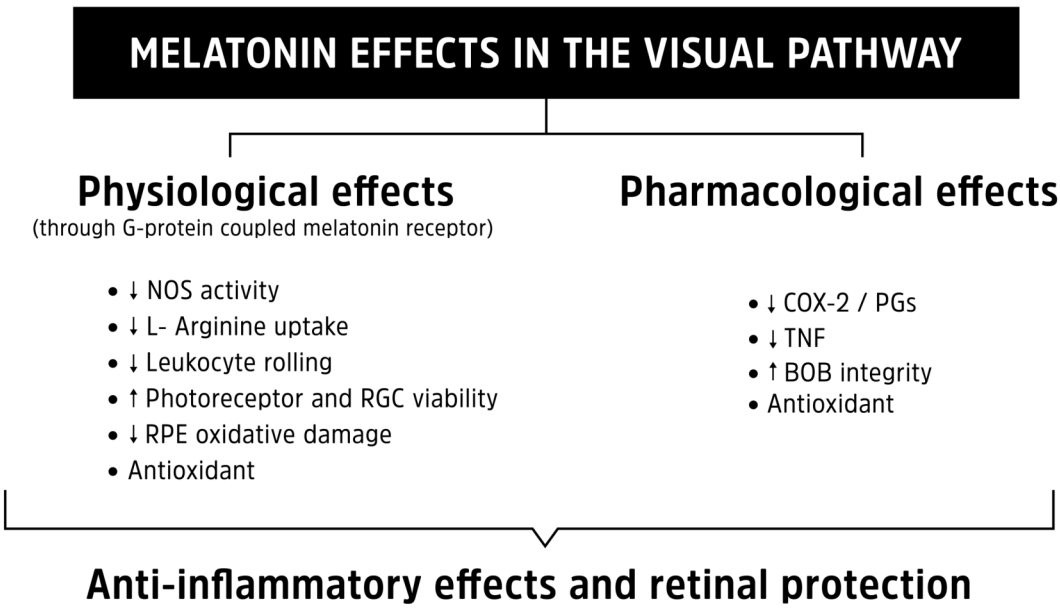

Fig. (1). Summary of receptor- and non-receptor-mediated effects of melatonin in the context of retinal and optic nerve protection against inflammation. Pharmacological effects refer to those effects that were elicited by pharmacological concentrations of melatonin, and it is still unknown whether physiological concentrations of melatonin could trigger these effects at retinal and optic nerve level, while physiological effects of melatonin refer to those effects that are mediated through melatonin receptors.

retinoschisis, and "retinitis pigmentosa-like" changes [4, 72]. Classical consequences of uveitis include eye hyperemia and pain, photophobia, blurred vision, floaters, and low visual acuity. The etiology of uveitis is wide, including general affections with secondary eye involvement, to primary ophthalmic disorders, such as ocular infections. However, in many cases, the specific cause is not recognized, thus uveitis is considered idiopathic [4, 72, 73]. In order to diagnose uveitis, a complete ophthalmologic examination is carried on, including visual acuity test, slit-lamp examination, intraocular pressure (IOP) measurement, and indirect fundus ophthalmoscopy [4, 72]. In addition, a complete medical history and laboratory tests may be of great value so that possible coexisting systemic diseases could be identified. Decreased vision is often due to posterior segment inflammation but not exclusively, as opacities in the vitreous, or even macular or optic nerve inflammation may result in a similar uveitic outcome. Patients perceive "floaters", clinically detected either by slit-lamp examination or fundoscopy, when inflammatory single or groups of cells aggregate in the vitreous cavity [4, 72]. The normally transparent retinal ophthalmoscopic aspect appears cloudy or whitish when inflamed. Choroid exclusive inflammation fundoscopically appears yellowish, whitish or as greyish well-defined patches. Histological findings include lymphocytes converging into the inflamed retinal vessels. Increased permeability of macular or optic nerve vessels, leads to edema and vision reduction. Macular cysts as a consequence of persistent macular edema, and optic atrophy, as a consequence of chronic optic nerve inflammation, both result in permanent visual incapacity [4, 73]. A uveitis hallmark is blood-ocular barrier (BOB) breakdown, causing leakage of proteins and infiltration of leukocytes into the aqueous humor, thus activating local and recruiting inflammatory cells [4].

