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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 system- leading to loss of homeostasis. [1] 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.[2] 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.[3] ROS, (mainly mitochondrial hydrogen peroxide as well as superoxide anion), induce the autophagy process to reduce oxidative damage.[4,5] 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.[6-9] Consequently, defective mitochondria gradually replace healthy organelles which finally leads to the death of senescence cells due to a lack of ATP.[3]

Furthermore, ROS can promote telomere erosion contributing to cellular aging and thus to the development of age-related diseases.[10-13] Indeed, Salpea and colleagues have shown a link between mitochondrial production of ROS and telomere attrition in type 2 diabetes.[12]

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.[14] 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.[1] 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.[15] 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).[1,15] This protein regulates the expression of genes encoding to pro-inflammatory cytokines and pro-oxidant proteins.[16-18] 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.[18]

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.[19,20] Therefore, melatonin concentrations display a circadian rhythm with low values during the daytime and high values at night.[21] Nocturnal levels of melatonin tend to decrease with age.[22,23] This fact can be explained by the deterioration of aged suprachiasmatic nucleus.[24,25] In addition, patients with age-related diseases such as Alzheimer and type 2 diabetes produce lesser melatonin than age-matched controls.[26,27]

Since many physiological processes display circadian rhythmicity, the reduction of melatonin levels might cause an internal desynchronization, altering body homeostasis.[28]

Melatonin effects are principally associated to its capacity to activate specific membrane receptors termed MT_1, MT_2 and the putative MT_3.[29-31] MT_1 and MT_2 receptors are members of the G protein-coupled hepta-helical receptor (GPCR) family. These receptors can couple to $G_{\alpha i}$ (pertussis toxin-sensitive), $G_{\alpha q}$ (pertussis toxin-insensitive) and $G_{\beta\gamma}$ proteins. Consequently, activation of MT_1/MT_2 receptors triggers different signal cascades (Fig. 1). Classically, melatonin inhibits adenylate cyclase but can also activate phospholipase C or inhibit guanylate cyclase.[32] 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).[32,33] In fact, several authors have suggested that the ERK/MAPK pathway, represents a major component of melatonin signaling during oxidative stress.[34-36] For a more detailed review of melatonin signaling see.[32,33]

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.[37-41]

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).[32,37-44] 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).[45-52] These genomic actions are mediated by redox-sensitive transcription factors such as nuclear factor erythroid

2-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).[47,48] Likewise, melatonin blocks NF-κB activity inhibiting the expression of pro-inflammatory genes such as NOS and cyclooxygenase-2 (COX-2) enzyme (Fig. 1).[47,48] For a specific review of genomics actions of melatonin see.[53]

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).[39,41] 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).[54,55]

Besides this, melatonin improves mitochondrial electron flux, stimulates de novo synthesis of respirasomal subunits, prevents Ca^{2+} overload in mitochondrial matrix, exerts antioxidant activity (e.g. to avoid cardiolipin peroxidation) (actions reviewed in[38,42,56,57]) and promotes mitochondrial biogenesis.[56-59] Again, AFMK and AMK metabolites contribute to the mitochondrial protection effect of melatonin.[41,60,61]

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.[62] Therefore, Guo and colleagues reported the pro-autophagy (and thus cytoprotective) effect of melatonin in ischemia/reperfusion-injured N2a cells.[63] Conversely, Teng and colleagues demonstrated the anti-autophagy (and thus neuroprotective) role of melatonin in neuronal cells of rats treated with arsenite.[59] 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.[64]

2.3 Melatonin and inflammation

Melatonin can also prevent the onset (due to its effects as antioxidant, antinitridergic, 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 *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.[38,42,79] By contrast, and as Carrillo-Vico and colleagues describe, under basal or immunosuppressive conditions melatonin promotes inflammation.[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.[81] Insulin/IGF-1 pathway regulates mitochondrial activity and energy metabolism, therefore it is involved in neuronal survival.[82-84] In fact, activation of this pathway improves mitochondrial function in lymphoblasts from patients with Huntington's disease.[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).[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.[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.[90]

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

modulates insulin secretion[100] and ameliorates insulin sensitivity, principally under insulin-resistant conditions such as in diabetes.[101]

In response to oxidative stress, SIRT1 reduces apoptosis induced by FoxO subtype 3 (FoxO3) factor.[93] Additionally, SIRT1 regulates the expression of antioxidant enzymes through a FoxO-dependent mechanism[102-104] and through mitochondrial NOS enzymes.[105] 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.[81,106]

In relation to the ability to reduce ROS load, sirtuins also inhibit the pro- oxidant and pro-inflammatory factor NF-κB.[95,107] In fact, Schug and colleagues suggest that modulators of sirtuins might be useful for the treatment of chronic inflammation and its associated diseases.[108]

As previously mentioned, oxidative stress can promote telomere attrition.[10-13] SIRT1 can reduce oxidative stress but also, maintains telomere stability (Table 1).[109,110]

Finally, sirtuins link cellular metabolism to the circadian molecular clock machinery. SIRT1 modulates circadian clock gene expression through its association to CLOCK/BMAL1-complex.[111] Furthermore, SIRT3 regulates rhythmicity of mitochondrial activity (Table 1).[112]

Melatonin can modulate SIRT1 expression and subsequently modify the levels of its acetylated substrates (Table 1).[113-117] Gutierrez-Cuesta and colleagues have shown the ability of melatonin to upregulate SIRT1 in SAMP8 mice.[114] In accordance with this result, melatonin induces SIRT1 expression in sleep-deprived rats[113] and in neuronal cultures from aged rat cerebellum.[117] Conversely, melatonin decreases SIRT1 expression under tumoral[115] and inflammatory conditions.[116] 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.[5,128] In the eye, autophagy plays a critical role in maintaining normal cellular function.[129] Indeed, alterations in the autophagy process contribute to the development of age-related ocular diseases.[129]

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.[130] 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.[130]

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.[131] 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.[132] Aged lens cells also present high ROS concentration that contributes to the loss of transparency.[132] 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.[127,133] Concurrently, autophagy and mitophagy processes attempt to restore lens homeostasis but

their failure can often result in more ROS, oxidation and finally in cataract formation.[134] Indeed, deletion of autophagy-related genes causes cataracts.[135]

In addition to age, other factors such as light exposure can also contribute to the oxidative stress-induced mitochondrial damage.[136]

3.1.1 The therapeutic potential of melatonin in cataracts

Melatonin, as a direct and indirect antioxidant, reduces cataract formation in rats (Fig 2).[137-142] This neurohormone reduces the level of lipid peroxidation, enhances the production of glutathione and increases the activity of antioxidant enzymes in rats with cataracts.[137,139,143]

In human lens epithelial cells (HLECs), melatonin inhibits H_2O_2-induced intracellular ROS generation and cell death through the activation of the PI3K/Akt signaling pathway (Fig. 1 and 2).[144] Bai and colleagues have suggested the possible therapeutic role of melatonin to prevent age-related cataracts by these receptor-mediated effects.[144] More recently, Babizhayev and Yegorov claimed a new approach for treating cataracts based on mitochondria-targeted antioxidants.[125] 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.[32] Indeed, MAPK pathway is a key component of melatonin signaling during oxidative stress (Fig. 1).[32] Moreover, MAPK signaling also plays a critical role in lens homeostasis.[145] 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.[145]

Regarding connexins, several authors have demonstrated its importance in cataract development.[145,146] Accumulation of ROS in age lenses alters gap junction function, probably, by oxidative degradation of connexins.[146,147] Wild-type and mutant connexins such as

CX50P88S – a connexin associated with cataracts – are degraded by autophagy.[148] Therefore, autophagy might contribute to these functional alterations of gap junction proteins.[148]

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).[62,149,150]

As mentioned before, ROS can also promote telomere erosion, which contributes to cellular aging process.[10-13] In this context, Huang and colleagues have demonstrated that the oxidative stressor H_2O_2 increases the rate of telomere shortening in HLECs cells.[151] Since aged lenses accumulate ROS, telomere length of lens epithelial cells can be a marker of aging and cataractogenesis.[152] 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).[42,113,114,117,118] Regarding sirtuins, Li and colleagues demonstrated that SIRT1 mRNA level decreases with age and the grade of human age-related cataract.[153] Indeed, a stabilizer of SIRT1-substrate interaction such as resveratrol,[154] prevents cataract formation in rats.[155] Conversely, Zheng and Lu observed a higher level of SIRT1 mRNA and protein in lens of cataract patients than in normal lens.[156]

3.2. Glaucoma

Glaucoma is the second most common cause of visual impairment (2% of global cases) and blindness (8% of global cases) worldwide.[131] 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.[157] The most prevalent type of glaucoma is the primary open-angle glaucoma whose prevalence increases exponentially with age.[158]

Additionally, Tezel and colleagues have suggested that an accelerated aging process accompanies glaucomatous neurodegeneration.[159] Indeed, the tissues involved in glaucoma - retina and trabecular meshwork (TM) - experiment remarkable changes during aging process.[160-165] Consequently, retinal ganglion cells (RGC) of aged mice are more vulnerable to injury than cells of young animals.[165] Regarding TM, their numbers of cells decrease with age and especially in glaucoma condition.[160,162] Furthermore, De la Paz and Epstein have reported an age-dependent decline of superoxide dismutase activity in trabecular tissue.[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.[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.[168-171]

Mitochondria of several ocular cells including TM cells and retinal ganglion cells present molecular and functional changes during glaucoma (reviewed in[172]). Glaucomatous TM cells present a defect in mitochondrial complex I and a higher mitochondrial permeability than normal cells.[173,174] This prompts the generation of more ROS, mitochondrial membrane depolarization as well as impairing intracellular calcium homeostasis.[173,174] Consequently, glaucomatous TM cells are especially vulnerable to calcium stress and thus to cell death.[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.[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.[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.[178,179]

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.[180,181] 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.[180,181]

High IOP has classically been considered the most important cause of glaucomatous damage and currently, it is the only modifiable factor.[157,182] 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.[183,184] 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.[185-189] Mitochondrial dysfunction has also been observed in cultured retinal ganglion cells submitted to elevated hydrostatic pressure.[190] Furthermore, high IOP triggers retinal glutamate receptor (mainly N-methyl-D-aspartate, NMDA receptor but also α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic 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.[191-193] 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.[194,195] 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.[196-198]

On the other hand, elevation of IOP in animals results in a marked activation of microglial and macroglial cells.[199-201] 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.[74,130,164,202-205]

Retinal hypoxia, which can be secondary to or independent of high IOP, is also implicated in the development of glaucoma.[179,206,207] In fact, Kergoat and colleagues have demonstrated that retinal ganglion cells are particularly sensitive to systemic hypoxic stress.[208] 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.[209] Additionally, hypoxic insult provokes glutamate release, which contributes to the retinal ganglion cells damage.[207]

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).[210,211] Indeed, a dysfunction of melatonin signaling triggers elevation of nocturnal IOP and loss of retinal ganglion cells (Fig. 2).[212] Additionally, exogenous melatonin and its analogues lower IOP in mammals, including humans (Fig. 2).[213-221] This ocular hypotensive effect seems to be mediated by ciliary body receptors (MT_2 and an unidentified receptor classically named MT_3) and final inhibition of ciliary chloride secretion.[29,31,222,223] 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.[224-226]

