The role and therapeutic potential of melatonin in age-related ocular diseases

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Abstract

The eye is continuously exposed to solar UV radiation and pollutants, making it prone to oxidative attacks. In fact, oxidative damage is a major cause of age-related ocular diseases including cataract, glaucoma, age-related macular degeneration and diabetic retinopathy. Since the nature of lens cells, trabecular meshwork cells, retinal ganglion cells, retinal pigment epithelial cells and photoreceptors is post-mitotic, autophagy plays a critical role in their cellular homeostasis. In age-related ocular diseases, this process is impaired, thus, oxidative damage becomes irreversible. Other conditions such as low-grade chronic inflammation and angiogenesis also contribute to the development of retinal diseases (glaucoma, age-related macular degeneration and diabetic retinopathy). As melatonin is known to have remarkable qualities such as antioxidant/antinitridergic, mitochondrial protector, autophagy modulator, anti-inflammatory and anti-angiogenic, it can represent a powerful tool to counteract all these diseases. The present review analyzes the role and therapeutic potential of melatonin in age-related ocular diseases, focusing on nitro-oxidative stress, autophagy, inflammation and angiogenesis mechanisms.

KEYWORDS: Melatonin; Aging; Aged-related ocular diseases; Cataract; Glaucoma, Age-related macular degeneration; Diabetic retinopathy.
1. INTRODUCTION

Aging involves a progressive deterioration of the body’s regulatory systems - immune, endocrine and nervous systems - leading to loss of homeostasis. The oxidative and inflammatory stress play a fundamental role in this decline. With age, the metabolic production of free radicals such as reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) exceeds the level of antioxidant enzymes. Therefore, ROS oxidise membrane lipids, DNA and proteins in the cellular senescence process. Since ROS are principally produced by the respiratory chain, mitochondria are the major sites of oxidation. This oxidative injury damages mitochondrial macromolecules, mainly DNA, that impairs mitochondrial function and generates more ROS. ROS, (mainly mitochondrial hydrogen peroxide as well as superoxide anion), induce the autophagy process to reduce oxidative damage. This process eliminates non-functional proteins as well as damaged organelles through their sequestration within an autophagosome and posterior degradation via lysosomes. Consequently, oxidatively-damaged mitochondria are degraded by a specialized form of autophagy called mitophagy. Nevertheless, during aging, mitophagy (mitochondria degradation) and biogenesis (mitochondria synthesis) are diminishing as well. Consequently, defective mitochondria gradually replace healthy organelles which finally leads to the death of senescence cells due to a lack of ATP.

Furthermore, ROS can promote telomere erosion contributing to cellular aging and thus to the development of age-related diseases. Indeed, Salpea and colleagues have shown a link between mitochondrial production of ROS and telomere attrition in type 2 diabetes.

Another condition that contributes to senescence progression is inflammation. During aging, pro-inflammatory compounds (e.g. coagulation factors and cytokines such as interleukin 6, IL-6) surpass anti-inflammatory compounds (e.g. transforming growth factor beta and cytokines such as IL-10) resulting in a chronic low-grade of inflammation. Senescence cells secrete pro-inflammatory factors which attract and activate immune cells, the so-called...
senescence-associated secretory phenotype (SASP). Immune cells produce new inflammatory compounds but also ROS/RNS that destroy senescent and/or abnormal cells as well as pathogens. Moreover, oxidized mitochondrial DNA and mitochondrial cardiolipin activate NLRP3 inflammasome (a high molecular weight protein complex) that promotes the maturation of pro-inflammatory cytokines such as IL-1β and IL-18. These cytokines bind to cell surface receptors triggering intracellular signal cascades, which finally activate transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). This protein regulates the expression of genes encoding to pro-inflammatory cytokines and pro-oxidant proteins. Consequently, NF-κB amplifies and prolongs inflammatory as well as oxidative response. On the other hand, ROS/RNS can also interact with NF-κB signaling pathway at different levels. For a more detailed review of ROS and NF-κB crosstalk see reference.

2. MELATONIN AND HUMAN AGING

2.1 Circadian rhythms and melatonin signaling

Melatonin is an indole-derived hormone secreted mainly by the pineal gland allowing the entrainment of the circadian and seasonal rhythms. Its production is also regulated by light/dark cycle via the suprachiasmatic nucleus, the master clock that generates 24 hour rhythms in mammals. Therefore, melatonin concentrations display a circadian rhythm with low values during the daytime and high values at night. Nocturnal levels of melatonin tend to decrease with age. This fact can be explained by the deterioration of aged suprachiasmatic nucleus. In addition, patients with age-related diseases such as Alzheimer and type 2 diabetes produce lesser melatonin than age-matched controls.

Since many physiological processes display circadian rhythmicity, the reduction of melatonin levels might cause an internal desynchronization, altering body homeostasis.
Melatonin effects are principally associated to its capacity to activate specific membrane receptors termed MT₁, MT₂ and the putative MT₃._MT₁ and MT₂ receptors are members of the G protein-coupled hepta-helical receptor (GPCR) family. These receptors can couple to G_{αi} (pertussis toxin-sensitive), G_{αq} (pertussis toxin-insensitive) and G_{βγ} proteins. Consequently, activation of MT₁/MT₂ receptors triggers different signal cascades (Fig. 1).

Classically, melatonin inhibits adenylate cyclase but can also activate phospholipase C or inhibit guanylate cyclase. Furthermore, melatonin can activate the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathways, as well as being able to regulate channels (e.g. inward rectifier potassium channels, Kir3.1/2). In fact, several authors have suggested that the ERK/MAPK pathway, represents a major component of melatonin signaling during oxidative stress. For a more detailed review of melatonin signaling see.

Additionally, melatonin and its metabolites (e.g. N1-acetyl-N2-formyl-5-methoxykynuramine, AFMK, and N1-acetyl-5-methoxykynuramine, AMK) possess added anti-aging effects including antioxidant, protection of mitochondria function as well as anti-inflammatory.

2.2 Melatonin and the nitro-oxidative stress

Antioxidant and antinitridergic effects of the indole-derived melatonin and its metabolites are mainly mediated by its ability to scavenge free radicals but also through specific signaling (see above) and transcription of stress-related genes (Table 1). Therefore, melatonin regulates the expression of genes encoding to antioxidant enzymes and to neuronal nitric oxide synthase (nNOS, isoform constitutively expressed) as well as inducible nitric oxide synthase (iNOS, isoform expressed during inflammatory processes). These genomic actions are mediated by redox-sensitive transcription factors such as nuclear factor erythroid
related factor 2 (Nrf2) and NF-κB. Melatonin activates Nrf2 factor under nitro-oxidative stress (stress induced by ROS and RNS) and thereby increases the expression of antioxidant enzymes (see Figure 1). Likewise, melatonin blocks NF-κB activity inhibiting the expression of pro-inflammatory genes such as NOS and cyclooxygenase-2 (COX-2) enzyme (Fig. 1). For a specific review of genomics actions of melatonin see.