No matter uveitis is one of the main causes of eye morbidity and visual disability, the complexity of the biochemical and immunological mechanisms implicated in its genesis and development is still elusive. It is unlikely that the most critical mechanisms are identified in studies limited to the clinical observation of ocular outcomes in human patients with uveitis. Consequently, the use of animal models that ensure far more detailed and invasive studies is of great interest [74]. In this sense, several animal models were developed, ranging from interleukin (IL)-1 induced uveitis, and experimental autoimmune uveitis (EAU), to endotoxin induced uveitis (EIU), among others, which add to the understanding of uveitis pathogenesis and new therapies testing [4, 75, 76]. EIU is induced by the administration of bacterial lipopolysaccharide (LPS), a component of Gram-negative bacterial outer membranes. Several lines of evidence support that uveitic damage is due to cytokines release by infiltrated leukocytes [4, 76-78] and other inflammatory mediators, like arachidonic acid (AA) metabolites (such as prostaglandin E2), ROS, and NO [79-82], among many others. ROS have been related to the inflammatory cascade linked to EIU in laboratory animals [4, 76, 83]. Retinal lipid peroxidation, started by free radicals, results in the formation of hydroperoxides that serve as amplification factors, inducing inflammatory cell chemotaxis [84]. An early event in uveitis development appears to be oxidative stress in photoreceptor mitochondria, which triggers phagocytic cells draw into this initial site of injury and the subsequent irreversible retinal damage [85]. In fact, evidence shows the anti-inflammatory activity of antioxidants and free radical scavengers which achieve eye protection against inflammatory mediated tissue damage [4, 86].

The activity of two cyclooxygenase (COX) isoforms is involved in the biosynthesis of prostaglandins (PGs). COX-1,

which is constitutively expressed in most tissues, catalyzes the synthesis of relatively small amounts of PGs which participate in the mediation and modulation of normal physiological functions. In an inflammatory scenario, cytokines, growth factors, and LPS rapidly induce COX-2, which results in a large amount of PGs [4, 87]. Small doses of PGs administered through a topic or intraocular way provoke some of the signs clinically seen after ocular inflammation, such as hyperemia, miosis, breakdown of the blood-aqueous barrier and increased IOP [87]. In addition, AA metabolites regulate vascular permeability, chemotaxis, thus contributing to uveitis amplification [4, 87, 88].

Studies from experimental models and human patients with uveitis relate oxygen/nitrogen reactive species in the inflammatory cascade. Multiple intraperitoneal injections of NG-nitro-l-arginine methyl ester, a well-known inhibitor of NOS, achieve a decrease in uveitic clinical signs in EIU according to Parks *et al*. [89], which points NO as an actor in experimental uveitis pathogenesis [4]. Furthermore, the increased production of NO has been implicated, at least in part, in EIU associated changes in vascular permeability and hemodynamics in rats. In addition, it has been demonstrated that at the peak of ocular inflammation following LPS injection, high levels of nitrite can be detected in the aqueous humor and vitreous of rats, as well as anterior segment iNOS activity [4, 90, 91]. *In situ* hybridization revealed that the main source of NO are epithelial cells of the iris-ciliary body and anterior segment, and retina infiltrating cells, such as macrophages and polymorphonuclear leukocytes [4, 92]. The efficacy of treating EIU with NOS inhibitors suggests that treatments reducing NO production or action could be of benefit and would provide new promises for human uveitis therapeutics [4].