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).[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).[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).[228] In fact, Nrf2 presents antioxidant as well as anti-inflammatory effects, improves mitochondrial function and stimulates autophagy.[229] This transcription factor reduces the effects of many $NO/ONOO^-$ cycle elements (e.g. oxidative stress, iNOS induction, inflammatory cytokines, mitochondrial dysfunction, excitotoxicity and intracellular calcium) in glaucomatous retinal ganglion cells and TM cells.[230] Regarding NO, several authors have demonstrated that NO plays an important role in IOP homeostasis (reviewed in[231]). Consequently, an inhibitor of NOS triggers TM contraction and reduces flow rates through TM.[232-234] Moreover, Ellis and colleagues have suggested that reduction of NO may increase outflow resistance through its effect on Schlemm's canal cells.[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.[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.[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.[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).[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).[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.[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.[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.[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.[249-251] Conversely, other authors have suggested that autophagy promotes retinal ganglion cell death and axonal degeneration.[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.[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.[252] Regarding TM, Pulliero and colleagues have demonstrated that aging promotes the autophagy processes in human TM.[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.[253,254] Nevertheless, since oxidative stress also impairs lysosomal activity and reduces lysosomal acidification, the autophagic flux is finally decreased.[253,254] More recently, Porter and colleagues demonstrated a mTOR-dependent dysregulation of autophagy pathway in human glaucomatous TM cells.[255] Melatonin can induce mTOR pathway and thereby play a role in this process (Fig. 2).[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.[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[258]). The disease affects the macular region of the eye containing photoreceptors, retinal pigment epithelium (RPE), Bruch´s membrane and choriocapillaris.[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.[260] Consequently, RPE dysfunction is a primary event in photoreceptor degeneration and in AMD development.[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.[263-268] Additionally, RPE cells present inadequate systems of antioxidant defense and mitochondrial DNA repair.[269-271] 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.[272,273] 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.[274-276]

Melatonin, as a potent antioxidant/mitochondrial protector, can counteract oxidative stress and increases viability of RPE cells as well as of photoreceptors (Fig. 2).[277-280] This indole-derived component scavenges free radicals but also regulates transcription of antioxidant genes via Nrf2 activation.[32,37-44]

Rosen and colleagues have reported that nocturnal melatonin production is decreased in AMD patients when compared to age-matched controls.[281] 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.[270,282] 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).[270,276,282,283] 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.[284]

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.[5] During aging, they degrade non-functional proteins or protein aggregates and damaged organelles.[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.[285] Phagocytosis of photoreceptor outer segments is another autophagy-related degradation system of RPE.[261] This process guarantees viability of photoreceptors and thus, visual function.[260,286,287] Scütt and colleagues have demonstrated that the impairing of mitochondrial activity diminishes the phagocytic and autophagic capacities of RPE cells.[288] Consequently, aged RPE cells accumulate damaged organelles such as mitochondria as well as non-functional or toxic proteins including the pigment lipofuscin.[261,289] The excessive accumulation of this pigment may be harmful to RPE cells, since it is a toxic photosensitizer.[290-292] In fact, many studies support the idea of a causal relationship between lipofuscin accumulation and AMD onset/progression.[291,293,294] Photooxidation products of the bisretinoids – component of lipofuscin – and of DHA, activate complement system.[294,295] Consequently, they may promote para-inflammation and drusen formation (extracellular deposits predominantly localized beneath RPE that impair its metabolic connection with choroid).[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)[130,296-299] play a reliable role in pathogenesis and progression of AMD (reviewed in[130,258]).

It is well known that the phagocytic and autophagic processes of RPE cells are controlled by circadian rhythms.[300-302] Some studies have demonstrated that melatonin, a key regulator of retinal circadian rhythms, regulates photoreceptor outer segment phagocytosis whereas others have not.[303,304] More recently, Yao and colleagues have demonstrated a circadian and light-dependent modulation of autophagy in photoreceptors.[302] Conversely, autophagy in

RPE cells is an independent-light process but occurs in response to photoreceptor phagocytosis.[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).[305-307] Additionally, melatonin induces melanosome (melanin granule) aggregation in RPE cells and therefore, protects them from light-induced apoptosis (Fig. 2).[308,309] Indeed, RPE melanin pigment absorbs light and scavenges light-induced free radicals acting as an antioxidant agent.[310] In the aging RPE, melanin experiments oxidative changes (it binds lipofuscin) and therefore, loses its photoprotective capacity.[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.[10-13] Indeed, Honda and colleagues demonstrated that RPE aged cells exhibit accelerated telomere shorting.[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).[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.[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.[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-α, 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.

Therefore, abnormal new blood vessels grow and invade vitreous humor that can finally produce blindness (stage of the disease called proliferative diabetic retinopathy).[326] 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.[327,328] Furthermore, oxidative stress underlies the pathogenesis of diabetic retinopathy.[328] In fact, diabetic retinopathy patients present higher levels of plasma lipid peroxidation than diabetes patients without diabetic retinopathy.[328] Retinal enzymatic (e.g. cytosolic NADPH oxidase and arginase activation) and non-enzymatic (mainly via mitochondrial damage) mechanisms produce excessive ROS in diabetic retinopathy.[329-331] 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[332]). Furthermore, inflammatory processes contribute to the development of diabetic retinopathy and so is considered a low grade chronic inflammatory disease.[333] Indeed, inflammatory mediators including iNOS, COX-2, VEGF and NF-κB are up-regulated in this condition.[333] NF-κB activation evokes cytokines release that can generate more ROS. Moreover, Romeo and colleagues suggested that the activation of NF-κB triggers apoptosis in retinal capillary cells.[334] Other sources of ROS and inflammation in diabetic retinopathy, are the photoreceptors.[335]

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.[332,336] This effect is due, at least in part, to the fact that DNA-binding activity of Nrf2 is also decreased.[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.[338-340] Consequently, the mitochondrial function is compromised and finally triggers apoptosis of capillary cells.[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).[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).[243,277-280] Furthermore, melatonin prevents the nitro-oxidative stress of diabetic retina[343-345] and high glucose-induced inflammatory response of RPE as well as retinal endothelial cells (Fig. 2).[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.[347] Various authors have also demonstrated that melatonin secretion is diminished in patients with proliferative diabetic retinopathy.[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[332]). Oxidative stress produces changes in the acetylation and methylation of histones present at the promoter of genes key in this disease (Reviewed in[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).[113-117,256] Furthermore, some of the sirtuins effects described above, have also been reported for melatonin.[243,344-346] Indeed, melatonin treatment of diabetic rats inhibits their MMP-9 expression and VEGF secretion (Fig. 2).[344,345] 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).[243] In humans, the treatment with melatonin also reduces the level of VEGF secreted by retinal endothelial and RPE cells subjected to high glucose concentration.[346] 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).[322,344,345] 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.[358,359] 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.[131] 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 [360]. In fact, these ocular pathologies present the nitro-oxidative damage, mainly at mitochondrial level, as a major cause.[124,125,178,179,262,328] 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.[128,163,250,261,356] 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.[5,129]

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.[130,209,361-364]

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.[217,284]

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.[56,57,365,366] Furthermore, Crooke and colleagues have demonstrated that the melatonin combination with classical anti-glaucoma drugs potentiates their effects on animals.[226] 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.[367,368] 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.[369] In this context, toxicological studies in humans have demonstrated the safe profile of melatonin, which is an important

requisite for drug combination (summarized in[370]). Conversely, melatonin exhibits poor pharmacokinetic properties (e.g. limited oral bioavailability and short plasma half-life) and low subtype receptor selectivity.[371-376] For these reasons, researchers try to develop new melatonin analogues which are more metabolically stable, subtype-selective, and with melatonin beneficial properties.[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.[369] In this sense, a reduced number of melatonin analogues (MCA-NAT, IIK7, agomelatine, INS48848, INS48862 and INS48852) have been tested for their *in vivo* ocular hypotensive effect.[213] Additionally, researchers have demonstrated the neuroprotective action of agomelatine, which is a melatonin analogue approved to treat depression.[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.

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REFERENCES

1. De La Fuente M , Miquel J An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. *Curr Pharm Des*. 2009;15:3003-3026.
2. Harman D Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956;11:298-300.
3. Miquel J, Economos AC, Fleming J et al. Mitochondrial role in cell aging. *Exp Gerontol*. 1980;15:575-591.
4. Mammucari C , Rizzuto R Signaling pathways in mitochondrial dysfunction and aging. *Mech Ageing Dev*. 2010;131:536-543.
5. Scherz-Shouval R , Elazar Z Regulation of autophagy by ROS: physiology and pathology. *Trends Biochem Sci*. 2011;36:30-38.
6. Barton GP, Sepe JJ, Mckiernan SH et al. Mitochondrial and Metabolic Gene Expression in the Aged Rat Heart. *Front Physiol*. 2016;7:352.
7. Cavallini G, Donati A, Taddei M et al. Evidence for selective mitochondrial autophagy and failure in aging. *Autophagy*. 2007;3:26-27.
8. Reznick RM, Zong H, Li J et al. Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis. *Cell Metab*. 2007;5:151-156.
9. Terman A The effect of age on formation and elimination of autophagic vacuoles in mouse hepatocytes. *Gerontology*. 1995;41 Suppl 2:319-326.
10. Liu L, Trimarchi JR, Navarro P et al. Oxidative stress contributes to arsenic-induced telomere attrition, chromosome instability, and apoptosis. *J Biol Chem*. 2003;278:31998-32004.
11. Richter T , Von Zglinicki T A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Exp Gerontol*. 2007;42:1039-1042.
12. Salpea KD, Talmud PJ, Cooper JA et al. Association of telomere length with type 2 diabetes, oxidative stress and UCP2 gene variation. *Atherosclerosis*. 2010;209:42-50.
13. Von Zglinicki T, Saretzki G, Docke W et al. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res*. 1995;220:186-193.
14. Franceschi C, Capri M, Monti D et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev*. 2007;128:92-105.
15. Franceschi C , Campisi J Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci*. 2014;69 Suppl 1:S4-9.
16. Barnes PJ , Karin M Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med*. 1997;336:1066-1071.
17. Freund A, Patil CK , Campisi J p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J*. 2011;30:1536-1548.
18. Morgan MJ , Liu ZG Crosstalk of reactive oxygen species and NF-kappaB signaling. *Cell Res*. 2011;21:103-115.
19. Moore RY , Eichler VB Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res*. 1972;42:201-206.
20. Stephan FK , Zucker I Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci U S A*. 1972;69:1583-1586.
21. Arendt J Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. *Rev Reprod*. 1998;3:13-22.
22. Iguichi H, Kato KI , Ibayashi H Age-dependent reduction in serum melatonin concentrations in healthy human subjects. *J Clin Endocrinol Metab*. 1982;55:27-29.
23. Touitou Y, Fevre M, Lagoguey M et al. Age- and mental health-related circadian rhythms of plasma levels of melatonin, prolactin, luteinizing hormone and follicle-stimulating hormone in man. *J Endocrinol*. 1981;91:467-475.