Regarding the antinitridergic effects of melatonin, it is noteworthy that its metabolite AMK also inhibits nNOS and cytosolic as well as mitochondrial (i-mtNOS) iNOS (Table 1). These enzymes catalyze the synthesis of nitric oxide (NO) a signaling molecule that participates in several cellular processes and is the source of RNS. In mitochondria, cytosolic/mitochondrial NO regulates the respiratory chain, the generation of free radicals and the mitochondrial apoptosis (Table 1). Besides this, melatonin improves mitochondrial electron flux, stimulates de novo synthesis of respirosomal subunits, prevents Ca$^{2+}$ overload in mitochondrial matrix, exerts antioxidant activity (e.g. to avoid cardiolipin peroxidation) (actions reviewed in ) and promotes mitochondrial biogenesis. Again, AFMK and AMK metabolites contribute to the mitochondrial protection effect of melatonin.

With regards to mitophagy (selective autophagy of mitochondria), melatonin can act as either pro- or anti- autophagy depending on cellular necessities and oxidative stress level. Therefore, Guo and colleagues reported the pro-autophagy (and thus cytoprotective) effect of melatonin in ischemia/reperfusion-injured N2a cells. Conversely, Teng and colleagues demonstrated the anti-autophagy (and thus neuroprotective) role of melatonin in neuronal cells of rats treated with arsenite. The potential role of melatonin in the regulation of autophagy/mitophagy during aging remains unknowns. Only, Caballero and coworkers observed that autophagic processes of aged animals were unaltered by melatonin treatment.
2.3 Melatonin and inflammation

Melatonin can also prevent the onset (due to its effects as antioxidant, antinitriidergic, mitochondrial protector, COX-2 inhibitor and anti-excitatory) and progression (due to its effects as direct and indirect immune modulator) of inflammation. Therefore, Melatonin and its metabolites can specifically prevent the gene expression and activation of COX-2, which regulates prostaglandins production and so are a key factor in the initiation of inflammatory process (Table 1).\(^{40,47,48}\) Additionally, this indole-derived compound has anti-excitatory properties, which also allows it to prevent neuroinflammation. Indeed, a possible cause of neuroinflammation is the excitotoxicity, a process of neuronal death originated by accumulation of neurotransmitter glutamate around the synaptic cleft. Excessive glutamate concentration increases NOS expression, principally nNOS, which produces high levels of NO.\(^{54,65}\) Finally, NO may induce apoptosis through DNA damage and mitochondrial respiration impairment.\(^{54,65}\) Since melatonin and its metabolites inhibit the expression of nNOS and iNOS, these compounds have anti-excitatory effects (Table 1).\(^{39,41,46,49,51}\) Furthermore, melatonin can modulate GABA, glutamate and glycine receptors thus acting as a direct anti-excitatory molecule (Table 1).\(^{66-73}\)

Not only do melatonin and its metabolites reduce the level of NO that produces immune-modulatory effects (effects summarized in\(^{74}\)) but they also reduces iNOS expression through the inhibition of NF-κB pathway (Table 1).\(^{46,53}\) As we previously commented, this pathway is essential to amplify inflammatory process and thus, melatonin might also act as an anti-inflammatory compound.\(^{16,38,42}\) Furthermore, melatonin is an immune modulator \textit{per se} and controls the release of various anti-inflammatory and also pro-inflammatory cytokines.\(^{38,42,75}\) This action is mediated by pineal and extra pineal melatonin (melatonin synthetized by immune cells) via membrane receptors (mainly MT\(_1\) receptors) as well as nuclear receptors of RZR/ROR family present in immune cells (Fig. 1).\(^{76-78}\) In this context, several authors have
reported that melatonin can act as an anti-inflammatory agent in chronic low-grade inflammation typical of aging.\textsuperscript{38,42,79} By contrast, and as Carrillo-Vico and colleagues describe, under basal or immunosuppressive conditions melatonin promotes inflammation.\textsuperscript{80}

### 2.4 Melatonin and its effect on insulin and sirtuins actions

The beneficial effects of melatonin, are not only limited to the aforementioned paths. The connection between melatonin and insulin/insulin-like growth factor 1 (IGF-1) as well as sirtuins signaling pathways provides additional anti-aging effects.\textsuperscript{81} Insulin/IGF-1 pathway regulates mitochondrial activity and energy metabolism, therefore it is involved in neuronal survival.\textsuperscript{82-84} In fact, activation of this pathway improves mitochondrial function in lymphoblasts from patients with Huntington's disease.\textsuperscript{85} Melatonin modulates insulin/IGF-1 receptor phosphorylation and regulates PI3K/AKT as well as ERK/MAPK pathways in isolated rat pancreatic islets (Fig. 1).\textsuperscript{86} Indeed, this indole-derived compound improves pancreatic functioning of senescence accelerated mouse (SAMP8) and counteracts insulin-resistance characteristic of metabolic syndrome as well as diabetes type 2.\textsuperscript{87-89}

Additionally, melatonin can indirectly regulate the autophagy process via PI3K/AKT modulation (Table 1). This critical enzyme of insulin/IGF-1 pathway activates the mammalian target of rapamycin (mTOR) signaling and thus suppresses autophagy.\textsuperscript{90}

Sirtuins are a family of NAD\textsuperscript{+}- dependent histone deacetylases.\textsuperscript{91,92} These enzymes, deacetylate histone and non-histone substrates such as Forkhead box O (FoxO) transcription factors\textsuperscript{93,94} and NF-\textsuperscript{kB},\textsuperscript{95} thereby sirtuins participate in several functions related with aging including energy metabolism, stress resistance, cell survival and circadian rhythms.\textsuperscript{96} Sirtuin subtype 1 (SIRT1) regulates the expression of IGF-1 and its receptor\textsuperscript{97} as well as the FoxO transcription factor which is a downstream target of insulin/IGF-1 pathway.\textsuperscript{93,94,98} Furthermore, it protects pancreatic beta cells by inhibition of NF-\textsuperscript{kB}.\textsuperscript{99}
modulates insulin secretion and ameliorates insulin sensitivity, principally under insulin-resistant conditions such as in diabetes.

In response to oxidative stress, SIRT1 reduces apoptosis induced by FoxO subtype 3 (FoxO3) factor. Additionally, SIRT1 regulates the expression of antioxidant enzymes through a FoxO-dependent mechanism and through mitochondrial NOS enzymes. Therefore, sirtuins are also involved in the control of free radical levels and mitochondrial biogenesis which are key factors in the aging process. Furthermore, sirtuin-mediated deacetylation is critical for autophagy regulation (Table 1). Indeed, SIRT1 promotes the expression of components of the autophagy process via deacetylation of transcription factors such as FoxO1 and FoxO3.

