The Role of the Retinal pigment epithelium in the pathogenesis of age-related macular degeneration: Is photobiomodulation a valid means of supplying therapy?

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Abstract

The retinal pigment epithelium (RPE) comprises a monolayer of cells located between the neuroretina and the choriocapillaries. The RPE serves several important functions in the eye including formation of the blood retina barrier, protection of the retina from oxidative stress, nutrient transport to and waste transport from the retina, subretinal ionic homeostasis, phagocytosis of photoreceptor outer segments, synthesis and release of growth factors and reisomerization of all-trans-retinal during the visual cycle. The RPE additionally facilitates establishment of ocular immune privilege. Age-related macular degeneration (AMD) is the leading cause of legal blindness in developed countries. Dysfunction of the RPE has been associated with the pathogenesis of AMD in relation to increased oxidative stress, mitochondrial destabilization and complement dysregulation. Photobiomodulation or near infrared light therapy which refers to non-invasive irradiation of tissue with light in the far-red to near-infrared light spectrum (630-1000 nm), is a promising intervention that specifically targets those key mechanisms of RPE dysfunction that are implicated in AMD pathogenesis. This procedure may therefore offer a safe potential therapy for AMD.

Keywords: retinal pigment epithelium, RPE, photobiomodulation, near infrared light, low light therapy, Age-related macular degeneration, AMD, pathogenesis

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Introduction

The retinal pigment epithelium (RPE) comprises a monolayer of cells located between the retinal photoreceptors and the fenestrated choriocapillaris, which is basally bordered by the elastin and collagen-rich Bruch's membrane (BM). It serves several important functions in maintaining photoreceptor health. This includes providing protective mechanisms such as forming the selective blood retina barrier to prevent toxin entry and absorption of light to protect against oxidative damage. The RPE maintains photoreceptor excitability by the delivery of nutrients, removal of wastes, careful regulation of subretinal ionic concentration,phagocytosis of shed photoreceptor outer segments (POS) as well as storage and recycling of retinoids during the visual cycle. Furthermore, the RPE contributes to the production and secretion of growth factors, in addition to providing ocular immune privilege.

Therefore, damage to the RPE can indirectly contribute to photoreceptor degeneration.

Age-related macular degeneration (AMD) accounts for 8.7% of worldwide blindness and is the leading cause of legal blindness in developed countries.[1] Dysfunction of the RPE, and, specifically, increased oxidative stress, mitochondrial destabilization and complement dysregulation in these cells, has been thought to contribute to the pathogenesis of AMD.

Photobiomodulation, also known as near infrared light (NIR) therapy, is known to increase cellular metabolism, energy supply and metabolic repair processes. Thus, in ocular diseases such as AMD, where RPE mitochondrial dysfunction, oxidative and complement mediated damage are attributed to the pathogenesis, the RPE may be a useful target in photobiomodulation.

The aim of this review is to summarize the structure and functions of the RPE and its role in the pathogenesis of age-related macular degeneration before discussing applications of photobiomodulation in AMD in particular relation to the RPE.

Structure

The RPE comprises a monolayer of cells in a hexagonal cuboidal arrangement; cell size varies as a function of retinal eccentricity from a 12 μm cross section at the fovea to approximately 60 μm in the peripheral.[2-6] The cell density of the RPE is therefore greater in the fovea compared to the equator.[7] With age, peripheral RPE density declines but is preserved at the fovea, postulated to be a result of inward migration of peripheral RPE. [8] Approximately 30-40 photoreceptors overlie each RPE cell. [7] The RPE projects microvilli apically that enshroud the POS.Long thin apical microvilli measuring 5-7 μm in length are projected from the RPE and enshroud the POS, facilitating transepithelial and retina-to-RPE transport. Similarly, the basal surface of the RPE contains complex infoldings to support molecular movement. This apical-basal polarity is also exhibited by the organization of RPE organelles and membrane proteins. Melanosomes are situated apically whilst the nucleus, mitochondria, endoplasmic reticulum, lysosomes and golgi apparatus are located towards the basal side. The polar distribution of different combinations of ion channels and pumps on either the apical or basal membrane enables the diffusion of certain ions in specific directions in order to transport nutrients to photoreceptors and remove retinal waste products as well as the maintenance of subretinal ionic homeostasis as elucidated below.

Blood Retinal Barrier

Tight junctions between adjacent RPE cells are attached to the actin cytoskeleton and form part of the outer blood retinal barrier. This prevents paracellular transport of large molecules, toxins, blood-borne products and water. As a result of the tight junctional barrier produced by the RPE, resistance to paracellular movement is 10 times higher than to transcellular movement.[9, 10] However, this resistance is plastic, as permeability of the tight junctions can change in the presence of diffusible factors produced from the neural retina, choroid or liver.

