

The potential of far-red to near-infrared light in glaucoma neuroprotection.

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ABSTRACT

Alternative treatment strategies are necessary to reduce the incidence of glaucoma, a group of eye conditions that progressively damage the optic nerve and impair vision. The aim of this review is to gain insight into the molecular mechanisms potentially exploitable to slow down the retinal ganglion cells (RGCs) death, a crucial element in the pathophysiology of all forms of glaucoma, and to stimulate adult optic nerve repair.

For this purpose, we focus our analysis both on far-red to near-infrared light photobiomodulation (PBM) as phototherapeutic agent recently proposed in RGCs and in nerve lamina region neural progenitor cell (ONLR-NPC) niche as a strategy of glaucoma neuroprotection. We discuss the impact of beneficial molecular effects of PBM on both the mitochondrial derangement and the alteration of ion fluxes considered important causes of RGCs damage, and on the stimulation of progenitor cells as the most promising approaches to prevent excessive neuronal cell loss. We describe the experimental evidences supporting the validity of PBM therapy, which although being a safe, non-invasive, inexpensive, and easy to administer procedure, has not yet been fully explored in the clinical practice of glaucoma treatment.

Keywords: glaucoma; photobiomodulation (PBM); far-red to near-infrared (FR/NIR) light therapy; low-level laser therapy (LLLT); retinal ganglion cells (RGC); mitochondrial dysfunction; nerve lamina region neural progenitor cell (ONLR-NPC) niche

1. Introduction

Glaucoma, one of the leading causes of blindness worldwide, is a multifactorial chronic optic neuropathy with distinctive and usually irreversible, but preventable visual field defects [1], characterized by optic nerve progressive changes with corresponding vision impairment [2]. The increased intraocular pressure (IOP) is considered the most important risk factor for glaucoma [2]. The prevalence of glaucoma reaches 6-8% of the population 50 years of age and over, and therefore it is a major public health issue; as the population in Europe and most developed countries is ageing, a fact that will lead to an increasing burden of vision impaired over the next decades.

There are two major types of glaucoma sharing the same signs and symptoms: primary, or idiopathic, and secondary glaucoma, both having two major subtypes (open-angle and angle-closure) according to the underlying anatomy and pathophysiology. Primary glaucoma results with no identifiable cause, whereas secondary glaucoma has an identifiable cause of increased intraocular pressure (IOP) that causes optic nerve damage. Open-angle glaucoma can be classified into primary open-angle glaucoma (POAG), characterized by an increased IOP with progression of the optic nerve, normal-tension glaucoma (NTG), characterized by normal IOP with progression and optic neuropathy, and secondary open-angle glaucoma, characterized by elevated IOP and/or optic neuropathy. Angle-closure glaucoma can be classified into primary angle-closure glaucoma (PACG), further classified into acute (closure of anterior chamber angle with a sudden increase in IOP) and chronic (closure of the anterior chamber angle with a gradual increase in IOP or development of peripheral anterior synechiae), whereas secondary closed-angle glaucoma is the closure of the anterior chamber angle with increased IOP [3].

Familial glaucoma is relatively rare, and only a few genes have thus far been validated as risk factors for glaucoma. Genes have been associated with adult-onset POAG (*MYOC*, *WDR36*, *OPTN*, *NTF4*), congenital glaucoma (*LTBP2*, *CYP11B1*), pseudoexfoliative glaucoma (*LOXLI*), and normal tension glaucoma (*OPTN*), although most POAG patients may not have any of these gene mutations or polymorphisms [4]. Glaucoma occurs at two time periods: early-onset exhibiting Mendelian inheritance (<40 years) and adult-onset forms that are inherited as complex traits (>40 years). The incidence of blindness is higher in primary angle-closure glaucoma despite it being less common than open-angle glaucoma [3].