In relation to the ability to reduce ROS load, sirtuins also inhibit the pro-oxidant and pro-inflammatory factor NF-κB. In fact, Schug and colleagues suggest that modulators of sirtuins might be useful for the treatment of chronic inflammation and its associated diseases.

As previously mentioned, oxidative stress can promote telomere attrition. SIRT1 can reduce oxidative stress but also, maintains telomere stability (Table 1).

Finally, sirtuins link cellular metabolism to the circadian molecular clock machinery. SIRT1 modulates circadian clock gene expression through its association to CLOCK/BMAL1-complex. Furthermore, SIRT3 regulates rhythmicity of mitochondrial activity (Table 1).

Melatonin can modulate SIRT1 expression and subsequently modify the levels of its acetylated substrates (Table 1). Gutierrez-Cuesta and colleagues have shown the ability of melatonin to upregulate SIRT1 in SAMP8 mice. In accordance with this result, melatonin induces SIRT1 expression in sleep-deprived rats and in neuronal cultures from aged rat cerebellum. Conversely, melatonin decreases SIRT1 expression under tumoral and inflammatory conditions. In any case, this modulation may explain some of melatonin
anti-aging actions. In fact, Lim and colleagues have shown that some of the anti-inflammatory properties of melatonin are dependent of SIRT1.\(^{116}\)

Another possible action of melatonin mediated by sirtuins is its influence on telomerase activity. Melatonin stimulates telomerase activity on normal cells\(^ {118}\) and SIRT1 is necessary for telomere elongation.\(^ {109,110}\) Conversely, and under tumoral conditions, melatonin inhibits the telomerase activity.\(^ {119,120}\)

3. MELATONIN AND AGING-RELATED EYE DISEASES

Melatonin as a regulator of physiological circadian rhythm processes, as an antioxidant, an anti-inflammatory agent and as a protector of mitochondria function, could represent a powerful tool to counteract age-related conditions including diseases of the eye. This indole-derived compound is also synthesized in human ocular tissues such as ciliary body and retina, which present specific melatonin receptors and therefore it might control the physiology of these ocular targets.\(^ {121}\) Additionally, melatonin and melanopsin (a regulator of ocular melatonin synthesis) are also produced in the human lens, although melatonin receptors have only been discovered in fiber cells of frog lens.\(^ {121-123}\)

Since the eye is continuously exposed to solar UV radiation and pollutants, this organ is prone to oxidative attacks. In fact, oxidative stress is involved in the development of many ocular diseases including cataract, glaucoma, age-related macular degeneration and diabetic retinopathy.\(^ {124}\) All these pathologies are associated with age and consequently, they may be the result of an imbalance between ocular ROS generation and the expression of antioxidant enzymes (see introduction section about cellular senescence process and reference\(^ {124}\)). In this context, mitochondria are essential in the onset and progression of age-related ocular diseases.\(^ {125-127}\) Ocular mitochondria are the source of antioxidant and protein repair systems but are also the main endogenous source of ROS.\(^ {127}\) Consequently, oxidatively-damaged mitochondria are unable to maintain redox balance and to repair damaged proteins.\(^ {127}\)
Furthermore, ROS produced by injured mitochondria induce autophagy/mitophagy process. In the eye, autophagy plays a critical role in maintaining normal cellular function. Indeed, alterations in the autophagy process contribute to the development of age-related ocular diseases.

All these oxidative stress-derived events play a particularly critical role in the onset as well as the progression of age-related retinal pathologies including glaucoma, age-related macular degeneration and diabetic retinopathy. Indeed, the retina is especially vulnerable to ROS damage due to its constant exposure to visible light that causes photooxidation, its extraordinary concentration of polyunsaturated fatty acids (mainly docosahexanoic acid, DHA), which are especially sensible to peroxidation, and its high metabolic activity. Additionally, oxidative stress triggers retinal para-inflammation process. This process tries to restore retina functionality during aging but if it fails and becomes chronic (low-grade inflammation) it can then contribute to the development of age-related retinal diseases.

3.1. Cataracts

According to a 2012 World Health Organization (WHO) report, cataracts are responsible for 33 per cent of visual impairment and 51 per cent of blindness worldwide. Cataracts are commonly associated with age. As the lens ages, fiber cells accumulate high protein concentration forming aggregates. These aggregates scatter light and impede its focus on the retina. Aged lens cells also present high ROS concentration that contributes to the loss of transparency. Glutathione and mitochondria antioxidant enzymes control redox balance in the lens. During aging process, glutathione levels decline and mitochondria are damaged. Therefore, cellular redox balance is broken down and protein repair systems fail. Concurrently, autophagy and mitophagy processes attempt to restore lens homeostasis but

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their failure can often result in more ROS, oxidation and finally in cataract formation. Indeed, deletion of autophagy-related genes causes cataracts.

In addition to age, other factors such as light exposure can also contribute to the oxidative stress-induced mitochondrial damage.

3.1.1 The therapeutic potential of melatonin in cataracts

Melatonin, as a direct and indirect antioxidant, reduces cataract formation in rats (Fig 2). This neurohormone reduces the level of lipid peroxidation, enhances the production of glutathione and increases the activity of antioxidant enzymes in rats with cataracts.

In human lens epithelial cells (HLECs), melatonin inhibits H$_2$O$_2$-induced intracellular ROS generation and cell death through the activation of the PI3K/Akt signaling pathway (Fig. 1 and 2). Bai and colleagues have suggested the possible therapeutic role of melatonin to prevent age-related cataracts by these receptor-mediated effects. More recently, Babizhayev and Yegorov claimed a new approach for treating cataracts based on mitochondria-targeted antioxidants. Consequently, and due to its antioxidant and mitochondria effects, melatonin seems to be a promising candidate in cataract management.

PI3K/Akt cascade can also be transactivated secondarily to MAPK pathway activation. Indeed, MAPK pathway is a key component of melatonin signaling during oxidative stress (Fig. 1). Moreover, MAPK signaling also plays a critical role in lens homeostasis. In fact, Shakespeare and colleagues have demonstrated that the constitutive activation of MAPK pathway increases connexin 50 - gap junction protein which mediates intercellular communication - activity producing cataract.

Regarding connexins, several authors have demonstrated its importance in cataract development. Accumulation of ROS in age lenses alters gap junction function, probably, by oxidative degradation of connexins. Wild-type and mutant connexins such as
CX50P88S – a connexin associated with cataracts – are degraded by autophagy. Therefore, autophagy might contribute to these functional alterations of gap junction proteins.

Melatonin can also regulate connexin expression as well as autophagy process under oxidative stress, and may thereby control intercellular communication mediated by gap junctions (Fig 2).