One such factor is hepatocyte growth factor[11] which has been implicated in chronic retinal detachment secondary to retina blood barrier breakdown.[12] During the development of the RPE, tight junctions tend to be leaky but these become progressively less permeable due to secretion of these diffusible factors from the neural retina.[13, 14]

Protection from oxidative damage

The RPE is responsible for the absorption of reflected and scattered light. This helps to both optimize image quality and protect the retina from oxidative damage caused by reactive free radicals which result from the combination of constant RPE exposure to light, the high RPE metabolic activity, the elevated local oxygen tension and the photo-oxidation of lipofuscin. [4, 5] The pigment melanin, which is stored and synthesized in melanosomes, absorbs and filters harmful light, particularly in the blue wavelength region of the spectrum which is known to cause photo-oxidation of lipofuscin, a lipid-protein pigment that accumulates with age and from phagocytosis of the POS.[15, 16] Melanin has been identified as having additional anti-oxidative properties, including counteracting singlet oxygen and scavenging reactive oxygen species (ROS).[17, 18] Melanin density increases towards the centre of the retina, reaching its peak at the fovea, contributing to the clinically darker appearance of the fovea and surrounding macula. Melanin within the RPE decreases uniformly after the age of 40,.[5] Another mechanism of defense against oxidative damage is the intrinsic antioxidant defence system of the RPE, which comprises both enzymes, such as catalase and superoxide dismutase, and non-enzymatic antioxidants such as α-tocopherol, β-carotene and ascorbate.[19-23] Glutathione has been implicated as another anti-oxidant in the RPE with functions including perioxidase-reduction of lipid hydroperoxides, maintenance of ascorbate in its reduced form as well as detoxification of reactive products of lipid peroxidation such as 4-hydroxynonenol.[24, 25]

Transport of nutrients, wastes and water

The close anatomical apposition of the RPE to the photoreceptors is necessary for facilitating the transcellular movement of nutrients, wastes, fluid and ions.

The delivery of nutrients, including glucose, ascorbate and fatty acids, from the choroidal vasculature to the photoreceptors occurs through transporters within the RPE membranes. Glucose is passively transported through numerous GLUT1 and GLUT3 transporters on both the apical and basal membranes. [26, 27]More recently, additional glucose transporters GLUT2 and GLUT5 have been identified on the apical and basolateral membranes in cultured human RPE cells.[28]

Ascorbate is a carbohydrate vitamin that is vital in hydroxylation reactions and biosynthetic pathways, especially in the production of collagen and glycosaminoglycans which are components of the interphotoreceptor matrix (IPM) and BM.[29-31]It is also an effective antioxidant and scavenger of superoxide radicals. The transcellular movement of ascorbic acid across the RPE occurs through active sodium-dependent transport that is reduced by presence of ouabain, 2,4-dinitrophenol and dehydroascorbic acid.[31]

Photoreceptor membranes, which comprise a significant portion of the omega 3 fatty acid docosahexanoic acid (DHA), are continuously renewed and added to the POS as photoreceptor tips are shed and phagocytized by the RPE.[32]Thus, photoreceptors require a constant supply of DHA. Initially, DHA is produced in the liver from the dietary precursor α-linelonic acid before being carried by lipoproteins in the circulation to the RPE. Transport through RPE membranes occurs through a concentration-dependent manner. [32-34] DHA acid has also been identified as a precursor to neuroprotectin 1, which protects RPE from

oxidative damage and may also play a role in RPE retinoid transport by modulating binding between interphotoreceptor binding protein (IRPB) and 11-cis-retinal.[35]

Lactic acid, a metabolic end product produced in excess by the retina and thought to be specifically released from POS and to lesser extent, photoreceptor inner segments has been proposed to act as a nutrient for RPE.[36] From the subretinal space, lactic acid enters the RPE apical membrane by active transport through lactate-H+ co-transporter monocorboxylate transporter 1 (MCT1) and the Na+-dependent cotransporter. Whether lactate acts as a fuel source for RPE cells or not, excess levels of this compound are removed through the RPE basolateral membrane via monocorboxylate transporter 3 (MCT3) and the Na+-lactate exchanger.[37, 38]

Constant transport of water away from the subretinal space to the choriocapillaris is required to maintain close apposition of the RPE and photoreceptors. Large volumes of subretinal fluid are generated from metabolic turnover in photoreceptors as well as intraocular fluid flux from the vitreous body towards the retina. Water travels across the RPE to the choriocapillaris by active transport through the aquaporin 1 channel, identified in studies of cultured rat and human RPE.[39, 40]Water transport is also facilitated by the transport of ions such as Cl- and K+ which will be elaborated below.

Transepithelial transport and maintenance of subretinal ion levels

The RPE plays a major role in preserving excitability of photoreceptors by tight regulation of subretinal ionic concentration through transepithelial transport of ions. The apical Na+/K+ ATPase pump, which results in outflow of Na+ and influx of K+, provides the energy gradient that is vital to transepithelial transport.[41-43]The Na+/K+ ATPase, together with the polarized distribution of other channels and transporters, produce a high subretinal sodium

concentration and high potassium concentration in the RPE cytosol. The elevated subretinal sodium is critical to the dark current which is produced by the entry of sodium ions through cGMP gated channels, resulting in depolarization of photoreceptors. Apical entry of sodium occurs through the Na+-K+-2Cl- cotransporter and exit occurs basolaterally through mechanisms that are unclear.[44]However, most intracellular sodium ions are recycled through the apical Na+/K+ ATPase to maintain the high subretinal sodium concentration.

