**Chapter 22**

# *Photobiomodulation in Animal Models of Retinal Injury and Disease*

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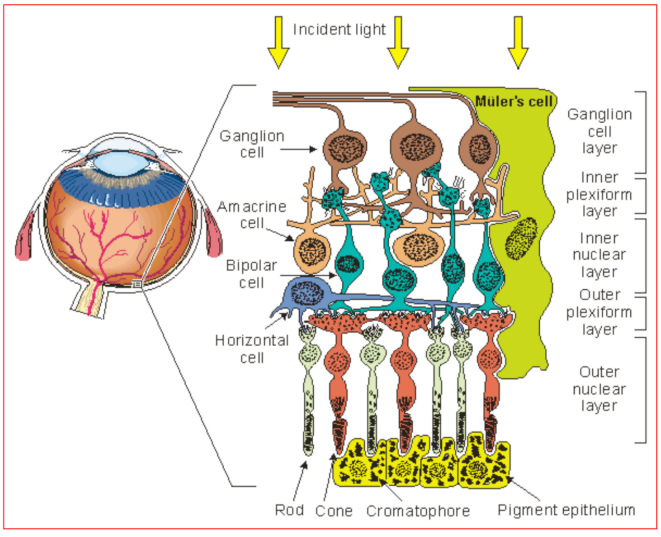
Running Title: PBM in the Eye

**Summary:**
Mitochondrial dysfunction, oxidative damage and inflammation play key roles in retinal aging and retinal degenerative disease. Mitochondrial repair and attenuation of oxidative stress are critical to the long-term survival of the retina. Therapeutic strategies directed towards improving mitochondrial integrity and function and reducing oxidative stress have considerable potential for the treatment of retinal disease. Low-intensity far-red to near-infrared (FR/NIR) light has been shown to act on mitochondria-mediated signaling pathways to preserve mitochondrial function, attenuate oxidative stress, reduce inflammation and prevent cell death. FR/NIR photons penetrate the brain, retina and optic nerve and this treatment, commonly known as photobiomodulation (PBM) has documented efficacy in the prevention and treatment of retinal degenerative diseases in experimental and clinical studies.

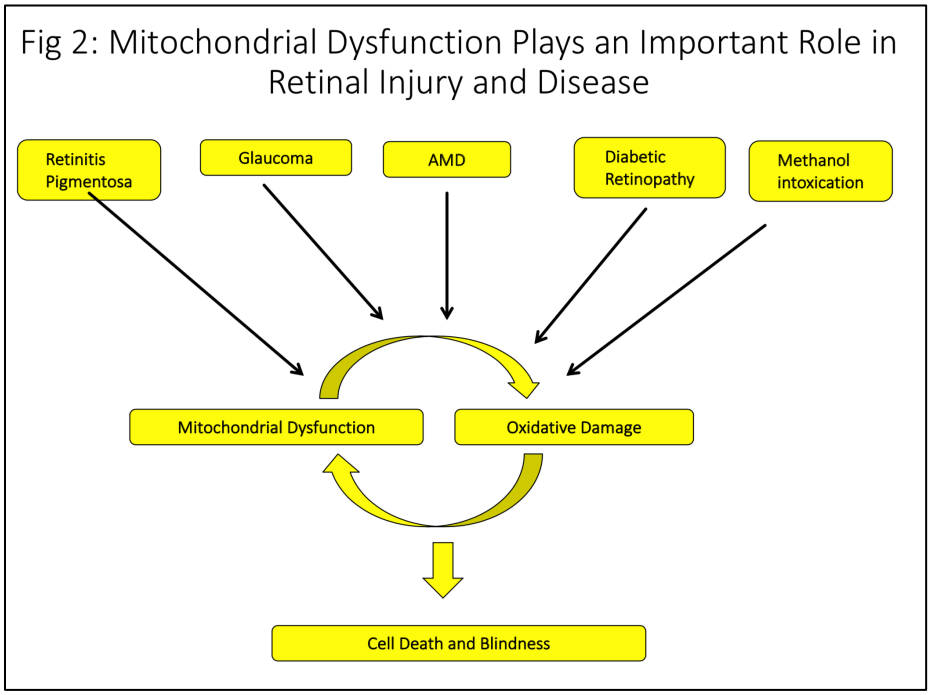
**Keywords:** photobiomodulation, retinal injury, retinal degeneration, mitochondrial dysfunction, electroretinogram, cytochrome c oxidase