The mechanisms causing glaucomatous neurodegeneration are not fully understood [5]. Various strategies have been proposed to delay or halt retinal ganglion cells (RGCs) death and thus prolong visual function; these include surgery and the employment of pharmacological agents. Surgical procedures and the use of topically applied substances are both aimed at lowering IOP and succeeded in reducing the rate of progression in certain subjects, nevertheless, some glaucomatous

patients do not benefit significantly from such treatments [6]. Therefore various substances have been proposed as add-on; these include antioxidants, nitric oxide synthase inhibitors and NO-donating agents, adenosine receptor antagonists, Rho-pathway inhibitors, PI3K/Akt activators, purinergic ligands, ATP-sensitive potassium channel (KATP) channel activators, glutamate antagonists, non-glucocorticoid steroidal compounds, cannabinoids, dopamine and serotonin receptors ligands, neurotrophic factors, histone deacetylase inhibitors [6,7], the use of stem cells and gene therapy [8].

As changes need to be made to reduce the incidence/burden of glaucoma and alternative treatment strategies are necessary, the aim of this review is to gain insight into the molecular mechanisms potentially exploitable to slow down the RGCs death. A major mechanism for such death was suggested to be mitochondrial dysfunction and the related oxidative stress; indeed, mitochondrial potentiation and preservation is actively studied together with the stimulation of progenitor cells as the most promising approaches to prevent excessive RGC loss. This review will discuss the impact of mitochondrial derangement and the alteration of ion fluxes among the causes of RGC damage. Because the use of red light has been proposed as a non-invasive procedure to protect RGCs from dying in diseases like glaucoma, suggesting a mitochondrial effect of red/near-infrared (NR) phototherapy [9], we focused our attention both on far-red to near-infrared light photobiomodulation (PBM) as phototherapeutic agent in RGCs and in nerve lamina region neural progenitor cell (ONLR-NPC) niche as strategy in glaucoma neuroprotection. We will highlight the multiple beneficial molecular effects of NR phototherapy.

Electronic search was performed using Scopus and PubMed from January 2011 to February 2021 selecting humans as species, classical article, review and medicine, biochemistry, genetics and molecular biology, and engineering as subject area in Scopus, and humans as species, classical article, review - in PubMed. Abstracts, case reports, conference presentations were excluded. To identify all relevant published studies, we combined the following medical subject headings (MeSH) terms or keywords: "glaucoma" AND "photobiomodulation" OR "far-red light" OR "near-infrared light" OR "low energy photon irradiation" AND "mitochondria" OR "mitochondrial respiratory chain activity" OR "redox signaling" OR "oxidative stress" AND/OR "retinal ganglial cells" OR "nerve lamina region neural progenitor cell niche". All publications were in English and limited to human subjects, with the only exception of 8 additional papers concerning *in vitro* and *in vivo* animal model studies retrieved from the reference lists of some selected articles. Indeed, the reference list of all retrieved articles was also reviewed and additional 6 articles were considered even if they were published before 2011. A total of 547 publications were retrieved through the research databases. After

excluding duplicated articles and publications that did not meet inclusion criteria, 61 articles remained, and were reviewed together with 2 patents retrieved using the following keywords: red light ocular treatments or visual system function (Figure 1).