The RPE also effectively compensates for ionic changes such as fluctuation in potassium levels during phototransduction. Potassium can enter the RPE apically through Na+/K+ ATPase or Na+2-K+-2Cl cotransporter and leave basolaterally or apically through K+ channels.[44] Whilst in the dark, the potassium concentration is 5 mM with net transepithelial efflux of potassium from the subretinal space to the choroid. Exposure to light causes the hyperpolarization of photoreceptors leading to the closure of cGMP gated Na+ channels in the outer segment membranes and reduced K+ efflux from the inner segment. Subsequently, the subretinal potassium concentration decreases to 2 mM which triggers hyperpolarization of the RPE apical membrane and activation of inward rectifying K+ channels on the apical membrane, causing K+ flow into the subretinal space . In the early phase of RPE hyperpolarization, the apical Na+-K+-2Cl- cotransporter reverses direction, further increasing K+ movement to the subretinal space, resulting in the restoration of subretinal potassium concentration.[45]

Chloride, a vital facilitator for fluid movement, enters through the RPE apical membrane via the Na+-K+-2Cl- cotransporter before exiting basolaterally via voltage dependent chloride and CIC-2 channels. The basolateral Cl-/HCO_{3-} exchanger, an important channel in pH regulation, transports chloride back into the RPE. An additional efflux channel for chloride in RPE cells is the CFTR channel on the basolateral aspect.[44] CFTR channels along with the CIC family channels have been found to be highly susceptible to oxidative stress in human

fetal and adult RPE cultures.[46, 47]Chloride channel dysfunction and altered fluid transport secondary to oxidative stress may play a role in AMD.[48]Chloride transport, similar to potassium transport in the RPE is regulated by calcium, cAMP, epinephrine and ATP.[47, 49] Transepithelial transport of HCO_{3-} is intrinsically associated with pH regulation of the subretinal space and the RPE cell itself. At high pH, HCO_{3-} travels apically into the RPE via the $Na+/HCO_{3-}$ transporter and leaves basolaterally in exchange with Cl- through the HCO_{3-} /Cl- exchanger. Conversely, at low pH HCO_{3-} enters basolaterally through the HCO_{3-}/Cl- exchanger before traveling to the subretinal space through the $Na+/HCO_{3-}$ transporter.[50]

Phagocytosis of POS

Photoreceptor membranes undergo a perpetual renewal process whereby the outer segment tips are shed and subsequently phagocytosed by RPE before new outer segments are constructed at the cilum. This is necessary for several reasons: to maintain photoreceptor excitability, to recycle nutrients, including DHA and retinal, and to prevent oxidative damage from photo-oxidation of damaged POS lipid and protein components. The turnover rate of a single rod POS is 7-12 days. It is estimated approximately 20-30,000 POS are phagocytosed daily following a circadian pattern with greatest activity after initial light exposure.[51, 52] The process of POS phagocytosis is triggered by interactions with ligand receptors including CD36[53, 54], the mannose-6-phosphate receptor[55], aVβ5 integrin[56], MerTK[57, 58], CD81[59] and L-type calcium channels[60]. Initially, specific binding of the POS to the RPE apical membrane leads to induction of a secondary messenger cascade. This results in the ingestion of the POS and subsequent fusion with a lysosome where enzymatic digestion occurs. The components from lysosomal digestion are either recycled back to photoreceptors as nutrients or transported into the choroid vasculature as waste.

Production and secretion of growth factors

In order to preserve the neural retina and choroid architecture, the RPE produces and secretes several protein mediators, cytokines and growth factors, including platelet-derived growth factor (PDGF), pigment epithelium derived growth factor (PEDF), vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), transforming growth factor β (TGF β), insulin-like growth-I (IGF-I), ciliary neurotrophic factor (CNTF), tissue inhibitor of metalloprotease (TIMP), lens epithelium-derived growth factor (LEDGF) and members of the interleukin family.[61-67] Photoreceptors are protected by the neuroprotectant growth factors PEDF, FGF, CNTF, LEDGF and IGF-I whilst PDGF modulates cell growth and healing. PEDF, which is secreted from the RPE apical membrane, has anti-angiogenic properties and helps maintain the fenestrations of the choriocapillaris endothelium. TGF β moderates inflammation and regulates extracellular matrix synthesis and turnover, a function shared by TIMP. VEGF, which is secreted from basal RPE, basically has an opposite role to PEDF and promotes angiogenesis and regulates the permeability of the choriocapillaris. VEGF overexpression has been implicated as a key player in the pathogenesis of choroidal neovascularisation (CNV) in AMD.[68, 69]The expression of VEGF is regulated by numerous factors, including mechanical stress, hypoxia advanced glycation end products (AGE), cytokines (interleukin 1 and TNF), vasopressor hormones (angiotensin II, vasopressin, growth factors (TGFβ, FGF, and PDGF) and L-type Ca^{2+} channels.[70]The RPE has been identified as the source of Somatostatin (SST), erythropoietin (EPO) and apolipoprotein A1 (apoA1) in the eye.[71] SST ,which regulates the release of growth hormone in the hypothalamus, is responsible for the modulation of intracellular Ca^{2+} pathways, nitric oxide function and glutamate release from photoreceptors. SST also reduces vascular proliferation by inhibiting IGF-I, VEGF, epidermal growth factor and PDFG. [71] EPO has neuroprotective

and angiogenic properties similar to VEGF.[72, 73] ApoA1, which is responsible for preventing lipid accumulation in the retina, also acts as an antioxidant.[74, 75]