**25.1 Introduction.** The retina is a neurosensory tissue lining the inner surface of the back of the eyeball. It is comprised of several cell layers that include the sensory neurons that respond to light (photoreceptors) and intricate neural circuits that perform the initial stages of image processing. (Kolb, 2003 and Sung and Chuang, 2010) *[Figure 1].* Retinal image processing occurs through circuits involving five classes of cells, photoreceptors, bipolar cells, amacrine cells, horizontal cells, and ganglion cells. These processes collectively amplify, extract, and compress signals to preserve relevant information before it gets transmitted to the midbrain and the thalamus through the axons of the ganglion cells that form the optic nerve.

Fig 1. Anatomy of the Human Retina



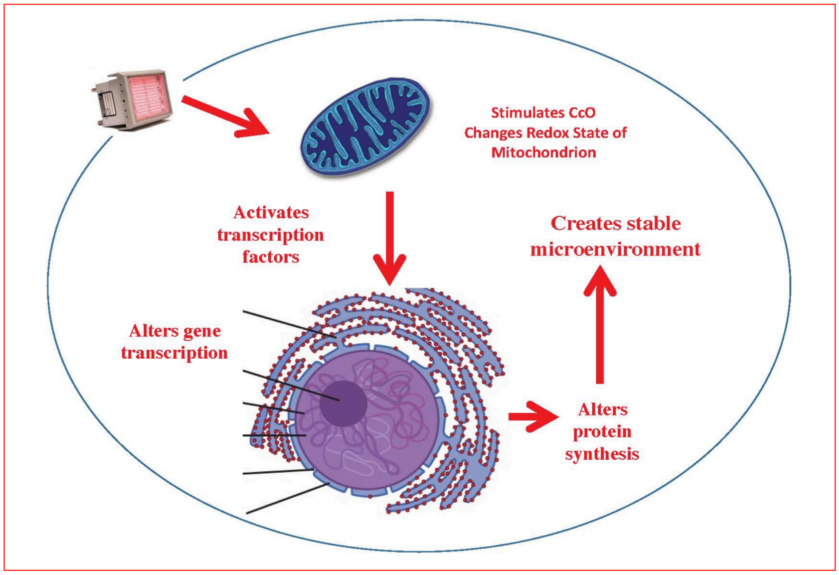
The retina is one of most bioenergetically active tissues in the human body. The inner layers of the retina including the retinal ganglion cells exhibit a high metabolic rate associated with all CNS neurons. The oxygen consumption rate of the photoreceptor layer is several times higher. Rod and cone photoreceptor cells within the retina are rich in mitochondria concentrated in their inner segments. These mitochondria provide the ATP required by the ionic pumps that drive the 'dark current'. Modulation of the "dark current" by light is the beginning of vision (Kolb, 2003, Sung and Chuang, 2010, Yu and Cringle, 2005). As a result, photoreceptors consume more oxygen per gram of tissue weight than any cell in the body, making the retina is one of the highest oxygen-consuming tissues in the human body (Yu and Cringle, 2005). This intense oxidative phosphorylation in photoreceptor cell inner segments, coupled with high concentrations of polyunsaturated fatty acids in their outer segments renders the retina susceptible to oxidative stress and lipid peroxidation (Winker, 1981). In addition, photosensitizers in the retina cause an increase in oxidative stress when exposed to visible light (Jarrett and Boulton, 2012). Normally, oxidative damage is minimized by endogenous antioxidants and repair systems. With aging and/or retinal disease there is an increase in mitochondrial dysfunction and oxidative damage. Mitochondrial dysfunction and oxidative damage to the neuronal and non-neuronal components of the retina has been implicated in many forms of retinal injury and degeneration (Eells et al., 2016, Gouras et al, 2016, Fisher and Ferrington, 2018 ). *[Figure 2].*




Mitochondrial repair and attenuation of oxidative stress are critical to the long-term survival of the retina. As a consequence, therapeutic strategies directed towards improving mitochondrial integrity and function and reducing oxidative stress have considerable potential for the treatment of retinal disease. Treatment using low-intensity far-red to near-infrared (FR/NIR) light has been shown to act on mitochondria-mediated signaling pathways to preserve mitochondrial function, attenuate oxidative stress, stimulate the production of cytoprotective factors and prevent neuronal death. (Eells et al., 2004; Chung et al., 2012) *[Figure 3].* FR/NIR photons penetrate the brain, retina and optic nerve and this treatment, commonly known as photobiomodulation (PBM) has documented efficacy in the treatment of retinal aging, injury

and retinal degenerative disease (Fitzgerald et al., 2013, Geneva, 2016). Research on photobiomodulation by FR/NIR light in animal models of retinal injury and disease is the focus of this chapter.