CURRENT THERAPY FOR UVEITIS

Corticosteroids have been used to treat uveitis since 1950, and are widely known for their robust anti-inflammatory effects [4, 93]. The mechanisms involved are complicated and only partly comprehended, including the direct binding between the glucocorticoid/receptor complex and genes involved in the inflammatory response, indirect activation of transcription factors as nuclear factor-kappa B (NFκB), inhibitory effects on a wide range of molecules associated to inflammation as chemokines, cytokines, AA metabolites, and adhesion molecules, and several anti-inflammatory mediators upregulation. Much as this wide-ranging pharmacodynamics manages an excellent anti-inflammatory effect, it also leads to significant undesired side effects [94]. Ophthalmic side effects (increased when topically or locally administered) comprise an increase in IOP and a more accelerated development of cataracts [95, 96]. Systemic side effects (more frequent when systemically administered) involve arterial hypertension, diabetes, Cushing's syndrome, osteoporosis, and disorders of sleep, mood, and appetite [97]. Other immunomodulatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) are used in uveitis therapy [98]. NSAIDs block the synthesis of PGs [99], suppress polymorphonuclear cell locomotion and chemotaxis [100], decrease expression of inflammatory cytokines [101], and may act as free radical scavengers [102]. In addition, several other drugs have been used for uveitis treatment, such as cyclosporine, antimetabolites (*e.g*., methotrexate), alkylating agents (*e.g*., cyclophosphamide), interferons, and anti-tumor necrosis factor (TNF) drugs (*e.g*., adalimumab), among many others [103]. These drugs that are often effective have also potential serious side effects and some of them may compromise protective immunity against pathogens. Therefore, there is a clear need for developing more effective, specific, and non-toxic strategies for uveitis treatment.

MELATONIN FOR UVEITIS TREATMENT

The effect of melatonin on experimental uveitis has been reported in two studies [104, 105]. In one of these studies, uveitis was induced in the guinea pig by injecting bovine serum albumin intravitreally, and melatonin, vitamin E, or aprotinin were administered i.p. after 3 days of intravitreal injection [104]. The authors show that melatonin, vitamin E, or aprotinin treated animals had similar leptin levels than control animals, and histopathological alterations and edema were decreased [28, 104]. In the other study, a single intravitreal injection of LPS was used to induce panuveitis in the golden hamster [105], and 2 h before LPS injection a pellet of melatonin was implanted subcutaneously. Several parameters such as BOB integrity, clinical signs, histopathologic outcomes, and retinal function were analyzed at day 1 and 8 post-injection. Melatonin decreases the effect of LPS on protein and cell leakage in the anterior segment, the occurrence of clinical signs, and protects the BOB ultrastructure [28]. Moreover, in animals injected with LPS a clear disruption of rod outer segment membranous disks is observed, whereas melatonin induces photoreceptor outer segment morphological preservation (Fig. **2**). Furthermore, melatonin prevents the visual dysfunction (ERG) in uveitic eyes [28]. The effect of melatonin seems to be mediated by the prevention of the increase in retinal NOS activity, TNF, and NFκB p50 and p65 subunits nuclear levels induced by LPS injection [28, 105]. As already mentioned, a uveitis hallmark is leukocyte infiltration into the aqueous humor [4]. In this line, a great number of evidences support that melatonin, acting through MT2 melatonin receptors, impairs the rolling and adhesion of leukocytes to the site of lesion [106, 107], suggesting the involvement of melatonin receptors in the anti-inflammatory properties of melatonin (Fig. **1**). Taken together, these results indicate that melatonin prevents the alterations induced by experimental uveitis in hamsters at clinical, biochemical, histological, ultrastructural, and functional level [28].

We have also examined the therapeutic effect of melatonin in experimental feline uveitis [108]. In this case, melatonin was orally administered once a day, from 24 h before until 45 days after the intravitreal injection of vehicle or LPS [108]. Also in this case, melatonin significantly decreases clinical signs and prevents the reduction in IOP and in the scotopic ERG a- and b-waves, and preserves BOB integrity in uveitic eyes [108]. At 45 days post-injection, the alterations at the middle portion of the retina and photoreceptors induced by LPS are prevented by melatonin [108]. These results support that melatonin might be useful for the treatment of feline uveitis. Later on, we studied the effect of melatonin administered after the onset of ocular