Reisomerization of all-trans-retinal and transport of retinoids in the visual cycle

The visual cycle is the fundamental process by which a photon is converted into an electrical signal in the retina. The triggering event of the visual cycle is the absorption of light by rhodopsin in the POS which results in one of its components, the chromophore 11-cis-retinal, to be converted to all-trans-retinal. This results in a conformational change in opsin, a G-protein coupled receptor component of rhodopsin, which activates the regulatory protein transducin to initiates signal transduction cascades leading to closure of cyclic GMP-gated cation channels, hyperpolarization of the photoreceptor cell membrane and, ultimately, decreased glutamate release. The RPE plays an integral role in the transport of retinoids as well as reisomerization of all-trans-retinal to 11-cis-retinal in the visual cycle. Following light exposure, all-trans-retinal is converted into all-trans-retinol by retinal dehydrogenase in the POS before being transported to the RPE by interstitial retinol-binding protein 3 (RBP3) .[76, 77] All-trans-retinal can also originate from the blood circulation, being carried by serum retinol binding protein, before entering the RPE cell through a receptor-mediated process via the RBP-transthyretin complex in the basolateral membranes.[78]Whilst in the RPE, all-trans-retinol is bound to cellular retinol binding protein and esterified to 11-cis-retinal in the presence of the catalyst lecithin retinol transferase and the chaperone protein RPE65. 11-cis-retinal is then transported by RBP3 to the POS where it combines with opsin to reform rhodopsin. The cycle is repeated following light exposure whereby the resulting all-trans-retinal is reconverted to its all-trans-retinol and cycled back to the RPE for reisomerization and oxidation.[78] [6]

Immune privilege

The RPE has a central role in maintaining immune privilege in the eye. Firstly, the blood retinal barrier formed by tight junctions between RPE cells creates a microenvironment which allows infiltration into the retina of immune system components to be carefully regulated. Secondly, the RPE itself is capable of secreting immunosuppressive factors, including TGF β, interleukin 11 and interferon β, to downregulate T cell activity.[79] Thirdly, the presence of Fas ligand on the membrane allows RPE cells to induce apoptosis in Fas expressing effector leukocyte cells.[80, 81] Furthermore, RPE cells which express MHC class I and II are able to act as antigen presenting cells in the eye. [82, 83] RPE cells can also synthesize or express numerous complement proteins and regulators. PCR analysis of RPE cells revealed that the RPE was capable of local production of complement 3(C3), complement factor B (CFB), complement factor H (CFH), complement factor D (CFD) and complement factor I (CFI). Complementary regulatory proteins membrane cofactor protein (MCP), decay accelerating factor (DAF) and CD59 are also expressed on the RPE membrane.[84]

Age-related macular degeneration

AMD is an ocular disease in association with aging that is characterized by decline in central vision. Classically, AMD is divided into two subtypes: “dry” and “wet” AMD. “Dry” or atrophic AMD is marked early by the deposition of yellow aggregates called drusen between the RPE and BM as well as pigmentary changes in the macula before progressing to late stage geographic atrophy (GA) defined by pathology of the RPE, choriocapillaris and overlying photoreceptors. There is currently no treatment for the atrophic form of AMD. “Wet” or neovascular AMD causes more rapid and dramatic loss of central vision and is characterized

by choroidal neovascularisation (CNV), the development of new blood vessels in the choroid that often leak into the retina, causing haemorrhage, retinal detachment and disciform scars. Anti-VEGF intravitreal injections are currently the main form of treatment for CNV. It is believed that the degeneration or injury of RPE cells is the earliest process in the pathogenesis of AMD, preceding photoreceptor loss that ultimately leads to vision loss[85-87] The following sections will describe the key mechanisms of RPE dysfunction that arises in the pathogenesis of both AMD subtypes.