24. Farajnia S, Michel S, Deboer T et al. Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. *J Neurosci*. 2012;32:5891-5899.
25. Swaab DF, Fliers E , Partiman TS The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res*. 1985;342:37-44.
26. O'brien IA, Lewin IG, O'hare JP et al. Abnormal circadian rhythm of melatonin in diabetic autonomic neuropathy. *Clin Endocrinol (Oxf)*. 1986;24:359-364.
27. Skene DJ, Vivien-Roels B, Sparks DL et al. Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease. *Brain Res*. 1990;528:170-174.
28. Armstrong SM , Redman JR Melatonin: a chronobiotic with anti-aging properties? *Med Hypotheses*. 1991;34:300-309.
29. Alarma-Estrany P, Crooke A , Pintor J 5-MCA-NAT does not act through NQO2 to reduce intraocular pressure in New-Zealand white rabbit. *J Pineal Res*. 2009;47:201-209.
30. Dubocovich ML, Delagrange P, Krause DN et al. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. *Pharmacol Rev*. 2010;62:343-380.
31. Vincent L, Cohen W, Delagrange P et al. Molecular and cellular pharmacological properties of 5-methoxycarbonylamino-N-acetyltryptamine (MCA-NAT): a nonspecific MT3 ligand. *J Pineal Res*. 2010;48:222-229.
32. Luchetti F, Canonico B, Betti M et al. Melatonin signaling and cell protection function. *FASEB J*. 2010;24:3603-3624.
33. Hardeland R Melatonin: signaling mechanisms of a pleiotropic agent. *Biofactors*. 2009;35:183-192.
34. Kimball SR, Abbas A , Jefferson LS Melatonin represses oxidative stress-induced activation of the MAP kinase and mTOR signaling pathways in H4IIE hepatoma cells through inhibition of Ras. *J Pineal Res*. 2008;44:379-386.
35. Luchetti F, Betti M, Canonico B et al. ERK MAPK activation mediates the antiapoptotic signaling of melatonin in UVB-stressed U937 cells. *Free Radic Biol Med*. 2009;46:339-351.
36. Zhu HQ, Cheng XW, Xiao LL et al. Melatonin prevents oxidized low-density lipoprotein-induced increase of myosin light chain kinase activation and expression in HUVEC through ERK/MAPK signal transduction. *J Pineal Res*. 2008;45:328-334.
37. Galano A, Tan DX , Reiter RJ On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. *J Pineal Res*. 2013;54:245-257.
38. Hardeland R, Cardinali DP, Brown GM et al. Melatonin and brain inflammaging. *Prog Neurobiol*. 2015;127-128:46-63.
39. Leon J, Escames G, Rodriguez MI et al. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. *J Neurochem*. 2006;98:2023-2033.
40. Mayo JC, Sainz RM, Tan DX et al. Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. *J Neuroimmunol*. 2005;165:139-149.
41. Tapias V, Escames G, Lopez LC et al. Melatonin and its brain metabolite N(1)-acetyl-5-methoxykynuramine prevent mitochondrial nitric oxide synthase induction in parkinsonian mice. *J Neurosci Res*. 2009;87:3002-3010.
42. Hardeland R Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms. *J Pineal Res*. 2013;55:325-356.
43. Ressmeyer AR, Mayo JC, Zelosko V et al. Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep*. 2003;8:205-213.
44. Tan DX, Manchester LC, Burkhardt S et al. N1-acetyl-N2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. *FASEB J*. 2001;15:2294-2296.

45. Deng WG, Tang ST, Tseng HP et al. Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. *Blood*. 2006;108:518-524.
46. Gilad E, Wong HR, Zingarelli B et al. Melatonin inhibits expression of the inducible isoform of nitric oxide synthase in murine macrophages: role of inhibition of NFkappaB activation. *FASEB J*. 1998;12:685-693.
47. Jung KH, Hong SW, Zheng HM et al. Melatonin downregulates nuclear erythroid 2-related factor 2 and nuclear factor-kappaB during prevention of oxidative liver injury in a dimethylnitrosamine model. *J Pineal Res*. 2009;47:173-183.
48. Jung KH, Hong SW, Zheng HM et al. Melatonin ameliorates cerulein-induced pancreatitis by the modulation of nuclear erythroid 2-related factor 2 and nuclear factor-kappaB in rats. *J Pineal Res*. 2010;48:239-250.
49. Leon J, Macias M, Escames G et al. Structure-related inhibition of calmodulin-dependent neuronal nitric-oxide synthase activity by melatonin and synthetic kynurenines. *Mol Pharmacol*. 2000;58:967-975.
50. Mauriz JL, Molpeceres V, Garcia-Mediavilla MV et al. Melatonin prevents oxidative stress and changes in antioxidant enzyme expression and activity in the liver of aging rats. *J Pineal Res*. 2007;42:222-230.
51. Reiter RJ, Acuna-Castroviejo D, Tan DX et al. Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. *Ann N Y Acad Sci*. 2001;939:200-215.
52. Rodriguez C, Mayo JC, Sainz RM et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res*. 2004;36:1-9.
53. Korkmaz A, Rosales-Corral S , Reiter RJ Gene regulation by melatonin linked to epigenetic phenomena. *Gene*. 2012;503:1-11.
54. Bolanos JP, Almeida A, Stewart V et al. Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J Neurochem*. 1997;68:2227-2240.
55. Tengan CH, Rodrigues GS , Godinho RO Nitric oxide in skeletal muscle: role on mitochondrial biogenesis and function. *Int J Mol Sci*. 2012;13:17160-17184.
56. Ramis MR, Esteban S, Miralles A et al. Protective Effects of Melatonin and Mitochondria-targeted Antioxidants Against Oxidative Stress: A Review. *Curr Med Chem*. 2015;22:2690-2711.
57. Tan DX, Manchester LC, Qin L et al. Melatonin: A Mitochondrial Targeting Molecule Involving Mitochondrial Protection and Dynamics. *Int J Mol Sci*. 2016;17.
58. Kato H, Tanaka G, Masuda S et al. Melatonin promotes adipogenesis and mitochondrial biogenesis in 3T3-L1 preadipocytes. *J Pineal Res*. 2015;59:267-275.
59. Teng YC, Tai YI, Huang HJ et al. Melatonin Ameliorates Arsenite-Induced Neurotoxicity: Involvement of Autophagy and Mitochondria. *Mol Neurobiol*. 2015;52:1015-1022.
60. Acuna-Castroviejo D, Escames G, Leon J et al. Mitochondrial regulation by melatonin and its metabolites. *Adv Exp Med Biol*. 2003;527:549-557.
61. Dragicevic N, Copes N, O'neal-Moffitt G et al. Melatonin treatment restores mitochondrial function in Alzheimer's mice: a mitochondrial protective role of melatonin membrane receptor signaling. *J Pineal Res*. 2011;51:75-86.
62. Coto-Montes A, Boga JA, Rosales-Corral S et al. Role of melatonin in the regulation of autophagy and mitophagy: a review. *Mol Cell Endocrinol*. 2012;361:12-23.
63. Guo Y, Wang J, Wang Z et al. Melatonin protects N2a against ischemia/reperfusion injury through autophagy enhancement. *J Huazhong Univ Sci Technolog Med Sci*. 2010;30:1-7.
64. Caballero B, Vega-Naredo I, Sierra V et al. Autophagy upregulation and loss of NF-kappaB in oxidative stress-related immunodeficient SAMP8 mice. *Mech Ageing Dev*. 2009;130:722-730.
65. Dawson VL , Dawson TM Nitric oxide neurotoxicity. *J Chem Neuroanat*. 1996;10:179-190.

66. Cheng XP, Sun H, Ye ZY et al. Melatonin modulates the GABAergic response in cultured rat hippocampal neurons. *J Pharmacol Sci*. 2012;119:177-185.
67. Chung SY , Han SH Melatonin attenuates kainic acid-induced hippocampal neurodegeneration and oxidative stress through microglial inhibition. *J Pineal Res*. 2003;34:95-102.
68. Escames G, Leon J, Lopez LC et al. Mechanisms of N-methyl-D-aspartate receptor inhibition by melatonin in the rat striatum. *J Neuroendocrinol*. 2004;16:929-935.
69. Paula-Lima AC, Louzada PR, De Mello FG et al. Neuroprotection against Abeta and glutamate toxicity by melatonin: are GABA receptors involved? *Neurotox Res*. 2003;5:323-327.
70. Prada C, Udin SB, Wiechmann AF et al. Stimulation of melatonin receptors decreases calcium levels in xenopus tectal cells by activating GABA(C) receptors. *J Neurophysiol*. 2005;94:968-978.
71. Saenz DA, Goldin AP, Minces L et al. Effect of melatonin on the retinal glutamate/glutamine cycle in the golden hamster retina. *FASEB J*. 2004;18:1912-1913.
72. Zhang M, Cao LH , Yang XL Melatonin modulates glycine currents of retinal ganglion cells in rat. *Neuroreport*. 2007;18:1675-1678.
73. Zhao WJ, Zhang M, Miao Y et al. Melatonin potentiates glycine currents through a PLC/PKC signalling pathway in rat retinal ganglion cells. *J Physiol*. 2010;588:2605-2619.
74. Bogdan C Nitric oxide synthase in innate and adaptive immunity: an update. *Trends Immunol*. 2015;36:161-178.
75. Cardinali DP, Esquifino AI, Srinivasan V et al. Melatonin and the immune system in aging. *Neuroimmunomodulation*. 2008;15:272-278.
76. Carlberg C , Wiesenberg I The orphan receptor family RZR/ROR, melatonin and 5-lipoxygenase: an unexpected relationship. *J Pineal Res*. 1995;18:171-178.
77. Carrillo-Vico A, Calvo JR, Abreu P et al. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. *FASEB J*. 2004;18:537-539.
78. Wiesenberg I, Missbach M , Carlberg C The potential role of the transcription factor RZR/ROR as a mediator of nuclear melatonin signaling. *Restor Neurol Neurosci*. 1998;12:143-150.
79. Cuesta S, Kireev R, Forman K et al. Melatonin improves inflammation processes in liver of senescence-accelerated prone male mice (SAMP8). *Exp Gerontol*. 2010;45:950-956.
80. Carrillo-Vico A, Lardone PJ, Alvarez-Sanchez N et al. Melatonin: buffering the immune system. *Int J Mol Sci*. 2013;14:8638-8683.
81. Jenwitheesuk A, Nopparat C, Mukda S et al. Melatonin regulates aging and neurodegeneration through energy metabolism, epigenetics, autophagy and circadian rhythm pathways. *Int J Mol Sci*. 2014;15:16848-16884.
82. Duarte AI, Santos MS, Oliveira CR et al. Insulin neuroprotection against oxidative stress in cortical neurons--involvement of uric acid and glutathione antioxidant defenses. *Free Radic Biol Med*. 2005;39:876-889.
83. Humbert S, Bryson EA, Cordelieres FP et al. The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. *Dev Cell*. 2002;2:831-837.
84. Yin F, Jiang T , Cadenas E Metabolic triad in brain aging: mitochondria, insulin/IGF-1 signalling and JNK signalling. *Biochem Soc Trans*. 2013;41:101-105.
85. Naia L, Ferreira IL, Cunha-Oliveira T et al. Activation of IGF-1 and insulin signaling pathways ameliorate mitochondrial function and energy metabolism in Huntington's Disease human lymphoblasts. *Mol Neurobiol*. 2015;51:331-348.
86. Picinato MC, Hirata AE, Cipolla-Neto J et al. Activation of insulin and IGF-1 signaling pathways by melatonin through MT1 receptor in isolated rat pancreatic islets. *J Pineal Res*. 2008;44:88-94.