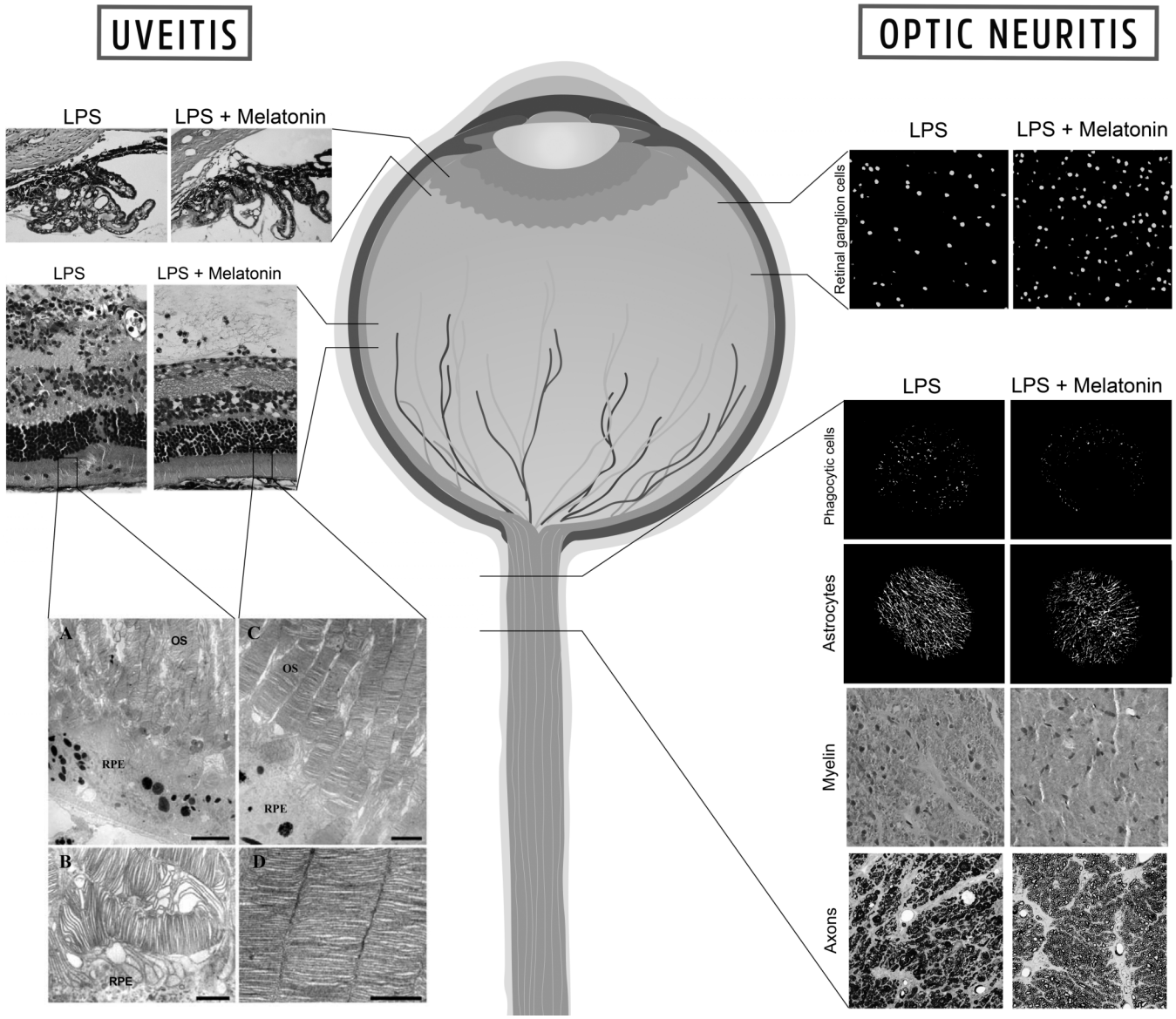

Fig. (2). Left panel: Effect of melatonin on experimental uveitis in golden hamster. Anterior chamber angle and retina sections (hematoxylin and eosin), obtained 24 h after vehicle or LPS injection in the absence or presence of melatonin. Note the intense inflammatory cell infiltration in anterior chamber structures after LPS injection, which was decreased in animals treated with melatonin. LPS injection provoked altered thickness of retinal layers, focal hemorrhages, and cellular infiltration, whereas an apparently normal morphology was observed in retinas from eyes injected with LPS in the presence of melatonin. At 8 days post-injection, LPS provoked a high disorganization of photoreceptor outer segments (A, B), whereas an ultrastructural preservation of photoreceptor outer segments was observed in the presence of melatonin (C, D). Right panel: Effect of melatonin on experimental ON in rats. At 21 days post-injection into the optic nerve, LPS induces phagocytic microglial activation (ED1-immunoreactivity), reactive gliosis (glial fibrillary acidic protein -immunoreactivity), demyelination (luxol fast blue staining), and axon (toluidine blue staining) and RGC (Brn3a-immunoreactivity) loss. Melatonin prevents all these optic nerve alterations.