Oxidative stress

Oxidative stress has been suggested as a potential integral mechanism of RPE injury in the pathogenesis of AMD. *In vitro* experiments where RPE cells are exposed to oxidative stress i.e. H_2O_2 have shown increased apoptosis, decreased proliferation and features of senescence.[88-90] Apoptotic RPE cells have also been identified in CNV membranes from AMD donor eyes.[91]

The underlying mechanism of oxidative stress induced apoptosis in AMD is thought to be an interference in the protective epidermal growth factor receptor/protein kinase B pathway as well as an activation of apoptosis-promoting factors such as caspase 3 and 9, as triggered by the release of cytochrome c from mitochondria.[89, 92] AMD donor eyes have histological evidence of established widespread oxidative damage in patients with advanced GA and also in eyes with CNV to a lesser extent.[93] Genetic evidence of the importance of RPE oxidative stress in the pathogenesis of AMD is supported by transgenic mice having a deletion of the superoxide dismutase 1 (SOD 1) gene. These mice develop clinical features of AMD, including drusen deposition, RPE atrophy and CNV.[94]

Carboxyethyl pyrole (CEP) residues, which are oxidized remnants of DHA, have been used as biomarkers in patients with AMD who have increased plasma CEP autoantibiodies and accumulation of CEP within the RPE and outer retina.[95] Mice that have been immunized with mouse serum albumin adducted with CEP developed AMD-like lesions similar to GA.[96] Subretinal injections of CEP modified human serum album have also been found to exacerbate angiogenesis in laser-induced CNV mouse models. [97]

A major source of oxidative stress in RPE cells is the formation of ROS during phagocytosis of POS. Extracellular H_2O_2 is presumably generated from the action of NAPH oxidase in a phagosome or from perioxidation of ingested POS lipids. Downregulation of the antioxidant enzyme catalase also occurs during phagocytosis, leading to increased susceptibility of RPE cells to ROS.[20]

Lipofuscin, autofluorescent collections of undegradable protein and lipids associated with aging, accumulate in lysosomes of postmitotic cells including RPE cells. Lipofuscin within RPE cells has been postulated to originate from insufficient RPE phagocytosis of POS and it is thought that this may also contribute to the decline in RPE phagocytic function with ageing.[99] N-retinyl-N-retinylidene ethanolamine (A2E), a component of lipofuscin, is susceptible to photo-oxidation from exposure to blue light, generating toxic A2E-epitopes that cause oxidative damage and subsequent caspase 3-mediated apoptosis of RPE cells.[100] [101]

Epidemiological studies associating cigarette smoking and sunlight to increased risk of developing AMD support the role of oxidative stress as a key factor in the pathogenesis of AMD.[102] Wang et al[103] demonstrated exposure to Benzo(a)Pyrene, the toxic element in

cigarette smoke, resulted in apoptosis of cultured RPE cells as well as ultrastructural changes akin to oxidative damage in mice.

The role of oxidative stress in AMD pathogenesis is also supported by the AREDS study which demonstrated high-dose antioxidants (vitamin C, vitamin E, and β-carotene) as well as zinc, which increases the activity of enzymes such as SOD and catalase, significantly reduced the progression of intermediate dry AMD to wet AMD.[104] The follow-up AREDS2 study in 2013 concluded that the antioxidants lutein and zeaxanthin were effective and safe alternatives to β-carotene which had, as a side-effect, been found to increase lung cancer in smokers.[105]

Mitochondrial dysfunction

Impaired mitochondrial function in the RPE appears to be a critical factor in AMD. Mitochondria are an endogenous source of oxidative stress. Mitochondrial injury or dysfunction results in impaired respiration which leads to the increased accumulation of ROS.[106] In a continuous cycle, oxidative stress in mitochondria can exacerbate the generation of ROS, eventually overwhelming RPE cells resulting in apoptosis. Mitochondrial DNA (MtDNA) is a more vulnerable target to oxidative damage than nuclear DNA (nDNA) given its proximity to the ROS-producing inner mitochondrial membrane, relatively poor repair capabilities and its lack of protective histones or other DNA-associated proteins. Furthermore, the coding regions of MtDNA are more predisposed to oxidative damage since there exist a large number of intronless regions with high transcription rates.[92] In *vitro* experiments, where RPE cells were exposed to oxidative stress demonstrated preferential damage to MtDNA over nDNA along with a decline in mtDNA repair and compromised

mitochondrial redox function.[90, 107] In human AMD donor eyes, there is evidence of increased macula specific mtDNA damage, mitochondrial heteroplasmic mutations and diminished mtDNA repair capacity in the RPE.[108] The association between AMD and RPE mitochondrial dysfunction was further illustrated in a proteomic analysis of AMD donor eyes demonstrating altered mitochondrial translation factors, decreased ATP synthase subunits and reduced mitochondrial heat shock protein 70 expression(mtHsp70).[109] Important functions of mtHSP70 include regulation of p53-mediated apoptosis, iron-sulfur cluster biogenesis, mitochondrial calcium regulation and ATP-dependent transport of nuclear-encoded proteins into the mitochondrial matrix.[109]

A2E in lipofuscin has been shown to induce apoptosis of RPE cells in a light dependent manner through a mitochondria-related mechanism. Upon exposure to blue light, A2E binds non-covalently to cytochrome oxidase C (COX), an essential mitochondrial enzyme in oxidative phosphorylation. Upon attachment, A2E exhibits its toxic effect by inhibiting COX causing impaired mitochondrial respiration, generation of ROS and formation of pro-apoptotic complexes involving cytochrome C.[110-112]