As mentioned before, ROS can also promote telomere erosion, which contributes to cellular aging process. In this context, Huang and colleagues have demonstrated that the oxidative stressor \( \text{H}_2\text{O}_2 \) increases the rate of telomere shortening in HLECs cells. Since aged lenses accumulate ROS, telomere length of lens epithelial cells can be a marker of aging and cataractogenesis. Again, melatonin as a powerful antioxidant and inducer of telomerase activity as well as of sirtuins (these last actions under normal conditions) may prevent telomere attrition (Fig 2). Regarding sirtuins, Li and colleagues demonstrated that SIRT1 mRNA level decreases with age and the grade of human age-related cataract. Indeed, a stabilizer of SIRT1-substrate interaction such as resveratrol prevents cataract formation in rats. Conversely, Zheng and Lu observed a higher level of SIRT1 mRNA and protein in lens of cataract patients than in normal lens.

3.2. Glaucoma

Glaucoma is the second most common cause of visual impairment (2% of global cases) and blindness (8% of global cases) worldwide. It agglutinates a group of eye diseases characterized by progressive loss of retinal ganglion cells and subsequent damage to the optic nerve that eventually results in blindness. The most prevalent type of glaucoma is the primary open-angle glaucoma whose prevalence increases exponentially with age.
Additionally, Tezel and colleagues have suggested that an accelerated aging process accompanies glaucomatous neurodegeneration.\textsuperscript{159} Indeed, the tissues involved in glaucoma - retina and trabecular meshwork (TM) - experiment remarkable changes during aging process.\textsuperscript{160-165} Consequently, retinal ganglion cells (RGC) of aged mice are more vulnerable to injury than cells of young animals.\textsuperscript{165} Regarding TM, their numbers of cells decrease with age and especially in glaucoma condition.\textsuperscript{160,162} Furthermore, De la Paz and Epstein have reported an age-dependent decline of superoxide dismutase activity in trabecular tissue.\textsuperscript{161}

Since glaucoma is an age-related disease, nitro-oxidative stress and particularly mitochondrial stress are also essential in its onset and progression. In fact, some authors have demonstrated the existence of a high level of stress proteins and their antibodies in the eyes and serum, respectively, of glaucomatous patients.\textsuperscript{166,167} Furthermore, elevated levels of iNOS activity have also been detected in astrocytes of the optic nerve head, endothelial cells of iris capillaries, aqueous humor and TM of glaucoma patients.\textsuperscript{168-171}

Mitochondria of several ocular cells including TM cells and retinal ganglion cells present molecular and functional changes during glaucoma (reviewed in\textsuperscript{172}). Glaucmatous TM cells present a defect in mitochondrial complex I and a higher mitochondrial permeability than normal cells.\textsuperscript{173,174} This prompts the generation of more ROS, mitochondrial membrane depolarization as well as impairing intracellular calcium homeostasis.\textsuperscript{173,174} Consequently, glaucomatous TM cells are especially vulnerable to calcium stress and thus to cell death.\textsuperscript{173}

Several authors have demonstrated that the apoptosis of retinal ganglion cells is also due, at least in part, to a decrease in their mitochondrial membrane potential and an increase in their membrane permeability.\textsuperscript{175,176} Furthermore, Wang and colleagues have suggested that in the early stages of glaucoma occurs a non-apoptotic cell death (called paraptosis), which is also triggered by mitochondrial damage.\textsuperscript{177} In addition to age, other factors such as light exposure, high intraocular pressure (IOP) and hypoxia can also contribute to the oxidative stress-induced mitochondrial damage.\textsuperscript{178,179}

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Regarding light exposure, some authors have demonstrated that visible light stimulates the production of mitochondrial ROS and induces intrinsic apoptotic pathway, which is associated with mitochondrial events, in retinal ganglion cells. Since this detrimental effect is more marked when cells are energetically comprised, the authors suggest that light acts as an additional source of stress for ganglion cells already submitted to mitochondrial damage by other factors such as age and high IOP.

High IOP has classically been considered the most important cause of glaucomatous damage and currently, it is the only modifiable factor. Elevation of IOP can occur due to an imbalance between aqueous humor secretion across the ciliary epithelium and its drainage, which is decreased with age and glaucoma, through TM. Some authors have demonstrated that the elevation of IOP in animals (models of chronic pressure-induced glaucoma) results in a high level of retinal lipid peroxidation, protein nitration as well as protein oxidation and mitochondrial membrane depolarization of retinal ganglion cells. Mitochondrial dysfunction has also been observed in cultured retinal ganglion cells submitted to elevated hydrostatic pressure. Furthermore, high IOP triggers retinal glutamate receptor (mainly N-methyl-D-aspartate, NMDA receptor but also α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA receptor) activation that permits the rapid influx of calcium ions impairing intracellular calcium homeostasis and at the same time increasing ROS/RNS generation. These effects also evoke mitochondrial dysfunction that finally leads to neuronal death by apoptosis and prompts calcium-dependent exocytosis of glutamate from injury neurons that induces further excitotoxic damage to adjacent neurons. Additionally, mitochondrial dysfunction also increases the sensitivity of neurons to glutamate excitotoxicity and therefore leads to a lower concentration of glutamate, which is sufficient to induce neuronal death.
On the other hand, elevation of IOP in animals results in a marked activation of microglial and macroglial cells. Activated glial cells contribute to age-related chronic low grade inflammation via the release of substances such as TNFα and NO that act as immune-modulators and as cell death mediators.

Retinal hypoxia, which can be secondary to or independent of high IOP, is also implicated in the development of glaucoma. In fact, Kergoat and colleagues have demonstrated that retinal ganglion cells are particularly sensitive to systemic hypoxic stress. Hypoxia induces the expression of the hypoxia inducible factor -1α gene and its gene targets vascular endothelial growth factor (VEGF) and NOS that mediate inflammation, cell death and disruption of the blood retinal barrier. Additionally, hypoxic insult provokes glutamate release, which contributes to the retinal ganglion cells damage.

### 3.2.1 The therapeutic potential of melatonin in glaucoma

Some authors have demonstrated that melatonin can counteract several etiopathogenic mechanisms for glaucomatous neurodegeneration, including elevation of IOP, nitro-oxidative stress, excitotoxicity and glial activation (Fig. 2).