87. Cuesta S, Kireev R, Garcia C et al. Beneficial effect of melatonin treatment on inflammation, apoptosis and oxidative stress on pancreas of a senescence accelerated mice model. *Mech Ageing Dev*. 2011;132:573-582.
88. Nishida S, Segawa T, Murai I et al. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. *J Pineal Res*. 2002;32:26-33.
89. Robeva R, Kirilov G, Tomova A et al. Melatonin-insulin interactions in patients with metabolic syndrome. *J Pineal Res*. 2008;44:52-56.
90. Heras-Sandoval D, Perez-Rojas JM, Hernandez-Damian J et al. The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. *Cell Signal*. 2014;26:2694-2701.
91. Imai S, Armstrong CM, Kaeberlein M et al. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature*. 2000;403:795-800.
92. Landry J, Sutton A, Tafrov ST et al. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci U S A*. 2000;97:5807-5811.
93. Brunet A, Sweeney LB, Sturgill JF et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science*. 2004;303:2011-2015.
94. Motta MC, Divecha N, Lemieux M et al. Mammalian SIRT1 represses forkhead transcription factors. *Cell*. 2004;116:551-563.
95. Yeung F, Hoberg JE, Ramsey CS et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J*. 2004;23:2369-2380.
96. Ramis MR, Esteban S, Miralles A et al. Caloric restriction, resveratrol and melatonin: Role of SIRT1 and implications for aging and related-diseases. *Mech Ageing Dev*. 2015;146-148C:28-41.
97. Sansone L, Reali V, Pellegrini L et al. SIRT1 silencing confers neuroprotection through IGF-1 pathway activation. *J Cell Physiol*. 2013;228:1754-1761.
98. Huang H , Tindall DJ Dynamic FoxO transcription factors. *J Cell Sci*. 2007;120:2479-2487.
99. Lee JH, Song MY, Song EK et al. Overexpression of SIRT1 protects pancreatic beta-cells against cytokine toxicity by suppressing the nuclear factor-kappaB signaling pathway. *Diabetes*. 2009;58:344-351.
100. Bordone L, Motta MC, Picard F et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol*. 2006;4:e31.
101. Sun C, Zhang F, Ge X et al. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell Metab*. 2007;6:307-319.
102. Cheng Y, Takeuchi H, Sonobe Y et al. Sirtuin 1 attenuates oxidative stress via upregulation of superoxide dismutase 2 and catalase in astrocytes. *J Neuroimmunol*. 2014;269:38-43.
103. Hasegawa K, Wakino S, Yoshioka K et al. Sirt1 protects against oxidative stress-induced renal tubular cell apoptosis by the bidirectional regulation of catalase expression. *Biochem Biophys Res Commun*. 2008;372:51-56.
104. Hori YS, Kuno A, Hosoda R et al. Regulation of FOXOs and p53 by SIRT1 modulators under oxidative stress. *PLoS One*. 2013;8:e73875.
105. Csiszar A, Labinskyy N, Pinto JT et al. Resveratrol induces mitochondrial biogenesis in endothelial cells. *Am J Physiol Heart Circ Physiol*. 2009;297:H13-20.
106. Ng F , Tang BL Sirtuins' modulation of autophagy. *J Cell Physiol*. 2013;228:2262-2270.
107. Kauppinen A, Suuronen T, Ojala J et al. Antagonistic crosstalk between NF-kappaB and SIRT1 in the regulation of inflammation and metabolic disorders. *Cell Signal*. 2013;25:1939-1948.
108. Schug TT, Xu Q, Gao H et al. Myeloid deletion of SIRT1 induces inflammatory signaling in response to environmental stress. *Mol Cell Biol*. 2010;30:4712-4721.
109. De Bonis ML, Ortega S , Blasco MA SIRT1 is necessary for proficient telomere elongation and genomic stability of induced pluripotent stem cells. *Stem Cell Reports*. 2014;2:690-706.
110. Palacios JA, Herranz D, De Bonis ML et al. SIRT1 contributes to telomere maintenance and augments global homologous recombination. *J Cell Biol*. 2010;191:1299-1313.

111. Asher G, Gatfield D, Stratmann M et al. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell*. 2008;134:317-328.
112. Peek CB, Affinati AH, Ramsey KM et al. Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. *Science*. 2013;342:1243417.
113. Chang HM, Wu UI , Lan CT Melatonin preserves longevity protein (sirtuin 1) expression in the hippocampus of total sleep-deprived rats. *J Pineal Res*. 2009;47:211-220.
114. Gutierrez-Cuesta J, Tajes M, Jimenez A et al. Evaluation of potential pro-survival pathways regulated by melatonin in a murine senescence model. *J Pineal Res*. 2008;45:497-505.
115. Jung-Hynes B, Schmit TL, Reagan-Shaw SR et al. Melatonin, a novel Sirt1 inhibitor, imparts antiproliferative effects against prostate cancer in vitro in culture and in vivo in TRAMP model. *J Pineal Res*. 2011;50:140-149.
116. Lim HD, Kim YS, Ko SH et al. Cytoprotective and anti-inflammatory effects of melatonin in hydrogen peroxide-stimulated CHON-001 human chondrocyte cell line and rabbit model of osteoarthritis via the SIRT1 pathway. *J Pineal Res*. 2012;53:225-237.
117. Tajes M, Gutierrez-Cuesta J, Ortuno-Sahagun D et al. Anti-aging properties of melatonin in an in vitro murine senescence model: involvement of the sirtuin 1 pathway. *J Pineal Res*. 2009;47:228-237.
118. Akbulut KG, Gonul B , Akbulut H The role of melatonin on gastric mucosal cell proliferation and telomerase activity in ageing. *J Pineal Res*. 2009;47:308-312.
119. Leon-Blanco MM, Guerrero JM, Reiter RJ et al. Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both in vivo and in vitro. *J Pineal Res*. 2003;35:204-211.
120. Martinez-Campa CM, Alonso-Gonzalez C, Mediavilla MD et al. Melatonin down-regulates hTERT expression induced by either natural estrogens (17beta-estradiol) or metalloestrogens (cadmium) in MCF-7 human breast cancer cells. *Cancer Lett*. 2008;268:272-277.
121. Alarma-Estrany PP, J. Melatonin receptors in the eye: location, second messengers and role in ocular physiology. *Pharmacol Ther*. 2007;113:507-522.
122. Alkozi HA, Wang X, Perez De Lara MJ et al. Presence of melanopsin in human crystalline lens epithelial cells and its role in melatonin synthesis. *Exp Eye Res*. 2017;154:168-176.
123. Wiechmann AF, Udin SB , Summers Rada JA Localization of Mel1b melatonin receptor-like immunoreactivity in ocular tissues of Xenopus laevis. *Exp Eye Res*. 2004;79:585-594.
124. Pinazo-Duran MD, Gallego-Pinazo R, Garcia-Medina JJ et al. Oxidative stress and its downstream signaling in aging eyes. *Clin Interv Aging*. 2014;9:637-652.
125. Babizhayev MA , Yegorov YE Reactive Oxygen Species and the Aging Eye: Specific Role of Metabolically Active Mitochondria in Maintaining Lens Function and in the Initiation of the Oxidation-Induced Maturity Onset Cataract--A Novel Platform of Mitochondria-Targeted Antioxidants With Broad Therapeutic Potential for Redox Regulation and Detoxification of Oxidants in Eye Diseases. *Am J Ther*. 2016;23:e98-117.
126. Barot M, Gokulgandhi MR , Mitra AK Mitochondrial dysfunction in retinal diseases. *Curr Eye Res*. 2011;36:1069-1077.
127. Brennan LA , Kantorow M Mitochondrial function and redox control in the aging eye: role of MsrA and other repair systems in cataract and macular degenerations. *Exp Eye Res*. 2009;88:195-203.
128. Pulliero A, Seydel A, Camoirano A et al. Oxidative damage and autophagy in the human trabecular meshwork as related with ageing. *PLoS One*. 2014;9:e98106.
129. Frost LS, Mitchell CH , Boesze-Battaglia K Autophagy in the eye: implications for ocular cell health. *Exp Eye Res*. 2014;124:56-66.
130. Xu H, Chen M , Forrester JV Para-inflammation in the aging retina. *Prog Retin Eye Res*. 2009;28:348-368.
131. Pascolini D , Mariotti SP Global estimates of visual impairment: 2010. *Br J Ophthalmol*. 2012;96:614-618.
132. Petrash JM Aging and age-related diseases of the ocular lens and vitreous body. *Invest Ophthalmol Vis Sci*. 2013;54:ORSF54-59.

133. Harding JJ Free and protein-bound glutathione in normal and cataractous human lenses. *Biochem J*. 1970;117:957-960.
134. Costello MJ, Brennan LA, Basu S et al. Autophagy and mitophagy participate in ocular lens organelle degradation. *Exp Eye Res*. 2013;116:141-150.
135. Morishita H, Eguchi S, Kimura H et al. Deletion of autophagy-related 5 (Atg5) and Pik3c3 genes in the lens causes cataract independent of programmed organelle degradation. *J Biol Chem*. 2013;288:11436-11447.
136. Ji Y, Cai L, Zheng T et al. The mechanism of UVB irradiation induced-apoptosis in cataract. *Mol Cell Biochem*. 2015;401:87-95.
137. Abe M, Reiter RJ, Orhii PB et al. Inhibitory effect of melatonin on cataract formation in newborn rats: evidence for an antioxidative role for melatonin. *J Pineal Res*. 1994;17:94-100.
138. Bardak Y, Ozerturk Y, Ozguner F et al. Effect of melatonin against oxidative stress in ultraviolet-B exposed rat lens. *Curr Eye Res*. 2000;20:225-230.
139. Karslioglu I, Ertekin MV, Taysi S et al. Radioprotective effects of melatonin on radiation-induced cataract. *J Radiat Res*. 2005;46:277-282.
140. Li ZR, Reiter RJ, Fujimori O et al. Cataractogenesis and lipid peroxidation in newborn rats treated with buthionine sulfoximine: preventive actions of melatonin. *J Pineal Res*. 1997;22:117-123.
141. Taysi S, Memisogullari R, Koc M et al. Melatonin reduces oxidative stress in the rat lens due to radiation-induced oxidative injury. *Int J Radiat Biol*. 2008;84:803-808.
142. Yagci R, Aydin B, Erdurmus M et al. Use of melatonin to prevent selenite-induced cataract formation in rat eyes. *Curr Eye Res*. 2006;31:845-850.
143. Shirazi A, Haddadi GH, Asadi-Amoli F et al. Radioprotective effect of melatonin in reducing oxidative stress in rat lenses. *Cell J*. 2011;13:79-82.
144. Bai J, Dong L, Song Z et al. The role of melatonin as an antioxidant in human lens epithelial cells. *Free Radic Res*. 2013;47:635-642.
145. Shakespeare TI, Sellitto C, Li L et al. Interaction between Connexin50 and mitogen-activated protein kinase signaling in lens homeostasis. *Mol Biol Cell*. 2009;20:2582-2592.
146. Gao J, Wang H, Sun X et al. The effects of age on lens transport. *Invest Ophthalmol Vis Sci*. 2013;54:7174-7187.
147. Berthoud VM , Beyer EC Oxidative stress, lens gap junctions, and cataracts. *Antioxid Redox Signal*. 2009;11:339-353.
148. Lichtenstein A, Minogue PJ, Beyer EC et al. Autophagy: a pathway that contributes to connexin degradation. *J Cell Sci*. 2011;124:910-920.
149. Kojima T, Mochizuki C, Mitaka T et al. Effects of melatonin on proliferation, oxidative stress and Cx32 gap junction protein expression in primary cultures of adult rat hepatocytes. *Cell Struct Funct*. 1997;22:347-356.
150. Lee HJ, Lee HJ, Sohn EJ et al. Inhibition of Connexin 26/43 and Extracellular-Regulated Kinase Protein Plays a Critical Role in Melatonin Facilitated Gap Junctional Intercellular Communication in Hydrogen Peroxide-Treated HaCaT Keratinocyte Cells. *Evid Based Complement Alternat Med*. 2012;2012:589365.
151. Huang XQ, Wang J, Liu JP et al. hTERT extends proliferative lifespan and prevents oxidative stress-induced apoptosis in human lens epithelial cells. *Invest Ophthalmol Vis Sci*. 2005;46:2503-2513.
152. Babizhayev MA, Vishnyakova KS , Yegorov YE Telomere-dependent senescent phenotype of lens epithelial cells as a biological marker of aging and cataractogenesis: the role of oxidative stress intensity and specific mechanism of phospholipid hydroperoxide toxicity in lens and aqueous. *Fundam Clin Pharmacol*. 2011;25:139-162.
153. Lin TJ, Peng CH, Chiou SH et al. Severity of lens opacity, age, and correlation of the level of silent information regulator T1 expression in age-related cataract. *J Cataract Refract Surg*. 2011;37:1270-1274.