Fig 3. FR/NIR Photons Stimulate Mitochondrial Cytochrome c Oxidase and Activate Protective Intracellular Pathways




**25.2 Methanol Intoxication.** Eells *et al.,* (2003) reported the first direct link between the actions of far-red light on mitochondrial bioenergetics *in vitro* and retinoprotection *in vivo* using an established model of retinal mitochondrial toxicity, methanol intoxication. Accumulation of formic acid generated from methanol oxidation in the course of methanol poisoning produces toxic injury to the retina and optic nerve, resulting in blindness (Eells et al., 2003). An acute toxic exposure to methanol results in an increase in tissue formic acid concentrations, metabolic acidosis and visual toxicity within 72 hours of ingestion (Seme et al.,1999). Formic acid is the toxic metabolite in methanol intoxication. Formic acid inhibits the key mitochondrial enzyme, cytochrome c oxidase, by reversibly binding to the same site as cyanide and azide (Seme et al., 1999, Eells et al., 2003, Wong-Riley et al., 2005). Studies were undertaken to test the hypothesis 670 nm PBM using light-emitting diode (LED) arrays would protect the retina against the toxic actions of methanol-derived formic acid in a rodent model of methanol toxicity and would improve the recovery of retinal function following methanol intoxication. Using the electroretinogram as a sensitive indicator of retinal function, these studies demonstrated that three brief 670 nm LED treatments (160 sec at 25 mW/cm$^2$ producing a fluence 4 J/cm$^2$ at the surface of the eye) (Spectralife, Quantum Devices Inc., Barneveld WI) treatments delivered at 5, 25, and 50 h of methanol intoxication, attenuated the retinotoxic effects of methanol-derived formate. The authors documented a significant protection against formate-induced retinal dysfunction during the course of intoxication and recovery of retinal function following intoxication in 670 nm light-treated, methanol-intoxicated rats *[Figure 4].* 670 nm light also protected the retina from the histopathologic changes induced by methanol-derived formate. These findings provided the first link between the actions of FR to NIR light on mitochondrial oxidative metabolism *in vitro* and retinoprotection *in vivo*. Moreover, they were the first studies to suggest that FR/NIR PBM had therapeutic potential for the treatment of retinal injury and disease.

**25.3 Bright-Light Induced Retinal Damage.** Another method of retinal damage is exposure to excessive white light which causes photoreceptor damage and death (Grimm and Reme, 2013). Oxidative damage produced by photo-oxidation of the photoreceptor outer segments is widely accepted as the initiating event in light-induced retinal damage (LD) (Demontis et al., 2002). The lesions produced by LD are characterized by photoreceptor cell death, retinal pigment epithelial (RPE) cell damage, Muller cell gliosis and disruption of the outer limiting membrane (OLM) (Hao et al., 2002). In addition to these structural changes, there is the induction of an inflammatory state characterized by an invasion of the outer retina by activated microglia (Levine et al., 2014). This progressive degeneration has been used to model many of the factors contributing to the expansion of the degenerative area, similar to the changes observed in AMD (Rutar et al, 2010, 2011 and 2012).

Protection by photobiomodulation against LD has been documented by several research groups (Qu et al., 2010, Albarracin et al. 2011, Albarracin and Valter, 2012). Albarracin *et al*. (2011), first showed that 670 nm light (9 J/cm$^2$) administered before, during or after bright light exposure ameliorated the damaging effects of LD. PBM protected photoreceptor function as measured by ERG responses and morphology. This protection involved a reduction in photoreceptor cell death and inflammatory stress biomarkers in the retina and a reduction in microglial and macrophage invasion (Albarracin and Valter, 2012). Pretreatment with 670 nm light proved to be most effective against LD compared to treatment during or after LD. However, animals treated with PBM post-LD also recovered photoreceptor function by one-month post-exposure. PBM also reduced cell stress responses and inflammation in the retina. PBM mitigated the upregulation of the stress marker, GFAP, in Müller glial cells and reduced microglial activation.