It is well known that the melatonergic system is involved in the circadian rhythms of IOP (Fig. 2). Indeed, a dysfunction of melatonin signaling triggers elevation of nocturnal IOP and loss of retinal ganglion cells (Fig. 2). Additionally, exogenous melatonin and its analogues lower IOP in mammals, including humans (Fig. 2). This ocular hypotensive effect seems to be mediated by ciliary body receptors (MT₂ and an unidentified receptor classically named MT₃) and final inhibition of ciliary chloride secretion. Furthermore, melatonergic compounds provide a sustained reduction in IOP through regulation of ciliary genes expression and potentiate the hypotensive action of classic anti-glaucomatous drugs such as timolol and brimonidine.
Belforte and colleagues have proved in a model of chronic pressure-induced glaucoma, that melatonin also increases retinal antioxidant defenses (e.g. it increases SOD activity and glutathione level) (Fig. 2).\textsuperscript{227} It also, reduces lipid peroxidation, NOS activity, the loss of retinal ganglion cells and the extracellular glutamate level (increasing glutamate uptake as well as glutamine synthetase activity and reducing glutaminase activity) (Fig. 2).\textsuperscript{227} These effects may be mediated, at least in part, by the transcription factor Nrf2, which plays a critical role in oxidative stress-induced retinal ganglion cell death (Fig. 2).\textsuperscript{228} In fact, Nrf2 presents antioxidant as well as anti-inflammatory effects, improves mitochondrial function and stimulates autophagy.\textsuperscript{229} This transcription factor reduces the effects of many NO/ONOO\textsuperscript{-} cycle elements (e.g. oxidative stress, iNOS induction, inflammatory cytokines, mitochondrial dysfunction, excitotoxicity and intracellular calcium) in glaucomatous retinal ganglion cells and TM cells.\textsuperscript{230} Regarding NO, several authors have demonstrated that NO plays an important role in IOP homeostasis (reviewed in\textsuperscript{231}). Consequently, an inhibitor of NOS triggers TM contraction and reduces flow rates through TM.\textsuperscript{232-234} Moreover, Ellis and colleagues have suggested that reduction of NO may increase outflow resistance through its effect on Schlemm’s canal cells.\textsuperscript{235} Curiously, melatonin as a NOS inhibitor could act on TM iNOS and thereby, increase outflow resistance (Fig. 2). In this sense, Alkozi and colleagues have found that the AH of individuals with high IOP presents higher melatonin concentration than normotensive subjects.\textsuperscript{236} Consequently, melatonin could act on ciliary cells but also it could contract TM and finally, be unable to lower IOP. Nevertheless, Chiquet and colleagues have found comparable melatonin concentration in AH as well as in plasma of glaucomatous and healthy individuals.\textsuperscript{237} Since TM plays a critical role in glaucoma onset and progression, it would be interesting to clarify and/or analyze in future studies the possible effect of melatonin on glaucomatous TM cells.

Several authors have demonstrated that SIRT1 may also prevent oxidative stress-induced TM DNA damage and retinal ganglion cell death as well as axonal degeneration.\textsuperscript{163,238-240}
Consequently, melatonin treatment regulating SIRT1 expression/activity, could be a useful strategy to prevent these effects and thus, glaucoma progression (Fig. 2)\textsuperscript{113-115,117}.

Additionally, and in experimental models of optic neuritis and retinal ischemia, melatonin treatment inhibits glial reactivity and reduces the expression of hypoxia inducible factor -1α, COX-2 and TNFα genes (Fig. 2).\textsuperscript{241,242} It is, thereby, also acting as a neuroprotective agent. In relation to these effects, Jiang and colleagues have demonstrated that melatonin decreases the production of VEGF by Müller cells, the main retinal glial cells, under high glucose concentration.\textsuperscript{243} Considering all these facts, the use of melatonin seems to be an appropriate strategy to prevent and treat glaucoma (Fig. 2).

### 3.2.2 A new therapeutic consideration: melatonin and autophagy

Since mitochondrial damage plays a critical role in glaucoma development, mitophagy and in general autophagy processes should also be important. Indeed, Coughlin and colleagues have detected a deficient mitophagy process in the optic nerve of glaucomatous mice.\textsuperscript{244} On the other hand, several authors have demonstrated that after optic nerve transection, elevation of IOP, ischemia or optic nerve crush, autophagy levels increase.\textsuperscript{245-249} Nevertheless, the role of autophagy in glaucoma neurodegeneration is still unclear. Several studies have suggested that autophagy promotes the survival of retinal ganglion cells after oxidative injury.\textsuperscript{249-251} Conversely, other authors have suggested that autophagy promotes retinal ganglion cell death and axonal degeneration.\textsuperscript{245,246,248} Alternatively, Park and colleagues have proposed that autophagy is initially activated in the dendrites of retinal ganglion cells where it promotes neuroprotection and afterwards it is activated in the cytoplasm where it provokes cell death.\textsuperscript{247}

Other cells key in glaucoma occurrence and progression such as glial and trabecular meshwork cells also experiment changes in the autophagy processes. Consequently, Tezel and colleagues have reported the overexpression of autophagy-related proteins in astrocytes.
of glaucomatous rats.\textsuperscript{252} Regarding TM, Pulliero and colleagues have demonstrated that aging promotes the autophagy processes in human TM.\textsuperscript{128} Additionally, some authors have demonstrated that porcine TM cells submitted to oxidative stress accumulate defective mitochondria as well as autophagic/lysosomal vacuoles and trigger autophagy induction.\textsuperscript{253,254} Nevertheless, since oxidative stress also impairs lysosomal activity and reduces lysosomal acidification, the autophagic flux is finally decreased.\textsuperscript{253,254} More recently, Porter and colleagues demonstrated a mTOR-dependent dysregulation of autophagy pathway in human glaucomatous TM cells.\textsuperscript{255} Melatonin can induce mTOR pathway and thereby play a role in this process (Fig. 2).\textsuperscript{256} Since alterations in TM autophagy may be critical for glaucoma onset and/or progression, it seems necessary to clarify this point.

### 3.3. Age-related macular degeneration

Macular diseases are the third most common cause of visual impairment (3.1 % of all visually impaired people) and blindness (6.6 % of all blind people) worldwide.\textsuperscript{257} Age-related macular degeneration (AMD) is a disease with a complex etiology including aging, genetic predisposition and environmental determinants (risk factors have been reviewed in\textsuperscript{258}). The disease affects the macular region of the eye containing photoreceptors, retinal pigment epithelium (RPE), Bruch´s membrane and choriocapillaris.\textsuperscript{259} RPE protects photoreceptors against photo-oxidative stress by absorption of light radiation and phagocytosis of their outer segment membranes. Additionally, and since RPE participates in the visual cycle (biochemical reactions that regenerate photoreceptor visual pigment), controls ion homeostasis in the subretinal space and supplies nutrients to photoreceptors, it maintains photoreceptor´s functions.\textsuperscript{260} Consequently, RPE dysfunction is a primary event in photoreceptor degeneration and in AMD development.\textsuperscript{261,262}
3.3.1 The therapeutic potential of melatonin in AMD: oxidative stress

Aged photoreceptors, choriocapillaris and RPE cells are subjected to intense oxidative insult because of their constant exposure to visible light, daily phagocytosis of photoreceptor outer segments, high local oxygen concentrations, presence of intracellular photosensitizers and choroidal blood photoactive compounds. Additionally, RPE cells present inadequate systems of antioxidant defense and mitochondrial DNA repair. Consequently, these cells and specially RPE cells accumulate oxidative damage with age (mainly mitochondrial and DNA damage) that alters their bioenergetics status, thus compromising cell function and viability. Certainly, during aging and particularly in AMD, the number/area of mitochondria as well as the content of mitochondrial electron transport chain proteins in RPE cells, is diminishing. Furthermore, several authors have demonstrated that in AMD disease, RPE cells accumulate a high level of mitochondrial DNA damage, even more than in age-matched cells.