154. Hou X, Rooklin D, Fang H et al. Resveratrol serves as a protein-substrate interaction stabilizer in human SIRT1 activation. *Sci Rep*. 2016;6:38186.
155. Doganay S, Borazan M, Iraz M et al. The effect of resveratrol in experimental cataract model formed by sodium selenite. *Curr Eye Res*. 2006;31:147-153.
156. Zheng T , Lu Y Changes in SIRT1 expression and its downstream pathways in age-related cataract in humans. *Curr Eye Res*. 2011;36:449-455.
157. Quigley HA Glaucoma. *Lancet*. 2011;377:1367-1377.
158. Leske MC Open-angle glaucoma -- an epidemiologic overview. *Ophthalmic Epidemiol*. 2007;14:166-172.
159. Tezel G, Luo C , Yang X Accelerated aging in glaucoma: immunohistochemical assessment of advanced glycation end products in the human retina and optic nerve head. *Invest Ophthalmol Vis Sci*. 2007;48:1201-1211.
160. Alvarado J, Murphy C , Juster R Trabecular meshwork cellularity in primary open-angle glaucoma and nonglaucomatous normals. *Ophthalmology*. 1984;91:564-579.
161. De La Paz MA , Epstein DL Effect of age on superoxide dismutase activity of human trabecular meshwork. *Invest Ophthalmol Vis Sci*. 1996;37:1849-1853.
162. Grierson I , Howes RC Age-related depletion of the cell population in the human trabecular meshwork. *Eye (Lond)*. 1987;1 (Pt 2):204-210.
163. Munemasa Y , Kitaoka Y Autophagy in axonal degeneration in glaucomatous optic neuropathy. *Prog Retin Eye Res*. 2015;47:1-18.
164. Tezel G The immune response in glaucoma: a perspective on the roles of oxidative stress. *Exp Eye Res*. 2011;93:178-186.
165. Wang AL, Yuan M , Neufeld AH Age-related changes in neuronal susceptibility to damage: comparison of the retinal ganglion cells of young and old mice before and after optic nerve crush. *Ann N Y Acad Sci*. 2007;1097:64-66.
166. Tezel G, Hernandez R , Wax MB Immunostaining of heat shock proteins in the retina and optic nerve head of normal and glaucomatous eyes. *Arch Ophthalmol*. 2000;118:511-518.
167. Tezel G, Seigel GM , Wax MB Autoantibodies to small heat shock proteins in glaucoma. *Invest Ophthalmol Vis Sci*. 1998;39:2277-2287.
168. Bagnis A, Izzotti A, Centofanti M et al. Aqueous humor oxidative stress proteomic levels in primary open angle glaucoma. *Exp Eye Res*. 2012;103:55-62.
169. Fernandez-Durango R, Fernandez-Martinez A, Garcia-Feijoo J et al. Expression of nitrotyrosine and oxidative consequences in the trabecular meshwork of patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2008;49:2506-2511.
170. Neufeld AH, Hernandez MR , Gonzalez M Nitric oxide synthase in the human glaucomatous optic nerve head. *Arch Ophthalmol*. 1997;115:497-503.
171. Rokicki W, Zaba M, Wygledowska-Promienska D et al. Inducible and endothelial nitric synthetase expression and nitrotyrosine accumulation in iris vasculature of patients with primary open-angle glaucoma: a pilot study. *Med Sci Monit*. 2015;21:76-81.
172. Yang XJ, Ge J , Zhuo YH Role of mitochondria in the pathogenesis and treatment of glaucoma. *Chin Med J (Engl)*. 2013;126:4358-4365.
173. He Y, Ge J , Tombran-Tink J Mitochondrial defects and dysfunction in calcium regulation in glaucomatous trabecular meshwork cells. *Invest Ophthalmol Vis Sci*. 2008;49:4912-4922.
174. He Y, Leung KW, Zhang YH et al. Mitochondrial complex I defect induces ROS release and degeneration in trabecular meshwork cells of POAG patients: protection by antioxidants. *Invest Ophthalmol Vis Sci*. 2008;49:1447-1458.
175. Munemasa Y, Ahn JH, Kwong JM et al. Redox proteins thioredoxin 1 and thioredoxin 2 support retinal ganglion cell survival in experimental glaucoma. *Gene Ther*. 2009;16:17-25.
176. Munemasa Y, Kitaoka Y, Kuribayashi J et al. Modulation of mitochondria in the axon and soma of retinal ganglion cells in a rat glaucoma model. *J Neurochem*. 2010;115:1508-1519.

177. Wang Y, Xu K, Zhang H et al. Retinal ganglion cell death is triggered by paraptosis via reactive oxygen species production: a brief literature review presenting a novel hypothesis in glaucoma pathology. *Mol Med Rep*. 2014;10:1179-1183.
178. Kong GY, Van Bergen NJ, Trounce IA et al. Mitochondrial dysfunction and glaucoma. *J Glaucoma*. 2009;18:93-100.
179. Tezel G Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. *Prog Retin Eye Res*. 2006;25:490-513.
180. Osborne NN, Lascaratos G, Bron AJ et al. A hypothesis to suggest that light is a risk factor in glaucoma and the mitochondrial optic neuropathies. *Br J Ophthalmol*. 2006;90:237-241.
181. Wood JP, Lascaratos G, Bron AJ et al. The influence of visible light exposure on cultured RGC-5 cells. *Mol Vis*. 2007;14:334-344.
182. Chen J, Runyan SA , Robinson MR Novel ocular antihypertensive compounds in clinical trials. *Clin Ophthalmol*. 2011;5:667-677.
183. Gabelt BT , Kaufman PL Changes in aqueous humor dynamics with age and glaucoma. *Prog Retin Eye Res*. 2005;24:612-637.
184. Mclaren JW Measurement of aqueous humor flow. *Exp Eye Res*. 2009;88:641-647.
185. Aslan M, Yucel I, Akar Y et al. Nitrotyrosine formation and apoptosis in rat models of ocular injury. *Free Radic Res*. 2006;40:147-153.
186. Ko ML, Peng PH, Ma MC et al. Dynamic changes in reactive oxygen species and antioxidant levels in retinas in experimental glaucoma. *Free Radic Biol Med*. 2005;39:365-373.
187. Mittag TW, Danias J, Pohorenec G et al. Retinal damage after 3 to 4 months of elevated intraocular pressure in a rat glaucoma model. *Invest Ophthalmol Vis Sci*. 2000;41:3451-3459.
188. Moreno MC, Campanelli J, Sande P et al. Retinal oxidative stress induced by high intraocular pressure. *Free Radic Biol Med*. 2004;37:803-812.
189. Tezel G, Yang X , Cai J Proteomic identification of oxidatively modified retinal proteins in a chronic pressure-induced rat model of glaucoma. *Invest Ophthalmol Vis Sci*. 2005;46:3177-3187.
190. Ju WK, Liu Q, Kim KY et al. Elevated hydrostatic pressure triggers mitochondrial fission and decreases cellular ATP in differentiated RGC-5 cells. *Invest Ophthalmol Vis Sci*. 2007;48:2145-2151.
191. Park YH, Broyles HV, He S et al. Involvement of AMPA Receptor and Its Flip and Flop Isoforms in Retinal Ganglion Cell Death Following Oxygen/Glucose Deprivation. *Invest Ophthalmol Vis Sci*. 2016;57:508-526.
192. Sattler R, Xiong Z, Lu WY et al. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science*. 1999;284:1845-1848.
193. Trump BF , Berezesky IK The mechanisms of calcium-mediated cell injury and cell death [correcgted]. *New Horiz*. 1996;4:139-150.
194. Ju WK, Kim KY, Angert M et al. Memantine blocks mitochondrial OPA1 and cytochrome c release and subsequent apoptotic cell death in glaucomatous retina. *Invest Ophthalmol Vis Sci*. 2009;50:707-716.
195. Ju WK, Lindsey JD, Angert M et al. Glutamate receptor activation triggers OPA1 release and induces apoptotic cell death in ischemic rat retina. *Mol Vis*. 2008;14:2629-2638.
196. Kanki R, Nakamizo T, Yamashita H et al. Effects of mitochondrial dysfunction on glutamate receptor-mediated neurotoxicity in cultured rat spinal motor neurons. *Brain Res*. 2004;1015:73-81.
197. Nguyen D, Alavi MV, Kim KY et al. A new vicious cycle involving glutamate excitotoxicity, oxidative stress and mitochondrial dynamics. *Cell Death Dis*. 2011;2:e240.
198. Novelli A, Reilly JA, Lysko PG et al. Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res*. 1988;451:205-212.
199. Inman DM , Horner PJ Reactive nonproliferative gliosis predominates in a chronic mouse model of glaucoma. *Glia*. 2007;55:942-953.

200. Ju KR, Kim HS, Kim JH et al. Retinal glial cell responses and Fas/FasL activation in rats with chronic ocular hypertension. *Brain Res*. 2006;1122:209-221.
201. Woldemussie E, Wijono M , Ruiz G Muller cell response to laser-induced increase in intraocular pressure in rats. *Glia*. 2004;47:109-119.
202. Liu B , Neufeld AH Expression of nitric oxide synthase-2 (NOS-2) in reactive astrocytes of the human glaucomatous optic nerve head. *Glia*. 2000;30:178-186.
203. Neufeld AH Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. *Surv Ophthalmol*. 1999;43 Suppl 1:S129-135.
204. Tezel G , Wax MB Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *J Neurosci*. 2000;20:8693-8700.
205. Yuan L , Neufeld AH Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. *Glia*. 2000;32:42-50.
206. He Z, Vingrys AJ, Armitage JA et al. The role of blood pressure in glaucoma. *Clin Exp Optom*. 2011;94:133-149.
207. Kaur C, Foulds WS , Ling EA Hypoxia-ischemia and retinal ganglion cell damage. *Clin Ophthalmol*. 2008;2:879-889.
208. Kergoat H, Herard ME , Lemay M RGC sensitivity to mild systemic hypoxia. *Invest Ophthalmol Vis Sci*. 2006;47:5423-5427.
209. Kaur C, Foulds WS , Ling EA Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. *Prog Retin Eye Res*. 2008;27:622-647.
210. Chiou GC , Mclaughlin MA Studies on the involvement of melatonergic mechanism in intraocular pressure regulation. *Ophthalmic Res*. 1984;16:302-306.
211. Rohde BH, Mclaughlin MA , Chiou LY Existence and role of endogenous ocular melatonin. *J Ocul Pharmacol*. 1985;1:235-243.
212. Alcantara-Contreras S, Baba K , Tosini G Removal of melatonin receptor type 1 increases intraocular pressure and retinal ganglion cells death in the mouse. *Neurosci Lett*. 2011;494:61-64.
213. Crooke A, Colligris B , Pintor J Update in glaucoma medicinal chemistry: emerging evidence for the importance of melatonin analogues. *Curr Med Chem*. 2012;19:3508-3522.
214. Ismail SA , Mowafi HA Melatonin provides anxiolysis, enhances analgesia, decreases intraocular pressure, and promotes better operating conditions during cataract surgery under topical anesthesia. *Anesth Analg*. 2009;108:1146-1151.
215. Martinez-Aguila A, Fonseca B, Bergua A et al. Melatonin analogue agomelatine reduces rabbit's intraocular pressure in normotensive and hypertensive conditions. *Eur J Pharmacol*. 2013;701:213-217.
216. Martinez-Aguila A, Fonseca B, Perez De Lara MJ et al. Effect of Melatonin and 5-Methoxycarbonylamino-N-Acetyltryptamine on the Intraocular Pressure of Normal and Glaucomatous Mice. *J Pharmacol Exp Ther*. 2016;357:293-299.
217. Pescosolido N, Gatto V, Stefanucci A et al. Oral treatment with the melatonin agonist agomelatine lowers the intraocular pressure of glaucoma patients. *Ophthalmic Physiol Opt*. 2015;35:201-205.
218. Pintor J, Martin L, Pelaez T et al. Involvement of melatonin MT(3) receptors in the regulation of intraocular pressure in rabbits. *Eur J Pharmacol*. 2001;416:251-254.
219. Pintor J, Pelaez T, Hoyle CH et al. Ocular hypotensive effects of melatonin receptor agonists in the rabbit: further evidence for an MT3 receptor. *Br J Pharmacol*. 2003;138:831-836.
220. Samples JR, Krause G , Lewy AJ Effect of melatonin on intraocular pressure. *Curr Eye Res*. 1988;7:649-653.
221. Serle JB, Wang RF, Peterson WM et al. Effect of 5-MCA-NAT, a putative melatonin MT3 receptor agonist, on intraocular pressure in glaucomatous monkey eyes. *J Glaucoma*. 2004;13:385-388.