There is also genetic evidence for the association between mitochondrial dysfunction and AMD. In human AMD patients, mtDNA lesions were increased significantly in all regions of the mitochondrial genome compared to age matched controls where age-related mtDNA lesions only occurred in the common deletion regions.[113] In addition, polymorphisms of ARMS2 gene, which encodes for an outer mitochondrial protein in RPE, is associated with significantly increased predisposition to AMD.[114, 115]

Complement dysregulation

Inappropriate activation of the complement system has been implicated in AMD pathogenesis. Biochemical analysis of drusen constituents reveals a significant number of complement activators (A2E, amyloid β, immunoglobulins, CRP, advanced glycation end products, cholesterol), complement components and inhibitors.[84]

The alternate complement pathway (AP) has been the main complement pathway implicated in AMD pathogenesis. Although there have been reports of classical pathway involvement, with variants of C2 genes and SERPING1 gene encoding c1 inhibitor being associated with AMD, this has not been confirmed in larger case-controlled studies.[116-118] Genetic variations of the genes encoding AP proteins CFH (inhibitor of AP) [119-121], CFB[116], CFI[122, 123] as well as C3[124] component are associated with higher susceptibility to AMD, indicating the significance of AP dysregulation in AMD pathogenesis. Haplotypes of CFH with deletion of CFH-related proteins protects against AMD.[125] Rodent models with genetic knockout of CFH display structural and functional retina degenerations similar to AMD. Increasing expression of human CFH in this model resulted in the inhibition of AP complement pathway, reduction of sub-RPE deposits as well as prevention of retinal and kidney damage caused by CFH deletion in a dose dependent manner.[126, 127] The process of AP-mediated RPE cell death which occurs in GA involves lysis of cells marked with C3b by membrane attack complex (MAC) that is modulated by extracellular calcium.[128]

The AP also has a critical role in the modulation of CNV in laser-induced models.[129] Complement components C3a, C5a, CFB and MAC induce CNV by upregulating RPE secretion of angiogenic factors including VEGF, TGF-β2 and β-fibroblast growth factor.[130, 131] Consequently, specific inhibition of AP reduced angiogenesis in CNV mouse models.[132]

The complement system may have a role in promoting the chronic local inflammatory process in AMD. C3a and C5a are known to have chemotactic properties and can increase RPE expression of the inflammatory cytokines interleukin-1β, interleukin-6, interleukin-8, MCP-1 and GM-CSF.[133]

There is evidence that oxidative stress can render RPE more susceptible to complement mediated damage.[128, 134] RPE cells exposed to oxidative stress exhibited reduced expression of the membrane bound complement inhibitors DAF, CD55 and CD59 and downregulation of CFH.[135-137] Additionally, mice immunized with CEP display increased deposition of C3d below the RPE.[96] In cultured human RPE cells, complement and oxidative stress synergistically increased VEGF secretion up to 100 fold.[135]

Photobiomodulation:

Photobiomodulation or NIR therapy refers to the non-invasive irradiation of tissue with light in the far-red to near-infrared light spectrum (630-1000 nm) with delivery methods varying from laser sources to light emitting diode (LED) devices.[138] Photobiomodulation originated in the 1960s shortly after the advent of the laser. Mester et al[139] were the first to demonstrate the positive effects of photobiomodulation. Using NIR lasers they were able to induce increased hair growth and wound healing in mice and treat non-healing skin ulcers in human patients. Since then the field of photobiomodulation has broadened to include treatment of a range of conditions including wound healing, diabetic ulcers, neurological pain, peripheral nerve injury, stroke and myocardial infarction.[140] More recently, focus has turned to the potential beneficial effects of photobiomodulation as a treatment for blinding retinal diseases. The success of photobiomodulation has been demonstrated in various retinal disease animal models including light-induced retinal degeneration[141, 142], age-related macular

degeneration[143], retinitis pigmentosa[144], diabetic retinopathy[145] and retinopathy of prematurity[146, 147] through improved ERG , decreased inflammatory markers and diminished cell loss.

Photobiomodulation is reported to have positive results in patients with age-related macular degeneration[148, 149], diabetic retinopathy[150], amblyopia[151] and retinitis pigmentosa[152], reflected in improvement of visual acuity and decreased visual field loss. However, these clinical studies range from interventional case series to a single case report. In addition, the mechanism of photobiomodulation particularly on amblyopia is unclear.