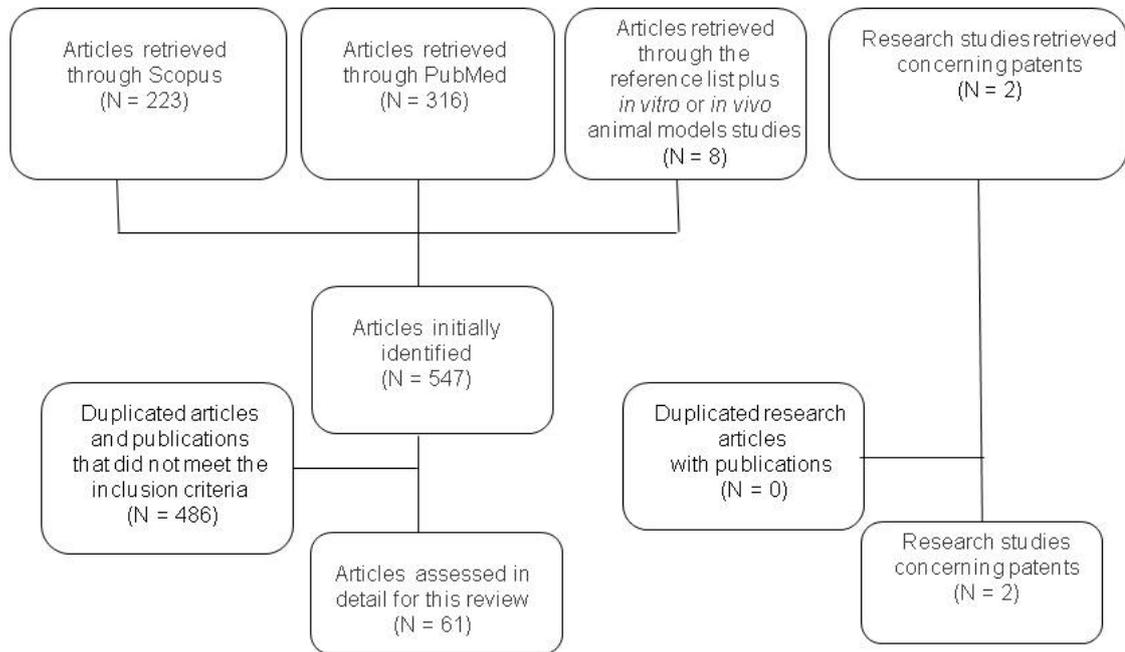


Figure 1. Search strategy for identifying scientific publications and clinical studies for this comprehensive review paper.

2. Pathophysiology of glaucoma: increased IOP, mitochondrial dysfunction and altered ion fluxes.

Older age and frailty, African descent, family history of glaucoma, diabetes [10], use of systemic or topical corticosteroids [11], myopia, ocular diseases, reduced central corneal thickness [12], trauma and surgery [13], smoking, ischemic event, hypertension and vasospasm [14] contribute to increase the eye pressure, the most important risk factor for glaucoma [2], which is thought to directly cause damage to neurons and to the optic nerve [15] as the disease is characterized by progressive degeneration of RGCs, the projection neurons of the eye, and of axons.

The balance between secretion of aqueous humor by the ciliary body and its drainage through two independent pathways - the trabecular meshwork (TM) and uveoscleral outflow pathway - is very important as the progressive resistance to outflow results in a gradual increase in IOP [11], which

triggers glaucomatous pathogenesis [16]. In patients with open-angle glaucoma, there is increased resistance to aqueous outflow through the trabecular meshwork. In contrast, the access to the drainage pathways is obstructed anatomically in patients with angle-closure glaucoma [11]. Notably, TM cells, as the endothelial like cells, lie in the collagen meshwork beams and were significantly decreased; they show both mitochondrial permeability transition pore alteration and mitochondrial release of calcium that are increased in glaucoma patients compared to healthy subjects [16]: the dysfunction of calcium regulation by these cells may cause failure control of IOP by TM tissue.

The chronic and progressive stress due to sensitivity to IOP is conveyed or transduced to the unmyelinated RGC axon segment as it passes through the optic nerve head in complex ways that include mechanical, inflammatory, and bioenergetic components [17]. However, glaucomatous RGCs and axon loss also occur in individuals with normal IOP, and patients whose IOP is effectively controlled by medical treatment often continue to suffer progressive neuron loss and visual field deterioration [18] suggesting other not fully understood mechanisms beyond pressure-mediated damage in neurodegeneration [5,15]. Indeed, there is the possibility that a pathophysiological stress, such as that induced by elevated IOP, triggers secondary immune or autoimmune responses, leading to RGC and axon damage after the initial insult is gone. In fact, evidence suggests that the transient elevation of IOP is sufficient to induce autoreactive T lymphocytes and T-cell infiltration into the retina in mice; the importance of this mechanism was demonstrated by the lack of T-cell autoreactivity in germ-free mice even after long-term experimentally induced IOP elevation. This T-cell infiltration leads to a prolonged phase of RGCs degeneration that persists also after IOP returns to a normal level [15], suggesting an autoimmune component in glaucoma.