Melatonin, as a potent antioxidant/mitochondrial protector, can counteract oxidative stress and increases viability of RPE cells as well as of photoreceptors (Fig. 2). This indole-derived component scavenges free radicals but also regulates transcription of antioxidant genes via Nrf2 activation.

Rosen and colleagues have reported that nocturnal melatonin production is decreased in AMD patients when compared to age-matched controls. Consequently, and considering the melatonin antioxidant effects, its decreased synthesis can contribute to the oxidative insult and finally occurrence of AMD. In fact, aged RPE cells have impaired Nrf2 signaling making them especially vulnerable to oxidative damage. Furthermore, and since some authors point to oxidative stress and Nrf2-mediated antioxidant defense as promising targets to prevent or treat AMD, exogenous melatonin might also be useful for this purpose (Fig. 2). Indeed, a preliminary study has demonstrated that the daily use of 3 mg of melatonin, during 3 months, protects the retina and delays macular degeneration.
3.3.2 A new therapeutic consideration in AMD: melatonin and autophagy

As previously mentioned, autophagy and its variants act as a second line of defense against oxidative damage.\textsuperscript{5} During aging, they degrade non-functional proteins or protein aggregates and damaged organelles.\textsuperscript{261} Therefore, the excessive accumulation of mitochondrial damage in AMD suggests a failure in these processes. Indeed, mitophagy – selective autophagy or degradation of mitochondria – diminishes in RPE cells in the early stages of AMD.\textsuperscript{285} Phagocytosis of photoreceptor outer segments is another autophagy-related degradation system of RPE.\textsuperscript{261} This process guarantees viability of photoreceptors and thus, visual function.\textsuperscript{260,266,286} Scütt and colleagues have demonstrated that the impairing of mitochondrial activity diminishes the phagocytic and autophagic capacities of RPE cells.\textsuperscript{288} Consequently, aged RPE cells accumulate damaged organelles such as mitochondria as well as non-functional or toxic proteins including the pigment lipofuscin.\textsuperscript{261,289} The excessive accumulation of this pigment may be harmful to RPE cells, since it is a toxic photosensitizer.\textsuperscript{290-292} In fact, many studies support the idea of a causal relationship between lipofuscin accumulation and AMD onset/progression.\textsuperscript{291,293,294} Photooxidation products of the bisretinoids – component of lipofuscin – and of DHA, activate complement system.\textsuperscript{294,295} Consequently, they may promote para-inflammation and drusen formation (extracellular deposits predominantly localized beneath RPE that impair its metabolic connection with choroid).\textsuperscript{258,294} Both, immuno-inflammatory events (e.g. complement, inflammasome and microglial/macrophage activation) and drusogenesis (drusen formation, triggered by reduced autophagy and complement activation)\textsuperscript{130,296-299} play a reliable role in pathogenesis and progression of AMD (reviewed in\textsuperscript{130,258}).

It is well known that the phagocytic and autophagic processes of RPE cells are controlled by circadian rhythms.\textsuperscript{300-302} Some studies have demonstrated that melatonin, a key regulator of retinal circadian rhythms, regulates photoreceptor outer segment phagocytosis whereas others have not.\textsuperscript{303,304} More recently, Yao and colleagues have demonstrated a circadian and light-dependent modulation of autophagy in photoreceptors.\textsuperscript{302} Conversely, autophagy in
RPE cells is an independent-light process but occurs in response to photoreceptor phagocytosis.\textsuperscript{302} The possible involvement of melatonin in these processes is unknown. Since failure in autophagy is a critical event in AMD pathogenesis, it seems necessary to clarify this point (Fig. 2).

In any case, retinal circadian clocks prevent melatonin secretion in the presence of light and thus protect photoreceptors against light-induced damage (Fig. 2).\textsuperscript{305-307} Additionally, melatonin induces melanosome (melanin granule) aggregation in RPE cells and therefore, protects them from light-induced apoptosis (Fig. 2).\textsuperscript{308,309} Indeed, RPE melanin pigment absorbs light and scavenges light-induced free radicals acting as an antioxidant agent.\textsuperscript{310} In the aging RPE, melanin experiments oxidative changes (it binds lipofuscin) and therefore, loses its photoprotective capacity.\textsuperscript{311} This fact confirms the protective role of melatonin and its potential to prevent/treat AMD disease (Fig. 2).

As previously mentioned, ROS can also promote telomere erosion that contributes to cellular aging process.\textsuperscript{10-13} Indeed, Honda and colleagues demonstrated that RPE aged cells exhibit accelerated telomere shorting.\textsuperscript{312} Since melatonin can modulate telomerase activity and retina contains active telomerase, its age-related deficiency can also contribute to the RPE cell damage (Fig. 2).\textsuperscript{42,118,313} In fact, some authors have suggested that melatonin may be useful in preventing or treating AMD by its capacity to modulate telomerase.\textsuperscript{314,315}

3.3.3 A novel therapeutic consideration: melatonin and angiogenesis

The more severe complication of AMD, choroidal neovascularization (the growth of new vessels derived of choriocapillaris into the subretinal space) is also triggered by ROS accumulation (principally mitochondrial ROS). ROS promotes the expression of pro-angiogenic mediators and thereby, participates in retinal angiogenesis.\textsuperscript{316} Atienzar-Aroca and colleagues have demonstrated that RPE cells, under oxidative stress, release a large amount of vascular endothelial growth factor (VEGF) receptor-loaded exosomes capable of
promoting angiogenesis (this factor regulates retinal vascular leakage and proliferation). \(^{317}\)

Furthermore, protein adducts formed by peroxidation of DHA and RPE lipofuscin induce angiogenesis by VEGF-independent pathways.\(^{293,318,319}\) Additionally, pro-angiogenic cytokines generated by lipofuscin promote accumulation of macrophages that may release new angiogenic promoters and then amplify the angiogenesis process.\(^{319,320}\) In fact, Wang and colleagues have recently suggested that the tumor necrosis factor alpha (TNF-\(\alpha\), pro-inflammatory cytokine generated mainly by macrophages) induces the expression of VEGF in RPE cells, through a mechanism ROS-dependent, and thus mediates choroidal neovascularization.\(^{321}\)

Melatonin also protects against the breakdown of the retinal-blood-barrier (BRB) that occurs in retinal neovascularization (Fig. 2).\(^{322}\) Indeed, melatonin reduces the level of VEGF as well as NO in retina and thus, prevents BRB breakdown (Fig. 2).\(^{322}\) These facts once again confirm the role of melatonin in the occurrence of AMD and that the administration of melatonin might be beneficial in treating this disease. Indeed, anti-VEGF strategy is routinely used to treat choroidal neovascularization.\(^{323}\) Since the choroidal neovascularization response to anti-VEGF therapy is variable, its combination with anti-inflammatory agents is frequent.\(^{324,325}\) Consequently, a combination of antiangiogenic agents with melatonin (with antioxidant/mitochondrial protector, antiangiogenic, anti-inflammatory as well as immunomodulatory effect) could improve the efficacy of AMD therapy.