At the molecular level, LD results in increased lipid peroxidation by photoreceptor outer segments resulting in morphological and functional damage (Liu et al., 2015). The oxidative stress caused by LD also increases gene expression of endogenous antioxidants. Natoli *et al*., (2010) explored the neuroprotective potential of FR/NIR pretreatment in retinas harvested from light-damaged rats using DNA microarray analysis. They showed that gene expression in pathways involved in inflammation and cell death were down-regulated by 670 nm light in LD retinas. In this study, experimental groups were protected against LD (1000 lux for 24 h) by pretreatment with 670 nm light (9 J/cm$^2$ at the eye, daily for 5 days). Quantitative real-time PCR analysis of 14 selected genes was used to validate the microarray results. LD caused the regulation of 175 entities (genes and ncRNAs) beyond criterion levels ($p<0.05$ in comparison with controls, fold-change >2). 670nm light pretreatment reduced the expression of 126 of these 175 LD-regulated entities below criterion. In addition, 670nm light altered 67 entities not regulated by LD. A high proportion of the entities regulated by LD (>90%) were known genes. By contrast, noncoding RNAs (ncRNAs) were prominent among the entities regulated by 670 nm light in its neuroprotective role (62%). One of the most highly modulated genes identified in this study was Ccl2, a potent chemokine involved in the recruitment of moncytes, T-cells and dendritic cells to sites of tissue injury. This chemokine family has also been implicated in the pathogenesis of AMD.

Müller glial cells play an important role in the maintenance and normal function of the photoreceptors and other elements of the retina. Albarracin and Valter, (2012) examined the effects of pretreatment with 670 nm red light on Müller cells using the bright light-induced rat model of retinal degeneration. Müller cell-specific markers were used to assess structural and functional changes in this cell type seven days later. Changes in gene (Edn2, LIF, TNF-$\alpha$) and protein (S100$\beta$, Vimentin, LIF, iNOS, GS, Cyclin-D1) levels and localization were evaluated using RT-qPCR and immunohistochemistry, respectively. 670 nm light

pretreatment ameliorated the bright light-induced alterations in the expression of Müller-cell specific markers for structure, stress, metabolism and inflammation. Thus, PBM preconditioning may promote neuroprotective effects in the retina from light-induced damage, possibly through pathways regulating the roles of Müller cells in maintaining retinal homeostasis.

PBM has been shown to downregulate TNF$\alpha$, a key pro-inflammatory cytokine, in two independent studies in the eye. Albarracin and Valter reported a reduction in TNF$\alpha$ gene expression in the retinas of LD rats pretreated with 670 nm PBM using PCR (Albarracin and Valter, 2012a, 2012b). In the second study, Kokkinopoulis *et al*. (2013), showed that 670 nm irradiation reduced TNF$\alpha$ immunoreactivity in the retinas of aged mice. They further showed a reduction in the recruitment of IBA-1 positive macrophages to the outer retina and a reduction in C3b and C3d immunoreactivity in Bruch's membrane. All of these changes are consistent with a down-regulation of inflammatory responses by PBM.

Complement activation is associated with the pathogenesis of age-related macular degeneration (AMD) (Zipfel et al., 2010). Complement activation also occurs following LD (Rutar et al., 2011, 2012). 670 nm light pretreatment (9 J/cm$^2$) reduced the expression of complement components and receptors in the retina following LD (Rutar et al., 2010). Moreover, there was a reduction in the recruitment of C-3 expressing microglia/macrophages in the retina following 670 nm light treatment and a concomitant reduction in the biomarker of oxidative damage 4-hydroxynonenal (4-HNE). These findings indicate the 670 nm light pretreatment attenuates oxidative damage to photoreceptors and reduces inflammation, which may reduce the stimulation of the complement cascade and, thus, protect photoreceptors.

**25.4 Diabetic Retinopathy.** Diabetic retinopathy is a common long-term complication of diabetes. This retinopathy is characterized by damage to the vasculature and neurons, and in severe cases, loss of vision itself (Rao and Dlouhy, 2012, Saliba et al., 2015). Although, the pathogenesis of diabetic retinopathy is incompletely understood, a reduction in hyperglycemia has been shown to exert positive effects on the development and progression of diabetic retinopathy. However, in many patients, maintenance of glycemic control is difficult, therefore effective therapies are needed to inhibit the retinopathy (Stewart, 2016).