Importantly, mitochondrial failure has been implicated in the etiology of retinitis pigmentosa and also retinal degeneration in glaucoma, in a way similar to neurodegenerative diseases such as Alzheimer's and Parkinson's disease. [19]. Similarities between glaucoma and mitochondrial optic neuropathies such as Leber's hereditary optic neuropathy and autosomal dominant optic atrophy exist; in all these illnesses a specific loss of RGCs is a common feature [20]. Barron et al. found a high density of voltage-gated Na⁺ channels and increased numbers of mitochondria in the pre-laminar optic nerve, which reflects the higher energy requirements for electrical conduction in unmyelinated axons in the pre-laminar and laminar optic nerve [21]. Due to the peculiar structural and energetic constraints, RGCs appear to be acutely susceptible to mitochondrial dysfunction, thus the death of RGCs is the hallmark of glaucomatous optic neuropathy.

Moreover, as mitochondrial function is also deemed to decline with aging in neurons, mitochondrial dysfunction, as either a cause or consequence of injury, renders RGCs sensitive to degeneration, which may be related to the increasing incidence of glaucoma with advancing age [22].

A further alteration observed in primary glaucoma - especially in the intermittent stage of angle closure glaucoma- is the change in quality and quantity of mitochondria in the iris tissue cells; for example, the disappearance of mitochondrial crest and vacuoles changes were reported. These morphological alterations are accompanied by an increase of intracellular reactive oxygen species (ROS) generation, higher intracellular calcium levels, and lower anti-oxidants expression in glaucomatous lamina cribrosa (LC) cells compared to that in normal cells, and are to some extent related to the pathogenesis of glaucoma [16]. Like all neurons of the central nervous system, RGCs depend on mitochondrial-generated ATP in order to preserve ionic gradients and survive [8], and any mitochondrial dysfunction will lead to cell damage. Such dysfunction can be initiated by the inhibition of enzyme processes which affect oxidative phosphorylation, leading to reduced ATP formation and increased oxidative stress, to finally result in cell death [8].

A decreased energy availability can have a strong impact on the maintenance of ion fluxes which are responsible for neuronal activity. As a consequence, or in addition, defects in ion channel activity can contribute to neuronal death. The expression and function of K^+ channels in Müller glia (critical for maintaining the proper physiology of RGCs) is altered in glaucoma and other retinal disorders [23]. The results of a recent study suggest that both K^+ and Na^+ homeostasis may be chronically altered in glaucoma [24]. In fact, it was demonstrated that the elevated pressure alters cation homeostasis and cation channel flux in Müller glia in vitro. The prolonged disruption of these gradients could contribute to the reported alterations in the electrophysiological properties of RGCs in glaucomatous retina [25,26]. Transient receptor potential vanilloid 4 (TRPV4) cation channels are important Ca^{2+} entry pathways sensitive to shear stress. Their activation triggers localized calcium influx and lowers IOP in rats and mice [27]. An interesting recent work carried out on TM cells showed that the mechanism of action of TRPV4 channels is mediated by the calcium-induced eNOS (endothelial NO synthase) activation and NO production [28]. The consequent vasodilation can explain the decrease of IOP observed in animals. The study demonstrated that the activity of TRPV4 channels is impaired in glaucoma, rendering TM cells insensitive to shear stress. Glaucoma associated functional impairment of TRPV4 channels may contribute to IOP elevation over time, leading to glaucomatous neurodegeneration. Upon calcium entry, its physiological levels are balanced by energy-dependent calcium efflux mechanisms, therefore mitochondrial activity must be strictly coupled to the activity of ion channels. If this balancing mechanism should fail, the excessive calcium influx could be detrimental to TM physiology and could justify the observations that link TRPV4 activity with TRPV4-dependent cytoskeletal remodeling, TM stiffness and outflow leading to elevated IOP [29].