222. Alarma-Estrany P, Crooke A, Mediero A et al. Sympathetic nervous system modulates the ocular hypotensive action of MT2-melatonin receptors in normotensive rabbits. *J Pineal Res*. 2008;45:468-475.
223. Huete-Toral F, Crooke A, Martinez-Aguila A et al. Melatonin receptors trigger cAMP production and inhibit chloride movements in nonpigmented ciliary epithelial cells. *J Pharmacol Exp Ther*. 2015;352:119-128.
224. Crooke A, Huete-Toral F, Martinez-Aguila A et al. Regulation of ocular adrenoceptor genes expression by 5-MCA-NAT: implications for glaucoma treatment. *Pharmacogenet Genomics*. 2011;21:587-589.
225. Crooke A, Huete-Toral F, Martinez-Aguila A et al. Involvement of carbonic anhydrases in the ocular hypotensive effect of melatonin analogue 5-MCA-NAT. *J Pineal Res*. 2012;52:265-270.
226. Crooke A, Huete-Toral F, Martinez-Aguila A et al. Melatonin and its analog 5-methoxycarbonylamino-N-acetyltryptamine potentiate adrenergic receptor-mediated ocular hypotensive effects in rabbits: significance for combination therapy in glaucoma. *J Pharmacol Exp Ther*. 2013;346:138-145.
227. Belforte NA, Moreno MC, De Zavalia N et al. Melatonin: a novel neuroprotectant for the treatment of glaucoma. *J Pineal Res*. 2010;48:353-364.
228. Himori N, Yamamoto K, Maruyama K et al. Critical role of Nrf2 in oxidative stress-induced retinal ganglion cell death. *J Neurochem*. 2013;127:669-680.
229. Pall ML , Levine S Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors. *Sheng Li Xue Bao*. 2015;67:1-18.
230. Pall ML Is Open-angle Glaucoma Caused by the NO/ONOO(-) Cycle Acting at Two Locations in the Eye? *Med Hypothesis Discov Innov Ophthalmol*. 2014;3:1-2.
231. Cavet ME, Vittitow JL, Impagnatiello F et al. Nitric oxide (NO): an emerging target for the treatment of glaucoma. *Invest Ophthalmol Vis Sci*. 2014;55:5005-5015.
232. Schneemann A, Dijkstra BG, Van Den Berg TJ et al. Nitric oxide/guanylate cyclase pathways and flow in anterior segment perfusion. *Graefes Arch Clin Exp Ophthalmol*. 2002;240:936-941.
233. Schneemann A, Leusink-Muis A, Van Den Berg T et al. Elevation of nitric oxide production in human trabecular meshwork by increased pressure. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:321-326.
234. Wiederholt M, Sturm A , Lepple-Wienhues A Relaxation of trabecular meshwork and ciliary muscle by release of nitric oxide. *Invest Ophthalmol Vis Sci*. 1994;35:2515-2520.
235. Ellis DZ, Sharif NA , Dismuke WM Endogenous regulation of human Schlemm's canal cell volume by nitric oxide signaling. *Invest Ophthalmol Vis Sci*. 2010;51:5817-5824.
236. Alkozi H, Sanchez-Naves J, De Lara MJ et al. Elevated intraocular pressure increases melatonin levels in the aqueous humour. *Acta Ophthalmol*. 2016.
237. Chiquet C, Claustrat B, Thuret G et al. Melatonin concentrations in aqueous humor of glaucoma patients. *Am J Ophthalmol*. 2006;142:325-327 e321.
238. Khan RS, Dine K, Das Sarma J et al. SIRT1 activating compounds reduce oxidative stress mediated neuronal loss in viral induced CNS demyelinating disease. *Acta Neuropathol Commun*. 2014;2:3.
239. Ren PL, Fan XJ, Yang XL et al. [SIRT1 promote GTM cell DSBs repair and resist cellular senescence]. *Sichuan Da Xue Xue Bao Yi Xue Ban*. 2014;45:572-577.
240. Zuo L, Khan RS, Lee V et al. SIRT1 promotes RGC survival and delays loss of function following optic nerve crush. *Invest Ophthalmol Vis Sci*. 2013;54:5097-5102.
241. Aranda ML, Gonzalez Fleitas MF, De Laurentiis A et al. Neuroprotective effect of melatonin in experimental optic neuritis in rats. *J Pineal Res*. 2016;60:360-372.
242. Park SW, Lee HS, Sung MS et al. The effect of melatonin on retinal ganglion cell survival in ischemic retina. *Chonnam Med J*. 2012;48:116-122.

243. Jiang T, Chang Q, Zhao Z et al. Melatonin-mediated cytoprotection against hyperglycemic injury in Muller cells. *PLoS One*. 2012;7:e50661.
244. Coughlin L, Morrison RS, Horner PJ et al. Mitochondrial morphology differences and mitophagy deficit in murine glaucomatous optic nerve. *Invest Ophthalmol Vis Sci*. 2015;56:1437-1446.
245. Deng S, Wang M, Yan Z et al. Autophagy in retinal ganglion cells in a rhesus monkey chronic hypertensive glaucoma model. *PLoS One*. 2013;8:e77100.
246. Knoferle J, Koch JC, Ostendorf T et al. Mechanisms of acute axonal degeneration in the optic nerve in vivo. *Proc Natl Acad Sci U S A*. 2010;107:6064-6069.
247. Park HY, Kim JH , Park CK Activation of autophagy induces retinal ganglion cell death in a chronic hypertensive glaucoma model. *Cell Death Dis*. 2012;3:e290.
248. Piras A, Gianetto D, Conte D et al. Activation of autophagy in a rat model of retinal ischemia following high intraocular pressure. *PLoS One*. 2011;6:e22514.
249. Rodriguez-Muela N, Germain F, Marino G et al. Autophagy promotes survival of retinal ganglion cells after optic nerve axotomy in mice. *Cell Death Differ*. 2012;19:162-169.
250. Lin WJ , Kuang HY Oxidative stress induces autophagy in response to multiple noxious stimuli in retinal ganglion cells. *Autophagy*. 2014;10:1692-1701.
251. Su W, Li Z, Jia Y et al. Rapamycin is neuroprotective in a rat chronic hypertensive glaucoma model. *PLoS One*. 2014;9:e99719.
252. Tezel G, Yang X, Luo C et al. An astrocyte-specific proteomic approach to inflammatory responses in experimental rat glaucoma. *Invest Ophthalmol Vis Sci*. 2012;53:4220-4233.
253. Liton PB, Lin Y, Luna C et al. Cultured porcine trabecular meshwork cells display altered lysosomal function when subjected to chronic oxidative stress. *Invest Ophthalmol Vis Sci*. 2008;49:3961-3969.
254. Porter K, Nallathambi J, Lin Y et al. Lysosomal basification and decreased autophagic flux in oxidatively stressed trabecular meshwork cells: implications for glaucoma pathogenesis. *Autophagy*. 2013;9:581-594.
255. Porter K, Hirt J, Stamer WD et al. Autophagic dysregulation in glaucomatous trabecular meshwork cells. *Biochim Biophys Acta*. 2015;1852:379-385.
256. Choi SI, Kim KS, Oh JY et al. Melatonin induces autophagy via an mTOR-dependent pathway and enhances clearance of mutant-TGFBIp. *J Pineal Res*. 2013;54:361-372.
257. Jonas JB, Bourne RR, White RA et al. Visual impairment and blindness due to macular diseases globally: a systematic review and meta-analysis. *Am J Ophthalmol*. 2014;158:808-815.
258. Parmeggiani F, Romano MR, Costagliola C et al. Mechanism of inflammation in age-related macular degeneration. *Mediators Inflamm*. 2012;2012:546786.
259. De Jong PT Age-related macular degeneration. *N Engl J Med*. 2006;355:1474-1485.
260. Strauss O The retinal pigment epithelium in visual function. *Physiol Rev*. 2005;85:845-881.
261. Ferrington DA, Sinha D , Kaarniranta K Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration. *Prog Retin Eye Res*. 2016;51:69-89.
262. Nowak JZ Oxidative stress, polyunsaturated fatty acids-derived oxidation products and bisretinoids as potential inducers of CNS diseases: focus on age-related macular degeneration. *Pharmacol Rep*. 2013;65:288-304.
263. Godley BF, Shamsi FA, Liang FQ et al. Blue light induces mitochondrial DNA damage and free radical production in epithelial cells. *J Biol Chem*. 2005;280:21061-21066.
264. Gottsch JD, Pou S, Bynoe LA et al. Hematogenous photosensitization. A mechanism for the development of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1990;31:1674-1682.
265. Jarrett SG, Lin H, Godley BF et al. Mitochondrial DNA damage and its potential role in retinal degeneration. *Prog Retin Eye Res*. 2008;27:596-607.

266. Jin GF, Hurst JS , Godley BF Rod outer segments mediate mitochondrial DNA damage and apoptosis in human retinal pigment epithelium. *Curr Eye Res*. 2001;23:11-19.
267. Miceli MV, Liles MR , Newsome DA Evaluation of oxidative processes in human pigment epithelial cells associated with retinal outer segment phagocytosis. *Exp Cell Res*. 1994;214:242-249.
268. Rozanowska M, Korytowski W, Rozanowski B et al. Photoreactivity of aged human RPE melanosomes: a comparison with lipofuscin. *Invest Ophthalmol Vis Sci*. 2002;43:2088-2096.
269. He Y , Tombran-Tink J Mitochondrial decay and impairment of antioxidant defenses in aging RPE cells. *Adv Exp Med Biol*. 2010;664:165-183.
270. Sachdeva MM, Cano M , Handa JT Nrf2 signaling is impaired in the aging RPE given an oxidative insult. *Exp Eye Res*. 2014;119:111-114.
271. Wang AL, Lukas TJ, Yuan M et al. Increased mitochondrial DNA damage and down-regulation of DNA repair enzymes in aged rodent retinal pigment epithelium and choroid. *Mol Vis*. 2008;14:644-651.
272. Feher J, Kovacs I, Artico M et al. Mitochondrial alterations of retinal pigment epithelium in age-related macular degeneration. *Neurobiol Aging*. 2006;27:983-993.
273. Nordgaard CL, Karunadharma PP, Feng X et al. Mitochondrial proteomics of the retinal pigment epithelium at progressive stages of age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2008;49:2848-2855.
274. Karunadharma PP, Nordgaard CL, Olsen TW et al. Mitochondrial DNA damage as a potential mechanism for age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2010;51:5470-5479.
275. Lin H, Xu H, Liang FQ et al. Mitochondrial DNA damage and repair in RPE associated with aging and age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:3521-3529.
276. Terluk MR, Kapphahn RJ, Soukup LM et al. Investigating mitochondria as a target for treating age-related macular degeneration. *J Neurosci*. 2015;35:7304-7311.
277. Fu Y, Tang M, Fan Y et al. Anti-apoptotic effects of melatonin in retinal pigment epithelial cells. *Front Biosci (Landmark Ed)*. 2012;17:1461-1468.
278. Liang FQ, Aleman TS, Zaixinyang et al. Melatonin delays photoreceptor degeneration in the rds/rds mouse. *Neuroreport*. 2001;12:1011-1014.
279. Liang FQ, Green L, Wang C et al. Melatonin protects human retinal pigment epithelial (RPE) cells against oxidative stress. *Exp Eye Res*. 2004;78:1069-1075.
280. Marchiafava PL , Longoni B Melatonin as an antioxidant in retinal photoreceptors. *J Pineal Res*. 1999;26:184-189.
281. Rosen R, Hu DN, Perez V et al. Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. *Mol Vis*. 2009;15:1673-1679.
282. Tokarz P, Piastowska-Ciesielska AW, Kaarniranta K et al. All-Trans Retinoic Acid Modulates DNA Damage Response and the Expression of the VEGF-A and MKI67 Genes in ARPE-19 Cells Subjected to Oxidative Stress. *Int J Mol Sci*. 2016;17.
283. Tokarz P, Kaarniranta K , Blasiak J Role of antioxidant enzymes and small molecular weight antioxidants in the pathogenesis of age-related macular degeneration (AMD). *Biogerontology*. 2013;14:461-482.
284. Yi C, Pan X, Yan H et al. Effects of melatonin in age-related macular degeneration. *Ann N Y Acad Sci*. 2005;1057:384-392.
285. Mitter SK, Song C, Qi X et al. Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. *Autophagy*. 2014;10:1989-2005.
286. D'cruz PM, Yasumura D, Weir J et al. Mutation of the receptor tyrosine kinase gene Mertk in the retinal dystrophic RCS rat. *Hum Mol Genet*. 2000;9:645-651.
287. Gal A, Li Y, Thompson DA et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet*. 2000;26:270-271.