Mechanisms of Photobiomodulation

In the far the red and near-infrared light spectrum, COX is the primary photoacceptor. By targeting COX, photobiomodulation modulates electron transfer in the reduction of oxygen during mitochondrial respiration, hence increasing the mitochondrial membrane potential and ATP synthesis. Ultimately, this triggers and enhances cellular repair processes and metabolism in photoreceptors, choroid and retinal pigment epithelium.[153] Evidence of COX as the primary target comes from Eels who reportedt photobiomodulation improved rat retinal function following methanol intoxication which is known to inhibit cytochrome oxidase activity.[154] NIR treatment of primary cultured neurones reversed the effect of tetrodotoxin by upregulating COX activity.[155] Desmettre et al.[156] found in choroidal layers, that transpupillary application of laser therapy induced increased expression of heat shock proteins, which are known to stimulate cellular metabolism and prevent premature cell death. Another potential mechanism of photobiomodulation is the unbinding of nitric oxide from COX as demonstrated by Karu et al.[157] Since nitric oxide inhibits mitochondrial respiration, its dissociation from COX would restore mitochondrial oxygen consumption, therefore increasing energy production and boosting cellular metabolic processes.[138] In vitro

experiments have reported that photobiomodulation therapy increases phagoctytosis and lysosomal activity, cellular processes important in the reduction of inflammation and enhancing repair of the retina.[158] These processes occur from photobiomodulation triggering downstream signalling cascades via ATP, cAMP, NO, ROS and Ca^{2+} resulting in eventual gene expression that promotes protein synthesis, anti-inflammatory processes, anti-oxidants, anti-apoptotic proteins as well as cell migration and proliferation.[159]Photobiomodulation usually stimulates mitochondrial ROS production at low levels however it may decrease ROS production during oxidative stress.[160] Does it just increase the efficiency of mitochondria or lower the level of ROS produced in normal respiration?

Applications in AMD

The pathogenesis of AMD as discussed above encompasses a complex interplay of oxidative stress, mitochondrial dysfunction and complement dysregulation, likely involving the RPE. Photobiomodulation may prove to be an effective treatment for AMD as there is evidence that it can significantly modulate all of these pathological processes.

Exposure of NIR light to human RPE cells in vitro stimulated a 56% increase in ATP and twofold increase in intracellular NO production at 5 hours post-exposure, presumably via unbinding of NO from COX leading to an enhancement in mitochondrial oxidative phosphorylation.[161] Photobiomodulation resulted in a six-fold increase in levels of growth promoting NF-KB, an 11-fold increase in levels of apoptosis suppressor protein Bcl-2 and a 70% decrease in levels of the apoptotic effector protein Bax. In RPE cultures exposed to the oxidative stressor H_2O_2 and challenged with POS, Fumar et al.[162] reported improvement of phagocytosis, increased expression of MerTK and reduced ROS production following treatment with 250 seconds of 670mm light for 4 consecutive days. Interestingly, mitochondrial membrane potential was not affected by NIR light in this study.

Photobiomodulation has also been demonstrated to protect RPE cells from the lethal effects of thermal laser.[163]

There is also considerable evidence of a beneficial effect of photobiomodulation in animal AMD models. In an aged mice model of AMD, Kokkinopoulos[164] reported that photobiomodulation could improve mitochondrial function of RPE cells and reduce inflammation. Following five treatments of NIR light exposure lasting 90 seconds, there was significantly increased mitochondrial membrane potential as well as reduced component C3d, proinflammatory cytokine TNF-α and reduced macrophage numbers. This was confirmed by Begum et al.[143] who reported similar results in CFH knockout mice. Mice exposed to NIR light 6 minutes twice daily for 2 weeks had significantly increased COX, decreased C3 complement deposition in the outer retina as well as reduced inflammatory markers vimentin and GFAP. There were also notable changes in the morphology of RPE macrophages and dendritic cells however there was no significant change in numbers. The relatively indirect delivery of photobiomodulation through supplemental environmental lighting in this study demonstrated the effectiveness of NIR light in penetrating tissue to reach the target. Calaza et al.[165] reported improved ATP levels in CFH mice following NIR light exposure supporting evidence that photobiomodulation improves oxidative phosphorylation. In a separate experiment, Kokkinopoulos[166] reported that photbiomodulation can drastically ameliorate inflammation from innate immunity. CFH mice exhibited decreased C3b expression in RPE/BM following regular 90 seconds NIR light exposure over 8 weeks. . Interestingly, TLR 2 and TLR 4 expression decreased in the inner nuclear but not in the in RPE layer. Expression of the systemic inflammatory marker calcitonin was also significantly in all layers. In a light induced model of atrophic AMD, 3 minutes of daily NIR light exposure over 5 days was reported to reduce complement propagation, C3 deposition, photoreceptor death

and oxidative stress.[167]

There have been several clinical studies investigating the effects of photobiomodulation on AMD patients including a prospective study by Ivandic and Ivandic[153] where 348 eyes of 203 patients with dry and wet type AMD were exposed to 40 seconds of transconjunctival 780 nm light from a semiconductor laser diode with 0.3 J/cm2 delivered to the macula four times over 2 weeks. The treatment group included 146 with cataracts with 182 without. The remaining 20 eyes of 10 patients received sham treatments and served as the control group. There was substantial improvement in both treated groups with 97% of patients with cataracts improving in visual acuity by a mean of 2 lines and 94.5% of the non-cataract patients by the same amount up to 36 months. An additional benefit was observed with reduced pigmentation and cystic drusen as well as improvement in metamorphopsia, dyschromatopsia and relative scotomas. Patients with wet AMD had reduced oedema and bleeding. No change was observed in the control group. The TORPA II study, which examined 42 eyes in 24 patients with dry AMD by Merry G et al.[168] consisted of delivering 88 seconds of 670 nm light at 4-7.7 J/cm2 through LED-based devices as well as 590 and 790 nm for 35 seconds delivering 0.1 J/cm2 in nine treatments over 3 weeks. Following photobiomodulation, there were statistically significant functional changes indicated by substantial improvement of contrast sensitivity and visual acuity at 3 weeks and 3 months as well as anatomical improvement exhibited by decrease in drusen volume and central drusen thickness. However, a control group was not used in the study. It is noteworthy to mention that in both studies there were no reported adverse effects or subjective discomfort by patients, illuminating an excellent safety profile and suggesting that NIR treatment is well tolerated.