The laboratory of Tim Kern has extensively investigated the therapeutic potential of PBM in diabetic retinopathy (Tang et al., 2013). The effects of 670 nm light treatment (240 sec, 25 mW/cm$^2$, 6 J/cm$^2$) on pathologic changes relevant to the development of diabetic retinopathy *in vivo* and *in vitro* were studied. In streptozotocin (STZ)-diabetic rats, 670 nm PBM attenuated diabetes-induced abnormalities of retinal function and reduced retinal ganglion cell (RGC) death. PBM also decreased retinal superoxide generation and inhibited diabetes-induced abnormalities in the electroretinogram (ERG), retinal ganglion cell (RGC) viability, superoxide generation, leukostasis, and expression of MnSOD and ICAM-1 *in vivo*. In cultured retinal cells incubated in diabetes-like concentrations of glucose (30 mM), PBM attenuated oxidative stress, expression of inflammatory biomarkers and improved cell survival in RGC5 (immortalized retinal ganglion cells) and 661W (immortalized photoreceptor-like cells) cells. The authors concluded that 670nm PBM is a simple adjunct therapy to attenuate the development of diabetic retinopathy.

In another series of studies, the Kern lab (Saliba et al., 2015) tested whether 670 nm PBM would exert beneficial effects in another species (mice), in the presence of heavy pigmentation (C57Bl/6J), as an intervention therapy. Their model system included diabetic mice treated with an inhibitor of the

antioxidant enzyme, heme oxygenase 1 (HO-1). These mice were then exposed to 670nm light while their eyes were blocked from direct exposure of the light. Mice treated with 670nm light showed both neuronal and vascular beneficial effects, and that this effect is mediated at least in part systemically.

**25.5 Retinitis Pigmentosa** Retinitis pigmentosa (RP) is a heterogeneous group of blinding retinal diseases with the common feature of photoreceptor degeneration that is commonly associated with single-gene mutations (Wang et al., 2005). Numerous investigations have provided considerable information regarding the genetic bases of these degenerations, and several previous studies have provided evidence that, in many forms of retinal dystrophy, oxidative damage to mitochondria is a step in the death of photoreceptors (Campochiaro et al., 2015).

The therapeutic efficacy and mechanism of action of 670 nm PBM was investigated in a rodent model of RP, the P23H rat (Kirk et al., 2013). In this rodent model of human disease, the transgene is a rhodopsin gene engineered to mimic a mutation that causes an autosomal dominant form of human RP common in North America. P23H rat pups were treated once per day during the critical period of photoreceptor development (between p10 and p25) with a 670 nm LED array (180 sec treatments at 50 mW/cm$^2$; fluence 9 joules/cm$^2$) (Quantum Devices Inc., Barneveld WI). Sham-treated rats were restrained, but not exposed to 670 nm light. In the first series of studies, rats were treated from postnatal day (p) 16-20. The status of the retina was determined at p22 by assessment of mitochondrial function, oxidative stress and cell death. In a second series of studies, rat pups were treated from p10-p25. Retinal status was assessed at p30 by measuring photoreceptor function by ERG and retinal morphology by Spectral Domain Optical Coherence Tomography (SD-OCT). 670 nm light treatment increased retinal mitochondrial cytochrome oxidase activity**.** 670 nm light treatment also attenuated photoreceptor cell loss and improved photoreceptor function. These data suggest that PBM protects photoreceptors in the developing P23H retina, by augmenting mitochondrial bioenergetics.

**25.6 Aging and Age-Related Macular Degeneration** Age-Related Macular Degeneration (AMD) is the most common cause of untreatable blindness among older adults worldwide (Ehrlich et al., 2008). Visual loss in AMD is due, in large part, to age-dependent impairment of retinal pigment epithelial (RPE) function. Although, the pathogenesis of AMD has not been elucidated, considerable evidence supports a role for mitochondrial dysfunction, oxidative stress and inflammation in its onset and development (Beckman and Ames, 1998, Goura et al., 2016). In addition to mitochondrial dysfunction and oxidative stress, inflammation is a common feature in both retinal aging and in AMD. The laboratory of Glen Jeffery in the Institute of Ophthalmology at University College in London has examined the effects of 670 nm light on retinal aging and on AMD in mouse models. They have shown 670 nm light treatment, in doses ranging from 4-7 J/cm$^2$ in the aged mouse retina increases mitochondrial membrane potential and reduces retinal inflammation (Kokkinopoulos et al., 2013).