### 3.4. Diabetic retinopathy

According to a 2012 World Health Organization (WHO) report, diabetic retinopathy is responsible for 1 per cent of visual impairment and blindness worldwide.\(^{131}\) Diabetic retinopathy is the most common ocular complication derived from diabetes mellitus. Chronic hyperglycemia of diabetes, and to a lesser extent hyperlipidemia as well as hypertension, induce changes in the permeability of retinal blood vessels and reduce retinal oxygenation.

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Therefore, abnormal new blood vessels grow and invade vitreous humor that can finally produce blindness (stage of the disease called proliferative diabetic retinopathy). Diabetes is an age-related disease (elderly adults present twice the prevalence compared to middle-aged or young adults) and thus, oxidative stress plays a prominent role in its development. Furthermore, oxidative stress underlies the pathogenesis of diabetic retinopathy. In fact, diabetic retinopathy patients present higher levels of plasma lipid peroxidation than diabetes patients without diabetic retinopathy. Retinal enzymatic (e.g. cytosolic NADPH oxidase and arginase activation) and non-enzymatic (mainly via mitochondrial damage) mechanisms produce excessive ROS in diabetic retinopathy. Additionally, auto-oxidation of glucose and metabolic abnormalities induced by hyperglycemia (e.g. activation of polyol as well as hexosamine pathways, protein kinase C activation and formation of advanced glycation endproducts - AGE) increase ROS production in diabetes (reviewed in). Furthermore, inflammatory processes contribute to the development of diabetic retinopathy and so is considered a low grade chronic inflammatory disease. Indeed, inflammatory mediators including iNOS, COX-2, VEGF and NF-кB are up-regulated in this condition. NF-кB activation evokes cytokines release that can generate more ROS. Moreover, Romeo and colleagues suggested that the activation of NF-кB triggers apoptosis in retinal capillary cells. Other sources of ROS and inflammation in diabetic retinopathy, are the photoreceptors.

3.4.1 The therapeutic potential of melatonin in diabetic retinopathy: nitro-oxidative stress

The impairment of the antioxidant defense system and mitochondrial DNA repair machinery also contributes to the oxidative burden of retina. Certainly, mitochondrial manganese superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase are
decreased in diabetic retina.\textsuperscript{332,336} This effect is due, at least in part, to the fact that DNA-binding activity of Nrf2 is also decreased.\textsuperscript{337}

Regarding DNA repair enzymes, both base excision repair as well as mismatch repair enzymes fail to repair damaged mitochondrial DNA and thus, its replication and transcription is impaired.\textsuperscript{338-340} Consequently, the mitochondrial function is compromised and finally triggers apoptosis of capillary cells.\textsuperscript{338-340}

Considering all these facts, the use of mitochondria-targeted antioxidants seems to be an appropriate strategy to treat diabetic retinopathy. In this context, melatonin acts as a mitochondria-targeted antioxidant and as a retinal cytoprotective agent (Fig. 2).\textsuperscript{56,277-280,341,342}

Certainly, melatonin offers protection from oxidative stress and increases the viability of retinal cells involved in diabetic retinopathy including RPE, photoreceptors, retinal ganglion cells and Müller cells (Fig. 2).\textsuperscript{243,277-280} Furthermore, melatonin prevents the nitro-oxidative stress of diabetic retina\textsuperscript{343-345} and high glucose-induced inflammatory response of RPE as well as retinal endothelial cells (Fig. 2).\textsuperscript{346}

In addition, melatonin may also play a role in the occurrence of diabetic retinopathy. In fact, do Carmo Buonfiglio and colleagues have reported that retinal melatonin synthesis and its content are reduced in diabetic rat retinas.\textsuperscript{347} Various authors have also demonstrated that melatonin secretion is diminished in patients with proliferative diabetic retinopathy.\textsuperscript{348,349} Since melatonin acts as an antioxidant/mitochondrial protector as well as an inflammatory agent, its deficiency may contribute to the progression of diabetic retinopathy.

3.4.2 New therapeutic possibility: melatonin and sirtuins

Epigenetic modifications also contribute to the mitochondrial damage in diabetic retinopathy (Reviewed in\textsuperscript{332}). Oxidative stress produces changes in the acetylation and methylation of histones present at the promoter of genes key in this disease (Reviewed in\textsuperscript{332}).
Consequently, sirtuins play an important role in the occurrence/progression of diabetic retinopathy. In this context, Kowluru and colleagues have demonstrated that diabetes-induced oxidative stress inhibits SIRT1 permitting NF-κB acetylation and thereby, the activation of matrix metalloproteinase-9 (MMP-9) transcription.\(^{330}\) MMP-9 protein triggers apoptosis in the early stages of disease and contributes to angiogenesis in a later phase.\(^{350}\)

Therefore, resveratrol - a stabilizer of SIRT1-substrate interaction - prevents extracellular glutamate accumulation in diabetic rat retinas through modulation of glutamate transporter GLAST and glutamine synthetase expression.\(^{154,351}\) This SIRT1-mediated effect permits the rescue of neuronal cells, which are affected in diabetic retinopathy, from death.\(^{351}\) Indeed, in diabetic retinopathy the glutamatergic system (glutamate receptors and transporters)\(^{352-354}\) and synaptic function (exocytotic proteins)\(^{353}\) are impaired and contribute to excitotoxicity as well as neuronal degeneration. In this context, melatonin as a modulator of sirtuins and anti-excitatory molecule, may be useful to prevent and treat diabetic retinopathy (Fig. 2).\(^{67,113-117}\)

On the other hand, overexpression of SIRT3 down-regulates pro-angiogenic mediators such as MMP-9 as well as VEGF and up-regulates the expression of autophagy-related genes.\(^{355}\)