Conclusion and future directions

The RPE is a vital component of the eye and its dysfunction has been implicated in the pathogenesis of AMD. Photobiomodulation is a promising non-invasive therapy that has demonstrated the capacity to ameliorate oxidative stress, mitochondrial dysfunction and complement dysregulation, all of which are key mechanisms of RPE dysfunction leading to AMD.

Clinical studies have indeed demonstrated some significant functional and anatomical improvements by photobiomodulation in AMD patients, however, there is a need for randomized control trials. The LIGHTSITE study which is currently enrolling in Canada, will be a 30-subject, randomized, double-masked, sham-controlled clinical trial examining similar outcomes from the TORPA II study.[168] As previous studies have used varying dosages, duration and frequency of NIR light with varying effectiveness, there is currently no consensus on treatment regimens. Therefore further studies are needed to establish effective treatment parameters.

Table 1- Applications of photobiomodulation in AMD

Experiment	Delivery	Dose	Frequency	Results
hTERT-RPE culture (Lavey et al.)[161]	671 and 637 nm light via LED, unspecified duration	2.88 J/cm^2	Unspecified	-x2 ↑NO -56% ↑ATP 5 hours post -x6 ↑NF-KB -x11 ↑BCl-2 -70% ↓Bax
Human RPE cultures exposed to H_2O_2 and POS (Fumar et al.)[162]	670 nm light via LED for 250 seconds	3.89 mW/cm2	Twice daily for 4 days	-↑phagocytosis -↑MerTK expression -↓ROS production
C57BL/6 mice (Kokkinopoulos et al.)	670 nm light via LED for 90 seconds	40 mw/cm^2	Five times over 35 hours	-↑mitochondrial membrane potential -↓C3d -↓TNF-α -↓macrophage numbers
CFH knockout mice (Begum et al.)	670 nm light via LED environmental lighting for 6 minutes	20 mw/cm^2	Twice daily for 14 days	-↑COX -↓C3 complement desposition in outer retina -↓Vimentin and GFAP
CFH knockout mice (Calaza et al.)	670 nm light via LED for 90 seconds	40 mw/cm^2	Daily for 5 days	-↑ATP
CFH knockout mice (Kokkinopoulos et al.)	670 nm light via LED for 90 seconds	40 mw/cm^2	4 times over 2 days for 1 and 8 weeks	-Shift of C3 deposition from neural retina to RPE/BM -↓C3b in RPE/BM. - ↓TLR 2 and TLR 4 expression inner nuclear layer -↓Calcitonin in all layers

Light induced rat model of atrophic AMD (Rutar et al.)	670 nm light via LED for 3 minutes	9 J/cm^2 (50 mw/cm^2)	Daily for 5 days	- ↓C1s, C2, C3, C4b, C3aR1, and C5r1 gene expression - ↓C3 deposition in ONL -↓C3 expression in subretinal microglia/monocytes -↓Oxidative damage marker 4-hydroxynonenal (4-HNE)
Prospective study of dry and wet AMD patients (Ivandic and Ivandic)	780 nm light via semiconductor laser diode to the macula for 40 seconds	0.3 J/cm^2 (7.5 mw/cm^2)	Twice weekly for 2 weeks	-Improvement in VA of 2 lines in 94.5-97% treated patients up to 36 months -↓pigment accumulations and cystic drusen as well as -↓metamorphopsia, ↓dyschromatopsia and ↓relative scotomas. ↓oedema and bleeding in wet AMD
TORPA II study-interventional longitudinal case series	-670 nm light via LED for 88 seconds -590 and 790 nm light via LED for 35 seconds	-50-80 mW/cm^2 (670 nm) -4 mW/cm^2 (790 nm) -0.6 mW/cm^2 (590 nm)	-Nine sessions over 3 weeks	-↑BCVA of 5.90 letters ($p < 0.001$) at 3-week treatment and 5.14 letters ($p < 0.001$) at 3 months -↑Contrast sensitivity improved significantly (log unit improvement of 0.11 ($p = 0.02$) at 3 weeks and 3 months (log unit improvement of 0.16 ($p = 0.02$) at three cycles per degree. -↓Drusen volume decreased by 0.024 mm^3 ($p < 0.001$) -↓central drusen thickness mean of 3.78 µm ($p < 0.001$),

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