inflammation in the golden hamster [74]. In this case, hamster eyes were intravitreally injected with LPS, and 10 mg/kg melatonin was i.p. administered once a day, starting 12 h or 24 h post-LPS injection. Both post-treatments with melatonin are similarly effective in reducing clinical symptoms, and cell and protein leakage. Melatonin decreases PGE2 and PGF2α levels in aqueous humor, and avoids the retinal dysfunction, protects the retinal structure, and reduces the increase in NOS activity, lipid peroxidation, and TNF levels induced by LPS [74]. Therefore, since melatonin is able of suppressing the ongoing inflammatory responses, it is tempting to speculate that melatonin could be effective in patients with fully established uveitis [74].

OPTIC NEURITIS

Optic neuritis (ON) is a neuropathy which affects mostly young adults (from 18 to 45 years of age) and children as

young as 4 years, and provokes primary inflammation, demyelination, and axonal injury in the optic nerve, that leads to RGC and vision loss [109-111]. Its annual incidence is ~ 5 in 100,000, and its estimated prevalence is 115 in 100,000 [112]. The pathognomonic clinical signs are: peri- or retro-ocular pain accentuated by eye movement, alterations in visual acuity, visual field, visual evoked potentials (VEPs), afferent pupillary response, and a reduction in color vision [109, 110, 113]. Edema of the optic nerve head (papillitis) may occur. [109, 110, 113]. Although the visual dysfunction may worsen in 1 or 2 weeks and usually begins improving along several weeks, vision loss could remain permanent in ~ 40% of the patients [109, 110, 112]. In fact, most patients show some persistent visual dysfunctions, such as alterations in visual acuity, pupillary response, color vision, contrast sensitivity, visual field, stereopsis, and VEPs [110, 114]. It has been suggested that vision loss induced by ON is ultimately caused by RGC loss [110, 115, 116].

ON may be associated to several infectious or autoimmune diseases [109, 110, 117], such as sarcoidosis, systemic lupus erythematosus, inflammatory bowel disease, and HIV [118-121], among others [110, 122, 123]. ON is closely linked to multiple sclerosis (MS). Indeed, in 25% of cases, ON is the initial symptom of MS and may occur during the disease in ~70%, particularly in the relapsing-remitting phase [109, 110]. Moreover, acute ON may occur as an isolated clinical event without a systemic contribution, and it is retrospectively diagnosed as primary ON [109, 110, 123]. The classically used model for ON studies is experimental autoimmune encephalomyelitis (EAE), a validated model of MS in humans, triggered by an immune-mediated demyelination mechanism, induced by immunization with different myelin antigens (*e.g.*, myelin basic protein, proteolipid protein, and myelin oligodendrocyte glycoprotein) [110, 124, 125], and that provokes optic nerve demyelination [109, 110]. However, besides optic nerve alterations, EAE is always associated with spinal cord and brain inflammation and demyelination. Therefore, EAE does not mimic the human primary form of ON. Additionally, the optic nerve damage is relatively unpredictable in EAE, with varying severities of ON [110, 126]. Other models were induced by the injection of lysolecithin, an oxidized low-density lipoproteins component, with detergent properties on myelin and myelinating cells. Although the injection of lysolecithin provokes local demyelination of the optic nerve from rats and primates [110, 126, 127], this model lacks the primary inflammatory component which characterizes human ON. The local injection of LPS induces inflammation in most tissues, affecting microglia and astrocytes in the central nervous system [110, 128-130]. Moreover, the local inflammatory response caused by the injection of LPS directly into the rat dorsal funiculus provokes demyelination [110, 131]. A new experimental model of primary ON through the microinjection of LPS into the rat optic nerve was developed [110]. LPS induces a significant reduction in VEPs and pupillary reflex (PLR), without affecting the ERG [110], as well as a decrease in anterograde transport of the cholera toxin β-subunit from the retina to the superior colliculus, and an early inflammatory response (increased cellularity, and Iba-1 and ED1-immunoreactivity), followed by astrocytosis, demyelination, and axon and RGC loss [110]. These results support that the injection of LPS into the optic nerve may constitute a new model of primary ON [110].