3. Photobiomodulation

The utility of red light had been valued in the practice of medicine since antiquity, and "re-discovered" at the end of the 1918th century by the Nobel Prize in Medicine and Physiology Finsen for his achievements of curing skin disorders by shining red light [30]. The therapy consists of series of brief illumination with low energy photon irradiation in the far-red (FR) to near-infrared (NIR) range of the electromagnetic spectrum (600-1000 nm); these methods are collectively termed photobiomodulation (PBM), and are carried out by use of a laser or a light emitting diode (LED). While lasers are capable of heat production that can induce tissue damage, LEDs generate negligible amounts of heat, thus reducing the risk of thermal injury [31].

The effectiveness of PBM on the target tissue is dependent on the parameters used such as light source, wavelength, energy density, light pulse structure, and the duration of the laser application [32]. Regarding the type of wave, whether continuous or pulsed, there are still conflicting opinions as to which is the best and which factors should determine the pulse parameters [33].

The long wavelengths allow for high tissue penetration and PBM therapy is currently applied to accelerate wound healing, reduce neurologic pain, healing after peripheral nerve injury, ischaemic stroke and heart attack [34]. One of the most recent applications of PBM offers a promising innovative and non-invasive therapeutic approach to a host of challenging sight-threatening retinal conditions including age-related macular degeneration, retinopathy of prematurity, diabetic retinopathy, Leber's hereditary optic neuropathy, amblyopia, methanol-induced retinal damage, and possibly others [30]. Indeed recent studies have demonstrated that FR/NIR photons penetrate diseased tissues including the retina having the most effective at inducing *in vivo* beneficial effects in cells that do not appear to have specialized photopigments [35].

4. Far-red to near-infrared photomodulation in RGCs mitochondrial protection and energy metabolism

Enhancing the viability of RGCs in order to maintain their function is a major goal of basic and translational research [36]. As mitochondria play an important role in the pathogenesis of primary open-angle glaucoma, various strategies targeting mitochondrial protection provide a promising way to delay the onset of glaucoma or protect RGCs against glaucomatous damage [8]. Particularly, experimental studies have shown that light of different wavelengths can directly affect, in a wavelength-specific manner, oxidative phosphorylation when it is absorbed by mitochondrial chromophores [36]. Indeed, some studies have shown that RGCs possess a high number of mitochondria and are sensitive to the exposure to visual red light (680-900 nm), which has been reported to be absorbed by cytochrome-c-oxidase (COX), a key enzymatic complex for cell

bioenergetics especially for nerve cells in the retina and brain. COX contains four redox metal centers, Cu_A, Cu_B, Hem a, and Hem a₃, where electrons are transferred sequentially; different light wavelength determines peaks of absorption for the complex: 620 nm (range 613.5–623.5 nm), 825 nm (range 812.5–846 nm), 760 nm (range 750.7–772.3 nm), and 680 nm (range 667.5–683.7 nm) which correspond to Cu_A reduced, Cu_A oxidized, Cu_B reduced, and Cu_B oxidized, respectively. In neural tissue COX is the most abundant metalloprotein complex and the exposure to light at wavelength peaks in its absorption spectrum (670 nm and 830 nm), highly correlates with its peaks in catalytic activity and with ATP content *in vitro* [37].

Many of the secondary mediators of PBM, among which ROS and nitric oxide (NO), are able to activate transcription factors and signaling pathways [38] demonstrating that redox equilibrium plays pivotal roles in cells' physiological and pathological events due to ROS's ability to activate or inactivate a variety of receptors, proteins, ions, and other signaling molecules. When the redox equilibrium is disturbed due to the excessive accumulation or depletion of ROS, many cellular signaling pathways are perturbed leading to cellular dysfunction and, subsequently, the development of various pathologies among which eyes diseases. In this contest, PBM may also function by increasing the bioavailability of NO by prompting its release from intracellular stores such as heme-containing proteins under normoxic conditions. Since NO functions as an inhibitor of the mitochondrial respiration, its dissociation from cytochrome C oxidase would restore mitochondria's oxygen consumption, which in turn should increase energy production and thus boost cellular metabolism [30]. Thus, it is currently accepted that red light enhances the mitochondrial function thereby improving mitochondrial energy metabolism and the consequent increased mitochondrial membrane potential ($\Delta\Psi$) and proton gradient (ΔpH); therefore, red light exerts a cytoprotective function by preventing apoptotic cell death [20,34], and, consequently, reducing the damage caused by a variety of conditions [9,39–41].