### 3.4.2 New therapeutic considerations in diabetic retinopathy: melatonin, autophagy and angiogenesis

Autophagy also plays an important role in the onset/progression of diabetic retinopathy, as commented. Shi and colleagues have demonstrated that inhibition of autophagy mediates the activation of NLRP3 inflammasome in RPE cells subjected to high glucose stress.\(^{356}\) Rapamycin, an activator of autophagy, also diminishes VEGF expression as well as oxidative stress in diabetic retinas by blocking the kinase mTOR.\(^{357}\)

All these data suggest that autophagy modulation (e.g. via sirtuins or mTOR) may be a promising therapeutic target for prevention/treatment of proliferative diabetic retinopathy. In this sense, melatonin can modulate sirtuins expression/activity and can induce autophagy via
mTOR-dependent pathway (Fig. 2). Furthermore, some of the sirtuins effects described above, have also been reported for melatonin. Indeed, melatonin treatment of diabetic rats inhibits their MMP-9 expression and VEGF secretion (Fig. 2). Jiang and colleagues have also demonstrated that high glucose-induced VEGF production of Müller cells, which are an important source of retinal VEGF, is reduced by melatonin treatment (Fig. 2). In humans, the treatment with melatonin also reduces the level of VEGF secreted by retinal endothelial and RPE cells subjected to high glucose concentration. Since melatonin reduces the level of VEGF and NO in hypoxic as well as diabetic rat retinas, melatonin can also prevent BRB breakdown (Fig. 2). These facts, once again, confirm the role of melatonin in the occurrence of proliferative diabetic retinopathy, suggesting that administration of melatonin might be beneficial to prevent and treat this disease.

4. EPILOGUE AND PERSPECTIVES

In 2012, the proportion of world population aged 60 or over was 11.5 per cent and United Nations estimates that this percentage will double by 2050 (http://unfpa.org/ageingreport/). Consistent with this trend, scientific community expects a higher prevalence of age-related conditions including eye diseases. Additionally, the World Health Organization (WHO) estimated that the percentage of people visually impaired or blind among the older population for 2010, were 65 and 82 per cent, respectively. Therefore, the researchers are trying to develop effective interventions to maintain ocular health and reduce the burden of age-related eye diseases, including cataract, glaucoma, age-related macular degeneration and diabetic retinopathy. The aforementioned diseases, share underlying molecular processes of aging and other age-related diseases. This fact agrees with the study of Johnson and colleagues that demonstrates the influence of common anti-aging pathways in human age-related diseases. In fact, these ocular pathologies present the nitro-oxidative damage, mainly at mitochondrial level, as a major cause. Furthermore,
several authors have demonstrated that autophagy is impaired in cataract, glaucoma, age-related macular degeneration and diabetic retinopathy diseases, suggesting that autophagy is a contributing factor to their evolution. This fact could be explained by the post-mitotic nature of the main cells involved in the mentioned ocular diseases, where autophagy plays a critical role in removing oxidized proteins or damaged mitochondria and restoring their cellular homeostasis.

Other conditions such as low grade chronic inflammation and angiogenesis also contribute to the development of age-related retinal diseases including glaucoma, age-related macular degeneration and diabetic retinopathy.

In this context, melatonin presents several direct and indirect (e.g. via Nrf2 or sirtuins modulation) actions including antioxidant/antinitridergic, mitochondrial protection, autophagy modulation, anti-inflammatory and anti-angiogenic, in ocular tissues. Consequently, this pleiotropic molecule can represent a powerful tool to prevent and counteract these complex age-related eye diseases. Indeed, some authors have demonstrated its beneficial effects, in human short- clinical studies, of age-related macular degeneration and glaucoma.

In addition to its pleiotropic actions, melatonin possesses an advantage over other possible candidates due to its amphiphilic character, which enables it to enter any fluid, cell or subcellular structures including mitochondria. Furthermore, Crooke and colleagues have demonstrated that the melatonin combination with classical anti-glaucoma drugs potentiates their effects on animals. Therefore, the combination of melatonin with other drugs could improve the efficacy of classical drugs in patients with the mentioned diseases. In fact, the combination therapy is a common strategy in glaucoma and age-related macular degeneration. Moreover, Rivara and colleagues, have reported that the current trend in melatonin invention disclosures is the co-administration of melatonin with other drugs to increase the efficacy of treatment and reduce side-effects. In this context, toxicological studies in humans have demonstrated the safe profile of melatonin, which is an important
requisite for drug combination (summarized in\textsuperscript{370}). Conversely, melatonin exhibits poor pharmacokinetic properties (e.g. limited oral bioavailability and short plasma half-life) and low subtype receptor selectivity.\textsuperscript{371-376} For these reasons, researchers try to develop new melatonin analogues which are more metabolically stable, subtype-selective, and with melatonin beneficial properties.\textsuperscript{369,377} Despite the emerging role of melatonin in age-related ocular pathologies, the bulk of recent patent applications is directed towards sleep disorders and/or circadian rhythm-related illnesses.\textsuperscript{369} In this sense, a reduced number of melatonin analogues (MCA-NAT, I1K7, agomelatine, INS48848, INS48862 and INS48852) have been tested for their \textit{in vivo} ocular hypotensive effect.\textsuperscript{213} Additionally, researchers have demonstrated the neuroprotective action of agomelatine, which is a melatonin analogue approved to treat depression.\textsuperscript{378} Consequently, it is also necessary to characterize in a more detailed way all the properties of these melatonin analogues to determine their efficacy to treat ocular diseases.

Considering all these factors, we can conclude that melatonin, as a single agent or in combination with other drugs, is an attractive pharmacological candidate for cataract, glaucoma, age-related macular degeneration and diabetic retinopathy. Further experimental evidences are needed to support the usefulness of melatonin analogues in these ocular age-related diseases.

\textbf{ACKNOWLEDGEMENTS}

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FIGURES AND TABLES

**FIGURE 1.** Schematic depiction of the multiple intracellular actions of melatonin involved in cellular aging processes. Green and red arrows indicate positive and negative interactions, respectively, between two consecutive steps. Dashed arrow indicates interaction not elucidated. MT₁/MT₂, melatonin membrane receptors; MT₃, putative melatonin receptor; IR/IGFR, insulin/insulin-like growth factor 1 receptor; AC, Adenylate cyclase; PLC, phospholipase C; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; AKT, protein kinase B; ERK, extracellular signal-regulated kinase; JNK, c-Jun amino-terminal kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor erythroid 2-related factor 2; FoxO, forkhead box-O transcription factor; CREB, cAMP response element-binding. The signaling pathways information has been compiled from: 18,32,53,86,379,380.

**FIGURE 2.** Overview of the confirmed and suggested actions of melatonin in age-related ocular diseases.

**TABLE 1.** Anti-aging effects of melatonin and its mediators.

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**TABLE 1. Anti-aging effects of melatonin and its mediators.**

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SIRT1 modulate autophagy process.

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Anti-inflammatory

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