CURRENT THERAPY FOR OPTIC NEURITIS

Corticosteroids are the current therapy for the treatment of ON. Results of the North American Optic Neuritis Treatment Trial (ONTT), show no improvement in visual acuity at 6 months after 3 days of 1 g per day intravenous methylprednisolone followed by 11 days of low-dose oral prednisolone versus placebo, although visual recovery was faster, and mild benefits were noted for some secondary outcomes (visual fields, contrast sensitivity and color vision) [132, 133]. Patients taking standard-dose (1 mg per kg) oral prednisolone did not differ from those taking placebo in visual outcomes, but there was an unexpected increased risk of ON recurrence for reasons that are still unclear. Intravenous methylprednisolone also delays the onset of clinically definite MS at 2 years [134], but this difference decreases over time [135]. In a Cochrane review, there was no evidence for long-term benefit for corticosteroid treatment in terms of visual acuity, contrast sensitivity or visual field test [136]. In a rat model of MS, corticosteroids given before induction of experimental allergic encephalomyelitis reduce the incidence of ON and preserve RGCs [137], but in another experimental model, methylprednisolone even increases RGC degeneration [138]. In humans, poor evidence for the efficacy of corticosteroids exists, and only results of uncontrolled studies examining very early treatment (within days of symptoms) have been published [109]. For other therapeutic approaches such as plasmaferesis or intravenous immunoglobulins, the evidence for a beneficial effect is still weak [109]. In summary, currently there are no therapeutic strategies able to improve the visual outcome in ON, and the development of therapies with the potential to prevent neuroaxonal loss following ON remains a significant unmet clinical need.

MELATONIN IN OPTIC NEURITIS

Recently, the therapeutic effect of melatonin on optic nerve and retinal alterations induced by experimental ON [139]. As previously described, LPS was injected into the optic nerve from adult male *Wistar* rats, and the contralateral optic nerve was injected with vehicle (sterile saline solution). One day before optic nerve injections, animals received a subcutaneous pellet of 20 mg melatonin, or were submitted to a sham procedure [139]. In these experimental conditions, melatonin induces visual function improvement, as shown by the preservation of two pathognomonic functional signs of ON (VEPs and PRL) [139]. Concomitantly with the preservation of visual functions, improved structural outcomes were observed, such as: reduced microglial/macrophage reactivity, astrocytosis, demyelination, and axon and RGC loss (Fig. **2**) [139]. A deficit in the axonal transport was described in mice with EAE-ON [140] and LPS-induced ON [110], whereas melatonin avoids the misconnection between the retina and the superior colliculus [139]. Microglial/ macrophage activation is a key component of the inflammatory response, and it has been shown that an early inflammatory response contributes to the late stages of brain injury with neurological function loss [139, 141], and that

microglial activation and progression towards a phagocytic state can lead to progressive neuron loss [139, 142]. Iba-1 represents an index of microglial/macrophage density, whereas abundance of the lysosomal antigen ED1 indicates microglial phagocytic activity [139, 143]. In LPS-injected optic nerves, melatonin treatment did not affect Iba-1(+) area, but it diminishes phagocytic microglial activation induced by experimental ON. In addition, melatonin significantly prevents the reactive gliosis and the occurrence of demyelination in the optic nerve induced by LPS [139]. Current data remain incapable of elucidating whether the benefit induced by melatonin primarily occurs in neurons, their axons, or glial elements. However, these results could suggest that the alterations in the crosstalk among neurons/axons/glial cells provoked by experimental ON could be prevented by melatonin [139]. Moreover, the protection of optic nerve axons could account for the preservation of RGCs [139]. The increase in inflammatory signal levels, such as TNF, COX-2 and iNOS, and oxidative damage has been associated with the pathophysiology of EAE-ON [139, 144, 145]. Consistently with reduced microglial activation, melatonin treatment prevents the increase in iNOS, COX-2, and TNF levels while the decrease in optic nerve lipid peroxidation, is consistent with its antioxidant activity [139]. Therefore, melatonin may behave as anti-inflammatory and antioxidant agent, and through these effects, it can reduce the severity of the damage induced by experimental ON [139]. In addition, the ability of physiological concentrations melatonin to suppress leukocyte migration *via* MT2 receptor [106, 107] and to reduce the expression of adhesion molecules on endothelial cells (through a NFκB -dependent mechanism) [146] may also contribute to its anti-inflammatory effect at the optic nerve level.