Additional studies investigated the effects of PBM in a mouse model of AMD, the complement factor H knockout mouse (*Cfh*$^{-/-}$) (Begum et al., 2013). In this model, retinal inflammation and AB deposition occur resulting in loss of retinal function. For these studies 670 nm PBM was by LED arrays mounted on the sides of the animal cages (360 seconds at a dose of 7.2 J/cm$^2$ twice per day for 14 days) rather than directly focused on the retina. PBM-treated animals exhibited significant increases in cytochrome c oxidase (CcO), a critical mitochondrial enzyme regulating oxidative phosphorylation. Complement component C3, an inflammatory marker in the outer retina was downregulated as were key biomarkers of retinal stress,

vimetin and glial fibrillary acidic protein (GFAP). The authors concluded that 670 nm PBM is effective in reducing retinal inflammation likely due to CcO activation in mice with a genotype similar to that in 50% of AMD patients even when brief exposures are delivered via environmental LED arrays. The efficacy revealed here supports current early stage clinical trials of 670 nm in AMD patients.

Studies by Calaza et al. (2015), tested the link between aging, retinal ATP, and complement system polymorphisms by examining ATP concentrations in normal aging eyes and those of complement factor H knockout mice (*Cfh*$^{-/-}$). The *Cfh*$^{-/-}$ mouse is a widely used murine model of AMD (Coffey et al., 2007). The authors observed a premature decline in retinal ATP in the aging *Cfh*$^{-/-}$ mouse and also changes in the expression of the mitochondrial heat shock protein, Hsp60. 670 nm PBM (40 mW/cm$^2$ for 90 seconds daily for 5 days; 3.6 J/cm$^2$ per treatment) corrected the ATP decline in *Cfh*$^{-/-}$ mice and shifted Hsp60 labeling pattern. The decline in retinal ATP concentrations in *Cfh*$^{-/-}$ mice occurs prior to the development of the ocular phenotype involving inflammation and photoreceptor dysfunction. These findings suggest that inflammation, Aβ deposition and reduced retinal function identified at 12 months in this mouse model, may be related to a reduction in mitochondrial bioenergetics that can be corrected by PBM.

**25.7 Retinopathy of Prematurity** Retinopathy of Prematurity (ROP) is a disorder of the developing retina and a serious sight threatening complication of supplemental oxygen therapy in premature neonates (Flynn et al., 1991). ROP is a two-stage disease resulting from abnormal development of the retinal vasculature caused by a disruption of the normal oxygen environment in the developing retina. In the first stage there are higher systemic oxygen concentrations than encountered *in utero* suppressing the development of the retinal vasculature. This is followed in the second stage by retinal hypoxia and the release of hypoxia-stimulated factors that stimulate neovascularization when the neonate begins breathing normal air (Flynn et al, 1991).

Current therapeutic interventions for ROP include laser photocoagulaton or cryotherapy to target the angiogenic aspect of the disease. These interventions are invasive, expensive and only partially effective. Natoli et al, (2013)., investigated the therapeutic efficacy of 670 nm light in two well-established rodent models of oxygen-induced retinopathy (OIR), hyperoxia exposed mice and hyperoxia exposed rats. These oxygen-induced retinopathy (OIR) models take advantage of the fact that normal retinal vascularization occurs *ex utero* in rodents, and thus replicates the incomplete vascular development of the retinal vasculature in premature infants.

Animals were treated with 670 nm light delivered by LED arrays at a dose of 9 J/cm$^2$ (50 mW/cm$^2$ x 180 sec) once daily during exposure to hyperoxia (p7-p17, mouse; p0 – p18, rat). PBM protected the retina from hyperoxia in both models. PBM reduced vaso-obliteration, neovascularization and retinal hemorrhage. PBM also preserved retinal vascular branching architecture and reduced neuronal cell death. In addition, the authors observed in the rat model that PBM reduced the incidence of lung pathology indicative of a systemic benefit. Although the mechanism of 670 nm PBM protection of the retinal vasculature in oxygen-induced retinopathy is unclear, the authors hypothesize that 670 nm light activates mitochondrial metabolism thus promoting the consumption of excess oxygen in the hyperoxic phase of the disease. They further suggest that PBM protects retinal glial cells and reduces oxidative stress.