The clinical use of far-red to near-infrared (FR/NIR) therapy to enhance mitochondrial function is supported by the successful attenuation of histopathological changes in animal retinas *in situ* [34,39,40,42,43]. Animal studies have specifically shown that optimum treatment conditions using red light (16.5 watts/m², 3000 lux, 625-635 nm for a short period of time) can attenuate an insult of raised IOP to the rat retina and the retinal dysfunctions [9] with no detectable harmful effects on lens, cornea and retina, dispelling previous doubts about therapeutic safety [44,45].

The beneficial effects of PBM on the neural and vascular elements of retina has also been supported by Tang et al. with both *in vitro* and *in vivo* studies. Indeed, daily 670 nm PBM treatment (6 J/cm²) resulted in a significant inhibition in the diabetes-induced death of cultured RGCs *in vitro*; this was possibly linked to the induction of superoxide production, in the preserved MnSOD

expression thus reducing oxidative stress, and in the decrease of leukostasis *in vivo*, ameliorating lesions of diabetic retinopathy [46]. Moreover Lu et al. showed that 670 nm light 9J/cm² once daily over 5 days reduces the Müller cell-mediated retinal inflammation both *in vivo* and *in vitro* [47] [48] offering a potential cellular mechanism for 670 nm light therapy in regulating inflammation associated with retinal degenerations. In fact, both the expression of COX5a and the mitochondrial membrane potential ($\Delta\Psi_m$) were increased, and the microglia/macrophage recruitment into the outer retina was reduced. This is of importance as non-neuronal components of the retina, the glial-derived Müller cells, are the supporting cells of the retina and play a key role in maintaining retinal homeostasis and in responding to retinal stress by the release of chemokines to recruit microglia and macrophages into the damaged retina where gliosis is a feature of eye disease progression. This highlights the value of low-level laser therapy (LLLT) as a novel paradigm to treat visual and supports the opinion that neuronal energy metabolism could constitute a major target for neurotherapeutics not only of the brain, but also of the eye [35].

Interestingly, calcium channels are modulated by red light, as demonstrated by recent works. In fact, it was demonstrated that PBM mitigated diabetes-induced calcium channel dysfunction across all retinal layers [49]. Moreover, another study reported that the 630-nm LED light can promote TRPV4 expression in synovial fibroblasts [50]. These observations suggest the utility of further studies investigating the effects of PBM on TRPV4 in glaucoma.

A schematic representation of the protective effects of PBM on cell metabolism is shown in Figure 2.