The autoimmune response in EAE-ON has been targeted by anti-inflammatory, immunomodulatory and immuno-suppressive agents, without neuroprotective properties [139, 147]. Since RGC loss seems to be a key cause of permanent visual function deficit in experimental and clinical ON, an ideal therapy should involve a combination of an immuno-suppressive, an anti-inflammatory, and a neuroprotective compound [139, 148]. Melatonin seems to be able to accomplish these requirements, since it prevents visual impairment, optic nerve structural alterations and RGC loss, and decreases inflammatory signal levels and oxidative damage triggered by LPS-induced ON [139]. However, the translational relevance of these results is limited since melatonin was administered before LPS injection. Therefore, we analyzed whether melatonin also preserves visual function when the treatment is initiated after the onset of ON (*i.e*., at 4 days post-injection of LPS) [139]. In these conditions, melatonin treatment significantly diminishes the reduction in VEPs and PLR induced by experimental ON, supporting that besides its preventive effect melatonin also reduces the progression of optic nerve damage [139]. In summary, these results show the capacity of melatonin to preserve visual functions, and reducing inflammation, astrocytosis, demyelination, and axon and RGC loss in experimental ON, likely acting through an anti-inflammatory and/or antioxidant mechanism. Thus, melatonin, a safe compound for human use even at high doses [149, 150], could constitute a potential therapy for primary ON.

CONCLUSION

Uveitis and ON, prevalent inflammatory diseases of the visual pathway with potentially blinding sequels remain a challenging field to ophthalmologists, since the current treatments are restricted by side effects and limited effectiveness [4]. Uveitis and ON are different diseases in many aspects, such as causes, risk factors, and affected cells, among others. However, both diseases seem to have in common some of the pathogenic mechanisms, such as oxidative and nitrosative damage, and PGs production. Melatonin, which behaves as an antioxidant and antinitridergic compound, also has the ability to reduce PGE2 and PGF2α levels in the retina and COX-2 levels in the optic nerve. As already mentioned uveitis and ON are mainly treated with corticosteroids. A long-term treatment with corticosteroids may induce both ocular and systemic complications [4], which could be reduced through the addition of other anti-inflammatory approaches. The results in hamster and cat models of uveitis, and in a rat model of ON strongly support that melatonin, with very low toxicity over a wide range of doses [149, 150], could be a new resource in the management of both inflammatory ocular diseases. In this line, a treatment with melatonin (alone or added to corticosteroids) may benefit patients with uveitis or ON and decrease corticosteroid-induced side effect [4, 139]. Furthermore, since melatonin not only prevents but also reduces the progression of uveitis and ON [74, 139], it could be recognized as a potentially useful anti-inflammatory therapy in ophthalmology.

LIST OF ABBREVIATIONS

AA	=	Arachidonic Acid
AA-NAT	=	Arylalkylamine N-Acetyltransferase
AFMK	=	N1-Acetyl-N2-Formyl-5-Methoxykynuramine
AH	=	Aqueous Humor
BOB	=	Blood-Ocular Barrier
COX	=	Cyclooxygenase
EAE	=	Experimental Autoimmune Encephalo-myelitis
EAU	=	Experimental Autoimmune Uveitis
EIU	=	Endotoxin Induced Uveitis
ERG	=	Electroretinogram
HIOMT	=	Hydroxyindole-O-Methyltransferase
IL	=	Interleukin
iNOS	=	Inducible Isoform of NO Synthase
IOP	=	Intraocular Pressure
LPS	=	Bacterial Lipopolysaccharide
MS	=	Multiple Sclerosis
MT1	=	Melatonin Receptors Type 1
MT2	=	Melatonin Receptors Type 2

NFκB	=	Nuclear Factor-Kappa B
NO	=	Nitric Oxide
NOS	=	Nitric Oxide Synthase
NSAIDs	=	Nonsteroidal Anti-Inflammatory Drugs
ON	=	Optic Neuritis
PGs	=	Prostaglandins
PLR	=	Pupil Light Reflex
RGC	=	Retinal Ganglion Cells
RPE	=	Retinal Pigment Epithelium
ROS	=	Reactive Oxygen Species
TNF	=	Tumor Necrosis Factor α
VEPs	=	Visual Evoked Potentials

CONFLICT OF INTEREST

Authors declare no financial or other relationships that might lead to a conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

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