288. Schutt F, Aretz S, Auffarth GU et al. Moderately reduced ATP levels promote oxidative stress and debilitate autophagic and phagocytic capacities in human RPE cells. *Invest Ophthalmol Vis Sci*. 2012;53:5354-5361.
289. Kaarniranta K, Tokarz P, Koskela A et al. Autophagy regulates death of retinal pigment epithelium cells in age-related macular degeneration. *Cell Biol Toxicol*. 2016.
290. Schutt F, Davies S, Kopitz J et al. Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci*. 2000;41:2303-2308.
291. Sparrow JR , Boulton M RPE lipofuscin and its role in retinal pathobiology. *Exp Eye Res*. 2005;80:595-606.
292. Sparrow JR, Nakanishi K , Parish CA The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells. *Invest Ophthalmol Vis Sci*. 2000;41:1981-1989.
293. Zhou J, Cai B, Jang YP et al. Mechanisms for the induction of HNE- MDA- and AGE-adducts, RAGE and VEGF in retinal pigment epithelial cells. *Exp Eye Res*. 2005;80:567-580.
294. Zhou J, Jang YP, Kim SR et al. Complement activation by photooxidation products of A2E, a lipofuscin constituent of the retinal pigment epithelium. *Proc Natl Acad Sci U S A*. 2006;103:16182-16187.
295. Hollyfield JG, Bonilha VL, Rayborn ME et al. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med*. 2008;14:194-198.
296. Scholl HP, Charbel Issa P, Walier M et al. Systemic complement activation in age-related macular degeneration. *PLoS One*. 2008;3:e2593.
297. Tseng WA, Thein T, Kinnunen K et al. NLRP3 inflammasome activation in retinal pigment epithelial cells by lysosomal destabilization: implications for age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2013;54:110-120.
298. Wang AL, Lukas TJ, Yuan M et al. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. *PLoS One*. 2009;4:e4160.
299. Wang Y, Hanus JW, Abu-Asab MS et al. NLRP3 Upregulation in Retinal Pigment Epithelium in Age-Related Macular Degeneration. *Int J Mol Sci*. 2016;17.
300. Besharse JC, Hollyfield JG , Rayborn ME Photoreceptor outer segments: accelerated membrane renewal in rods after exposure to light. *Science*. 1977;196:536-538.
301. Lavail MM Rod outer segment disk shedding in rat retina: relationship to cyclic lighting. *Science*. 1976;194:1071-1074.
302. Yao J, Jia L, Shelby SJ et al. Circadian and noncircadian modulation of autophagy in photoreceptors and retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 2014;55:3237-3246.
303. Besharse JC , Dunis DA Methoxyindoles and photoreceptor metabolism: activation of rod shedding. *Science*. 1983;219:1341-1343.
304. Grace MS, Chiba A , Menaker M Circadian control of photoreceptor outer segment membrane turnover in mice genetically incapable of melatonin synthesis. *Vis Neurosci*. 1999;16:909-918.
305. Organisciak DT, Darrow RM, Barsalou L et al. Circadian-dependent retinal light damage in rats. *Invest Ophthalmol Vis Sci*. 2000;41:3694-3701.
306. Sugawara T, Sieving PA, Iuvone PM et al. The melatonin antagonist luzindole protects retinal photoreceptors from light damage in the rat. *Invest Ophthalmol Vis Sci*. 1998;39:2458-2465.
307. Wiechmann AF , O'steen WK Melatonin increases photoreceptor susceptibility to light-induced damage. *Invest Ophthalmol Vis Sci*. 1992;33:1894-1902.
308. Pang SF , Yew DT Pigment aggregation by melatonin in the retinal pigment epithelium and choroid of guinea-pigs, Caviaporcellus. *Experientia*. 1979;35:231-233.
309. Seagle BL, Rezai KA, Kobori Y et al. Melanin photoprotection in the human retinal pigment epithelium and its correlation with light-induced cell apoptosis. *Proc Natl Acad Sci U S A*. 2005;102:8978-8983.

310. Sarna T Properties and function of the ocular melanin--a photobiophysical view. *J Photochem Photobiol B*. 1992;12:215-258.
311. Sarna T, Burke JM, Korytowski W et al. Loss of melanin from human RPE with aging: possible role of melanin photooxidation. *Exp Eye Res*. 2003;76:89-98.
312. Honda S, Hjelmeland LM , Handa JT Oxidative stress--induced single-strand breaks in chromosomal telomeres of human retinal pigment epithelial cells in vitro. *Invest Ophthalmol Vis Sci*. 2001;42:2139-2144.
313. Lau BW, Tsao GS, So KF et al. Expression of telomerase reverse transcriptase in adult goldfish retina. *J Mol Neurosci*. 2007;32:160-167.
314. Blasiak J, Reiter RJ , Kaarniranta K Melatonin in Retinal Physiology and Pathology: The Case of Age-Related Macular Degeneration. *Oxid Med Cell Longev*. 2016;2016:6819736.
315. Rastmanesh R Potential of melatonin to treat or prevent age-related macular degeneration through stimulation of telomerase activity. *Med Hypotheses*. 2011;76:79-85.
316. Wilkinson-Berka Jennifer l, Rana I, Armani R et al. Reactive oxygen species, Nox and angiotensin II in angiogenesis: implications for retinopathy. *Clinical Science*. 2013;124:597-615.
317. Atienzar-Aroca S, Flores-Bellver M, Serrano-Heras G et al. Oxidative stress in retinal pigment epithelium cells increases exosome secretion and promotes angiogenesis in endothelial cells. *J Cell Mol Med*. 2016;20:1457-1466.
318. Ebrahem Q, Renganathan K, Sears J et al. Carboxyethylpyrrole oxidative protein modifications stimulate neovascularization: Implications for age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2006;103:13480-13484.
319. Higgins GT, Wang JH, Dockery P et al. Induction of angiogenic cytokine expression in cultured RPE by ingestion of oxidized photoreceptor outer segments. *Invest Ophthalmol Vis Sci*. 2003;44:1775-1782.
320. Espinosa-Heidmann DG, Suner IJ, Hernandez EP et al. Macrophage depletion diminishes lesion size and severity in experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci*. 2003;44:3586-3592.
321. Wang H, Han X, Wittchen ES et al. TNF-alpha mediates choroidal neovascularization by upregulating VEGF expression in RPE through ROS-dependent beta-catenin activation. *Mol Vis*. 2016;22:116-128.
322. Kaur C, Sivakumar V, Yong Z et al. Blood-retinal barrier disruption and ultrastructural changes in the hypoxic retina in adult rats: the beneficial effect of melatonin administration. *J Pathol*. 2007;212:429-439.
323. Vedula SS , Krzystolik MG Antiangiogenic therapy with anti-vascular endothelial growth factor modalities for neovascular age-related macular degeneration. *Cochrane Database Syst Rev*. 2008:CD005139.
324. Amoaku WM, Chakravarthy U, Gale R et al. Defining response to anti-VEGF therapies in neovascular AMD. *Eye (Lond)*. 2015;29:721-731.
325. Yang S, Zhao J , Sun X Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. *Drug Des Devel Ther*. 2016;10:1857-1867.
326. Crawford TN, Alfaro DV, 3rd, Kerrison JB et al. Diabetic retinopathy and angiogenesis. *Curr Diabetes Rev*. 2009;5:8-13.
327. Corriere M, Rooparinesingh N , Kalyani RR Epidemiology of diabetes and diabetes complications in the elderly: an emerging public health burden. *Curr Diab Rep*. 2013;13:805-813.
328. Gurler B, Vural H, Yilmaz N et al. The role of oxidative stress in diabetic retinopathy. *Eye (Lond)*. 2000;14 Pt 5:730-735.
329. Kanwar M, Chan PS, Kern TS et al. Oxidative damage in the retinal mitochondria of diabetic mice: possible protection by superoxide dismutase. *Invest Ophthalmol Vis Sci*. 2007;48:3805-3811.

330. Kowluru RA, Santos JM , Zhong Q Sirt1, a negative regulator of matrix metalloproteinase-9 in diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2014;55:5653-5660.
331. Narayanan SP, Rojas M, Suwanpradid J et al. Arginase in retinopathy. *Prog Retin Eye Res*. 2013;36:260-280.
332. Kowluru RA , Mishra M Oxidative stress, mitochondrial damage and diabetic retinopathy. *Biochim Biophys Acta*. 2015;1852:2474-2483.
333. Kern TS Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res*. 2007;2007:95103.
334. Romeo G, Liu WH, Asnaghi V et al. Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes*. 2002;51:2241-2248.
335. Du Y, Veenstra A, Palczewski K et al. Photoreceptor cells are major contributors to diabetes-induced oxidative stress and local inflammation in the retina. *Proc Natl Acad Sci U S A*. 2013;110:16586-16591.
336. Kowluru RA, Kern TS , Engerman RL Abnormalities of retinal metabolism in diabetes or experimental galactosemia. IV. Antioxidant defense system. *Free Radic Biol Med*. 1997;22:587-592.
337. Zhong Q, Mishra M , Kowluru RA Transcription factor Nrf2-mediated antioxidant defense system in the development of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2013;54:3941-3948.
338. Madsen-Bouterse SA, Zhong Q, Mohammad G et al. Oxidative damage of mitochondrial DNA in diabetes and its protection by manganese superoxide dismutase. *Free Radic Res*. 2010;44:313-321.
339. Mishra M , Kowluru RA Retinal mitochondrial DNA mismatch repair in the development of diabetic retinopathy, and its continued progression after termination of hyperglycemia. *Invest Ophthalmol Vis Sci*. 2014;55:6960-6967.
340. Santos JM, Tewari S , Kowluru RA A compensatory mechanism protects retinal mitochondria from initial insult in diabetic retinopathy. *Free Radic Biol Med*. 2012;53:1729-1737.
341. Jiang T, Chang Q, Cai J et al. Protective Effects of Melatonin on Retinal Inflammation and Oxidative Stress in Experimental Diabetic Retinopathy. *Oxid Med Cell Longev*. 2016;2016:3528274.
342. Kaur C, Sivakumar V, Robinson R et al. Neuroprotective effect of melatonin against hypoxia-induced retinal ganglion cell death in neonatal rats. *J Pineal Res*. 2013;54:190-206.
343. Baydas G, Tuzcu M, Yasar A et al. Early changes in glial reactivity and lipid peroxidation in diabetic rat retina: effects of melatonin. *Acta Diabetol*. 2004;41:123-128.
344. Ozdemir G, Ergun Y, Bakaris S et al. Melatonin prevents retinal oxidative stress and vascular changes in diabetic rats. *Eye (Lond)*. 2014;28:1020-1027.
345. Salido EM, Bordone M, De Laurentiis A et al. Therapeutic efficacy of melatonin in reducing retinal damage in an experimental model of early type 2 diabetes in rats. *J Pineal Res*. 2013;54:179-189.
346. Xie M, Hu A, Luo Y et al. Interleukin-4 and melatonin ameliorate high glucose and interleukin-1beta stimulated inflammatory reaction in human retinal endothelial cells and retinal pigment epithelial cells. *Mol Vis*. 2014;20:921-928.
347. Do Carmo Buonfiglio D, Peliciari-Garcia RA, Do Amaral FG et al. Early-stage retinal melatonin synthesis impairment in streptozotocin-induced diabetic wistar rats. *Invest Ophthalmol Vis Sci*. 2011;52:7416-7422.
348. Chen H, Liu X, Zhu W et al. SIRT1 ameliorates age-related senescence of mesenchymal stem cells via modulating telomere shelterin. *Front Aging Neurosci*. 2014;6:103.
349. Hikichi T, Tateda N , Miura T Alteration of melatonin secretion in patients with type 2 diabetes and proliferative diabetic retinopathy. *Clin Ophthalmol*. 2011;5:655-660.
350. Kowluru RA, Zhong Q , Santos JM Matrix metalloproteinases in diabetic retinopathy: potential role of MMP-9. *Expert Opin Investig Drugs*. 2012;21:797-805.