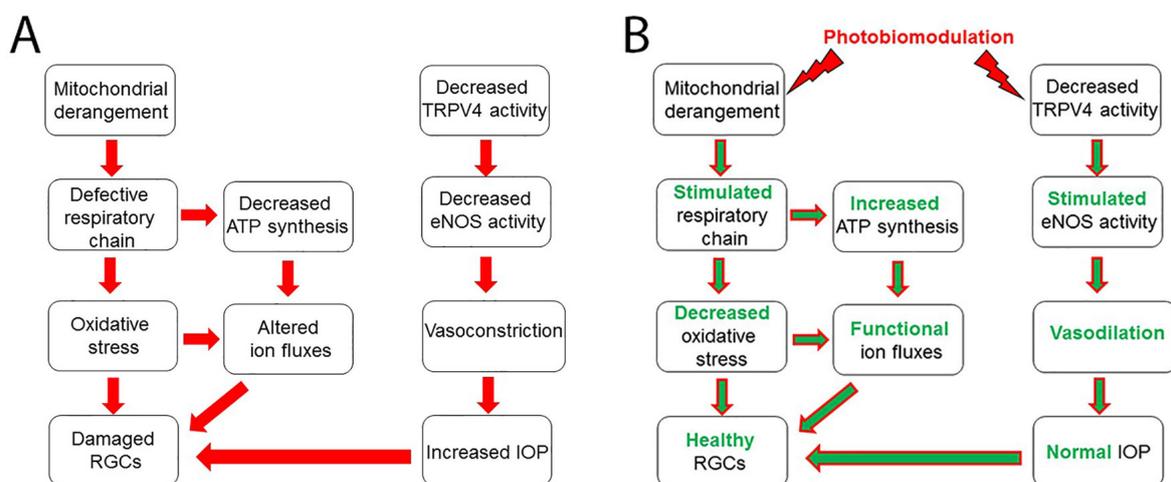


Figure 2. A schematic representation of the protective effects of PBM on cell metabolism.

5. Far-red to near-infrared photomodulation in neuronal differentiation

Many studies have found that mesenchymal stem cells (MSCs) can be used in the treatment of neurological diseases including spinal cord injury, Alzheimer's disease, and stroke as the differentiation of MSCs could be multi-directional and is regulated by many factors *in vivo* [51]. However, the lack of guidance and regulation might lead to uncontrolled proliferation and may even promote tumor formation; this remains a major safety concerns in the use of stem cells for therapeutic intervention. Also in glaucoma, the regenerative potentialities of MSC could be precious to substitute damaged RGCsm but their differentiation should be tightly controlled.

Photobiomodulation therapy (PBMT) in the neurological area acts in neuronal tissues as a complementary treatment of traumatic brain degeneration/injury, spinal cord trauma, and in the process of peripheral nerve regeneration [37]. Due to the photochemical and photobiological effects of PBM at the cellular level, there is a relationship between the improvement of trophic conditions and the reduction of inflammatory processes, closely related to a more efficient nervous regeneration, ATP production, enhanced secretion of neural factors, the increase of vascular network and collagen synthesis to facilitate neural differentiation and regeneration [37,52].

The potential role of PBM on stimulating cell differentiation has been described in various *in vitro* human models. Indeed, 810 nm wavelength was used in stimulating dental pulp stem cell [53], and red (660 nm) and near-infrared (810 nm) red light were demonstrated to be more effective than blue and green (415 nm and 540 nm) to induce differentiation of human adipose-derived stem cells (hASC) into osteoblasts when cultured in osteogenic medium. In fact after green/blue stimulation intracellular calcium and ATP production were lower compared to those observed after red light, and associated with higher level of ROS [54]. Recently, irradiation with laser light at 808-nm delivering energy densities from 0 to 10J/cm² has been demonstrated to induce neuronal rather than glial differentiation of human umbilical cord mesenchymal stem cells in early stage [55].

Also neuromodulation is achievable by photostimulation; indeed, when human Schwann cells, the glial cells of peripheral neural system, were treated with a diode laser at 810 nm, 50 mW (two different energies: 1 J/cm² and 4 J/cm²) for three consecutive days, cell proliferation and the nerve growth factor (NGF) gene expression on day 20 were stimulated [52].

However, emerging evidences suggest a plausible role also for blue light in neural stem cells (NSCs), the quiescent adult cells residing in specific regions of the mammalian brain; these, in fact, constitutively express blue/red light-sensitive photoreceptors. Indeed, Wang et al. showed a 4.3-fold increase in proliferation and 2.7-fold increase in differentiation toward astrocyte phenotype of the abovementioned NSCs upon low-power blue monochromatic light exposure (455 nm, 300 μW/cm²);

indeed, the melanopsin (Opn4)/transient receptor potential channel 6 (TRPC6) non-visual opsin might serve as a key photoreceptor response to blue light irradiation. This suggests that also the blue light-triggered system of NSCs could enable nongenetic and non-invasive neuromodulation with therapeutic potential for central nervous system diseases [56].