**25.8 Optic Nerve Injury**

Optic nerve injury can be induced by intravitreal injection of rotenone to inhibit mitochondrial complex I (Zhang et al., 2002). Using this rodent model of toxic optic neuropathy, Rojas *et al*. (2008) reported on the neuroprotective actions of 633nm light. Pigmented rats received single bilateral intravitreal doses of rotenone or rotenone plus different dosage regimens of 633 nm light (3.6 J/cm$^2$ for 3 or 6 days). Treatment effects were evaluated using behavioral testing, histology and neurochemistry. Rotenone induced a decrease in visual function compared with vehicle-treated controls. Behavioral impairment correlated with a decrease in retinal and visual pathway metabolic activity, retinal nerve fiber layer thickness and ganglion cell layer cell density. These changes were prevented by light treatments given after rotenone in a dose-dependent manner (the most effective total dose was six 3.6 J/cm$^2$ doses of 633 nm delivered once per day for 6 days following rotenone treatment). Whole-brain cytochrome oxidase and superoxide dismutase activities were also increased in light-treated subjects in a dose-dependent manner, suggesting an *in vivo* transcranial effect of PBM. In whole-brain membrane isolates, PBM prevented the rotenone-induced decrease in cell respiration. The results show that PBM can effectively prevent the neurotoxic effects of rotenone suggesting therapeutic benefits for neurodegenerative disorders associated with mitochondrial dysfunction.

Another model of optic nerve injury involves examination of secondary degeneration through partial transection of the optic nerve (ON) (Fitzgerald et al., 2010). Traumatic injury to the central nervous system (CNS) is often accompanied by the spreading damage of secondary degeneration, resulting in further loss of neurons and function. In this model of secondary degeneration, axons of retinal ganglion cells in the ventral ON are spared from initial dorsal injury, however they are vulnerable to secondary degeneration mediated by oxidative stress (Fitzgerald et al., 2010, 2013, Cummins et al., 2013). Using this partial injury model, Fitzgerald et al. (2010), have demonstrated that PBM (WARP10 LED array, 670 nm) reduced oxidative stress in areas of ON vulnerable to secondary degeneration. Visual function was also restored to normal by 670 nm light treatment as assessed using optokinetic nystagmus and the Y-maze pattern discrimination task thus providing evidence that 670-nm light attenuates oxidative stress and improves function in the CNS after traumatic injury *in vivo*.

**25.9 Glaucoma.** Glaucoma is a leading cause of irreversible blindness worldwide (Weinreb et al., 2014). It is associated with retinal ganglion cell (RGC) degeneration leading to damage to the optic nerve and visual field loss (Weinreb, *et al*., 2014). Intraocular pressure (IOP) is currently the only modifiable risk factor for glaucoma, although RGC and vision loss can continue in patients despite well-controlled IOP (Coleman and Kodjebacheva, 2009). Studies in a rodent model of glaucoma produced by elevated IOP have provided evidence that 670 nm light focused through the pupil can attenuate the negative effects induced by ischemia (Del Olmo-Aguado et al., 2016). Elevation of IOP induces a state of retinal ischemia and results in increased expression of stress proteins including GFAP, HO-1 and mTORC1 culminating in RGC cell death. These negative effects of ischemia were significantly reduced by 670 nm light treatment (165 mW/cm$^2$). The authors conclude that low fluences of 670 nm light focused on the retina for a short period of time are sufficient to attenuate an insult of raised IOP to the rat retina.

**25.10 Conclusions and Future Directions.** Taken as a whole these studies in experimental models of retinal injury and disease show that FR/NIR PBM improves mitochondrial function, reduces oxidative stress and modulates inflammatory mediators leading to decreased apoptosis and retinoprotection.

Moreover, an increasing number of clinical investigations also support the therapeutic efficacy of FR/NIR PBM in the treatment of AMD and Diabetic retinopathy (Tang et al, 2014; Merry et al, 2017). Further studies are necessary to characterize the effect of PBM on the human retina and to define safe protocols for the application of this novel therapy to mechanistically complex diseases.

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