351. Zeng K, Yang N, Wang D et al. Resveratrol Prevents Retinal Dysfunction by Regulating Glutamate Transporters, Glutamine Synthetase Expression and Activity in Diabetic Retina. *Neurochem Res*. 2016;41:1050-1064.
352. Bai N, Aida T, Yanagisawa M et al. NMDA receptor subunits have different roles in NMDA-induced neurotoxicity in the retina. *Mol Brain*. 2013;6:34.
353. Baptista FI, Castilho AF, Gaspar JM et al. Long-term exposure to high glucose increases the content of several exocytotic proteins and of vesicular GABA transporter in cultured retinal neural cells. *Neurosci Lett*. 2015;602:56-61.
354. Semkova I, Huemmeke M, Ho MS et al. Retinal localization of the glutamate receptor GluR2 and GluR2-regulating proteins in diabetic rats. *Exp Eye Res*. 2010;90:244-253.
355. Mao XB, You ZP, Wu C et al. Potential suppression of the high glucose and insulin-induced retinal neovascularization by Sirtuin 3 in the human retinal endothelial cells. *Biochem Biophys Res Commun*. 2017;482:341-345.
356. Shi H, Zhang Z, Wang X et al. Inhibition of autophagy induces IL-1beta release from ARPE-19 cells via ROS mediated NLRP3 inflammasome activation under high glucose stress. *Biochem Biophys Res Commun*. 2015;463:1071-1076.
357. Ozdemir G, Kilinc M, Ergun Y et al. Rapamycin inhibits oxidative and angiogenic mediators in diabetic retinopathy. *Can J Ophthalmol*. 2014;49:443-449.
358. Akpek EK , Smith RA Overview of age-related ocular conditions. *Am J Manag Care*. 2013;19:S67-75.
359. Burch JB, Augustine AD, Frieden LA et al. Advances in geroscience: impact on healthspan and chronic disease. *J Gerontol A Biol Sci Med Sci*. 2014;69 Suppl 1:S1-3.
360. Johnson SC, Dong X, Vijg J et al. Genetic evidence for common pathways in human age-related diseases. *Aging Cell*. 2015;14:809-817.
361. Bai Y, Ma JX, Guo J et al. Muller cell-derived VEGF is a significant contributor to retinal neovascularization. *J Pathol*. 2009;219:446-454.
362. Querques G, Rosenfeld PJ, Cavallero E et al. Treatment of dry age-related macular degeneration. *Ophthalmic Res*. 2014;52:107-115.
363. Rodrigues GB, Abe RY, Zangalli C et al. Neovascular glaucoma: a review. *Int J Retina Vitreous*. 2016;2:26.
364. Tezel G, Yang X, Luo C et al. Oxidative stress and the regulation of complement activation in human glaucoma. *Invest Ophthalmol Vis Sci*. 2010;51:5071-5082.
365. Ceraulo L, Ferrugia M, Tesoriere L et al. Interactions of melatonin with membrane models: portioning of melatonin in AOT and lecithin reversed micelles. *J Pineal Res*. 1999;26:108-112.
366. Shida CS, Castrucci AM , Lamy-Freund MT High melatonin solubility in aqueous medium. *J Pineal Res*. 1994;16:198-201.
367. Englander M , Kaiser PK Combination therapy for the treatment of neovascular age-related macular degeneration. *Curr Opin Ophthalmol*. 2013;24:233-238.
368. Webers CA, Beckers HJ, Nuijts RM et al. Pharmacological management of primary open-angle glaucoma: second-line options and beyond. *Drugs Aging*. 2008;25:729-759.
369. Rivara S, Pala D, Bedini A et al. Therapeutic uses of melatonin and melatonin derivatives: a patent review (2012 - 2014). *Expert Opin Ther Pat*. 2015;25:425-441.
370. Bonnefont-Rousselot D , Collin F Melatonin: action as antioxidant and potential applications in human disease and aging. *Toxicology*. 2010;278:55-67.
371. Andersen LP, Werner MU, Rosenkilde MM et al. Pharmacokinetics of oral and intravenous melatonin in healthy volunteers. *BMC Pharmacol Toxicol*. 2016;17:8.
372. Demuro RL, Nafziger AN, Blask DE et al. The absolute bioavailability of oral melatonin. *J Clin Pharmacol*. 2000;40:781-784.
373. Dubocovich ML Pharmacology and function of melatonin receptors. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 1988;2:2765-2773.

374. Gooneratne NS, Edwards AY, Zhou C et al. Melatonin pharmacokinetics following two different oral surge-sustained release doses in older adults. *J Pineal Res*. 2012;52:437-445.
375. Markantonis SL, Tsakalozou E, Paraskeva A et al. Melatonin pharmacokinetics in premenopausal and postmenopausal healthy female volunteers. *J Clin Pharmacol*. 2008;48:240-245.
376. Mistraletti G, Sabbatini G, Taverna M et al. Pharmacokinetics of orally administered melatonin in critically ill patients. *J Pineal Res*. 2010;48:142-147.
377. Mor M, Rivara S, Pala D et al. Recent advances in the development of melatonin MT(1) and MT(2) receptor agonists. *Expert Opin Ther Pat*. 2010;20:1059-1077.
378. Chumboatong W, Thummayot S, Govitrapong P et al. Neuroprotection of agomelatine against cerebral ischemia/reperfusion injury through an antiapoptotic pathway in rat. *Neurochem Int*. 2017;102:114-122.
379. Li W, Khor TO, Xu C et al. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. *Biochem Pharmacol*. 2008;76:1485-1489.
380. Van Heemst D Insulin, IGF-1 and longevity. *Aging Dis*. 2010;1:147-157.

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. MT_1/MT_2, melatonin membrane receptors; MT_3, 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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Melatonin function	Possible mediators	References	Mediators function	References
Regulator of physiological circadian rhythm processes	Sirtuins: melatonin induces SIRT1 expression in SAMP8 mice, in sleep-deprived rats and in neuronal cultures of aged rats.	113,114,117	SIRT1 negatively regulates CLOCK/BMAL1-complex.	111
			SIRT3 acetylates and actives oxidative enzymes and respiration in isolated mitochondria. Thereby, it regulates rhythmicity of mitochondrial respiration.	111,112
Antioxidant/protector of mitochondria function	Antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase): melatonin increases the expression and activity of antioxidant enzymes under oxidative stress conditions and in the liver of aging rats. Melatonin enhances antioxidant enzyme gene expression via Nrf2 activation.	47,50,52	Antioxidant enzymes removal free radicals such as superoxide anion and hydrogen as well as lipid peroxides.	380
	Nitric oxide synthases (NOS): melatonin and its metabolite AMK inhibit nNOS, iNOS and i-mtNOS enzymes. These effects have been tested in murine macrophages stimulated with endotoxin, in parkinsonian mice and in a rat model of dimethylnitrosamine-induced liver injury. Melatonin inhibits iNOS gene expression via NF-κB inactivation.	31,33,45-47,49	NOS enzymes catalyze the synthesis of NO, a molecule that regulates respiratory chain, generation of free radicals and mitochondrial apoptosis.	55
	Insulin/IGF-1 signaling (IIS) pathways: melatonin induces phosphorylation of insulin/IGF-1 receptors and modulates PI3K/AKT and ERK/MAPK pathways. These effects have been demonstrated in isolated pancreatic islets of neonate and adult rats as well as in an insulin-secreting cell line.	37,86	IIS pathways regulate mitochondrial biogenesis and function. PI3K/AKT pathway control the expression of genes involved in stress resistance and glucose as well as lipid metabolism. PI3K/AKT pathway can also modulate autophagy. IIS can also activate indirectly SIRT1 through its control of mitochondrial NAD^+ level.	82,84,90,381
	Sirtuins: melatonin induces SIRT1 expression in SAMP8 mice, in sleep-	33,113,114	SIRT1 reduces apoptosis in the presence of stress stimuli.	93

	deprived rats and in neuronal cultures of aged rats.		SIRT1 increases the expression levels of antioxidant enzymes such as superoxide dismutase and catalase.	102-104
			SIRT1 induces mitochondrial biogenesis by upregulation of endothelial NOS.	105
			SIRT1 modulate autophagy process.	81,106
			SIRT1 regulates the expression of IGF-1 and its receptor as well as the activity of FoxO transcription factor that is a downstream target of IIS pathway. Furthermore, it protects pancreatic beta cells, modulates insulin secretion and improve insulin sensitivity under insulin-resistant conditions.	93,94,97,99-101
Anti-inflammatory	Cyclooxygenase-2 (COX-2): melatonin and its metabolites prevent specifically the activation of COX-2 in murine macrophages activated with endotoxin. Furthermore, melatonin suppresses NF-κB binding to DNA and therefore decreases the levels of macrophage COX-2 transcript.	32,45	COX-2 enzyme regulates prostaglandins production that is key in the initiation of inflammatory process.	382
	Glutamatergic system: melatonin increases glutamate clearance and its conversion to glutamine in golden hamster retina. Additionally, it inhibits *N*-methyl-D-aspartate and kainate receptors activation in rats.	67,68,71	High levels of glutamate around the synaptic cleft cause excitotoxicity.	383,384
	Nitric oxide synthases (NOS): melatonin and its metabolite AMK inhibit nNOS, iNOS and i-mtNOS enzymes. These effects have been tested in murine macrophages stimulated with endotoxin, in parkinsonian mice and in a rat model of dimethylnitrosamine-induced liver injury Melatonin inhibits iNOS gene expression via NF-κB inactivation.	31,33,45-49	In the presence of high levels of glutamate, NO synthesis may be excessive and thereby NO may become neurotoxic. NO is also an immune modulator.	54,65 74
	GABAergic and glycinergic systems: melatonin enhances these systems.	66,69,72,73	Possible compensation of over-excitation by enhancing inhibitory	385

			synaptic transmission.	
	Sirtuins: melatonin induces SIRT1 expression in SAMP8 mice, in sleep-deprived rats and in neuronal cultures of aged rats.	113,114,117	SIRT1 inhibits NF-kB transcription factor and thereby it can abolish inflammatory response.	16,18,95,107, 108
Telomere attrition	Sirtuins: melatonin induces SIRT1 expression in SAMP8 mice, in sleep-deprived rats and in neuronal cultures of aged rats.	113,114,117	SIRT1 maintains telomere stability.	109,110