These findings are of notable relevance as Bernstein et al. have recently demonstrated that the optic nerve lamina region (ONLR) contains a neural progenitor cell (NPC) niche, which may have a role in both postnatal optic nerve development and in adult optic nerve support and repair [57]. Indeed, it is well known that ONLR has a number of unusual characteristics: it inhibits intraocular myelination, enables postnatal optic nerve myelination of growing axons, modulates the fluid pressure differences between eye and brain, and is the primary lesion site in the age-related disease open angle glaucoma. **It remains to be investigated whether PBM therapy with red light can effectively stimulate NPC and at least in part regenerate the damaged tissue in glaucoma.**

6. Existing patents for ocular treatments using laser red light

Nowadays, it is well known that *in vivo*, LLLT led to a significant improvement in visual acuity in adolescent and adult patients with amblyopia caused by ametropia or strabismus when area of the macula was irradiated through the conjunctiva from 1 cm distance for 30 sec with laser light (780 nm, 292 Hz, 1:1 duty cycle; average power 7.5 mW; spot area 3 mm, repeated on average 3.5 times) [58]. Moreover, two patents were filed concerning LLLT. Particularly, the one by LumiThera describes a device and a method for non-invasive multi-wavelength photobiomodulation for ocular treatments [59] encompassing symptoms of acute or chronic ocular syndromes as glaucoma, age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa, central serous retinopathy, non-arteritic anterior ischemic optic neuropathy, Leber's hereditary optic neuropathy, but also eyelids, wrinkles, keratitis, viral and bacterial infections, and many more. The irradiance with multiple wavelengths provided by multiple laser sources from 25 nm to 900 nm is also pulsed at frequencies from 1 to 100 Herz, or from 100 Hz to 100 kHz during 1 millisecond up to 150 seconds. Furthermore, a method of treating visual system disease is disclosed in this first patent. In the other one, sponsored by Department of Defense Advanced Research Project Agency (USA), the aim was to restore vision after methanol toxicity. It is based on the use of LEDs developed by NASA and consists in (a) exposing a component of a patient's visual system to light treatment, wherein the light treatment is characterized by wavelength of between 630-1000 nm and power intensity between 10-90 mW/cm for a time of 1-3 minutes, and (b) observing restoration of visual system function [60].

7. The future of clinical trials for the treatment of glaucoma

Glaucoma is generally a slowly progressive disease, as a rule; hence, there have been concerns regarding the feasibility of conducting clinical trials to evaluate the efficacy of new treatments for slowing or preventing vision loss [61]. It is generally believed that such trials would require very large sample sizes or long follow-up. Nevertheless, clinical trials for glaucoma neuroprotection are not impossible. When treatment efficacy is evaluated by (1) difference in incidence of pointwise event-based progression and (2) difference in rate of visual field mean deviation (MD) change between groups using linear mixed models (LMMs) versus conventional event-based survival analysis the feasibility of future glaucoma clinical trials can be improved substantially [62].

The studies described in this review highlight the relevance of some intracellular pathways which could be novel therapeutic targets, with the intent of lowering IOP on one side, and stimulating tissue regeneration on the other side. The potentiation of mitochondrial activity associated with ion flux control could be proposed as a metabolic approach worth of being pursued, and the therapy with red light could be used with this aim.

If PBM usefulness could be established through large clinical trials, it would offer a non-invasive and inexpensive approach, easy to deliver by medical providers, or even by patients themselves, to prevent or slow down the progress of retinal pathologies [30], including glaucoma.

7. Conclusion

As justly said by Dr. Michael R. Hamblin "after decades confined to the scientific wasteland, PBM may be finally emerging into the light of day (pun intended)" [63]. Indeed, as various prophylactic and therapeutic strategies targeting mitochondrial protection might provide a promising way to delay the onset of glaucoma, PBM could be useful for ameliorate mitochondrial function and/or stimulating progenitor cells to slow down the RGCs death in glaucoma. Moreover, PBM has been documented to have minimal risk, to be non-invasive, inexpensive, and easy to administer.

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