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# Targeting mitochondrial dysfunction as in aging and glaucoma

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**Neurons depend on their mitochondria for optimum function and become susceptible with age. Mitochondrial function is gradually impaired during aging because more electrons are converted to reactive oxygen species rather than being converted to ATP. Retinal ganglion cell mitochondria are additionally affected in glaucoma because of reduced oxygen delivery. Thus, targeting neuronal mitochondria to enhance their function as in glaucoma and aspects associated with aging provides potential ways of attenuating degenerating diseases. A substance worthy of mention is rapamycin, which affects regulated in development and DNA damage 1 (REDD1), and is known to enhance mitochondrial function. REDD1 appears to be prominent in retinal ganglion cells. An alternative exciting non-invasive approach is to use red light therapy that enhances mitochondrial function.**

## Introduction

A body of evidence now exists to support the notion that mitochondrial dysfunction accounts for the initiation of glaucoma and subsequent progressive loss of vision [1–9]. Mitochondria are known to perform a number of tasks in cells. These include maintaining homeostasis and being involved in numerous metabolic functions including oxidative energy metabolism, controlling intracellular calcium levels for mediating signalling and regulating neuronal excitability and synaptic transmission. Mitochondria are also associated with specific functions in defined cell types, such as in the production of urea in liver and kidney cells. However, in certain cell types such as fibroblasts, sufficient ATP to maintain function can be generated through mitochondria-independent processes like glycolysis [10]. Neurons, as opposed to most dividing cells like fibroblasts, have an absolute requirement for mitochondrial function to maintain survival, because they need an abundance of ATP to regulate ion gradients across their membranes for the generation of membrane potentials. Approximately 90% of their mitochondria-generated ATP is used to maintain membrane dynamics for neuronal survival and even a brief period of oxygen or glucose deprivation results in impaired mitochondrial function, loss of action potentials and subsequent death [11,12].

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Neurons in particular, but in some cases other cell types (e.g. photoreceptors) too, are absolutely dependent on ATP formed by mitochondria oxidative phosphorylation due to limited glycolytic capacity [13,14]. Logic therefore suggests that the number of neuronal mitochondria might reflect their importance for a defined type of neuron or cell. Significantly, in the retina mitochondria are particularly concentrated in the unmyelinated portion of mammalian retinal ganglion cell (RGC) axons [15–17], indicating that in this part of the neuron many mitochondria are necessary to provide energy for transmission of information to the brain. Retinal photoreceptors also have a significant abundance of mitochondria within their inner segments probably to provide the energy required for phagocytosis and outer segment renewal to occur [18].

## Age-related mitochondrial changes

A variety of studies suggest a correlation between lifespan and the aging of mitochondria. For example, cells from young rats undergo rapid senescence and degeneration when microinjected with mitochondria extracted from fibroblasts derived from old rats [19]. As mitochondria age they undergo a number of changes, including increased disorganisation of mitochondrial structure, decline in mitochondrial oxidative phosphorylation, increased production of reactive oxygen species (ROS), accumulation of mtDNA mutations and increased oxidative damage to DNA, lipids and proteins

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[20,21]. Increased mitochondrial ROS production with aging appears to be of special importance. Graphs showing an inverse relationship in a variety of different animal species between lifespan and increased formation of mitochondrial superoxide dismutase [22] and hydrogen superoxide [23], as well as enhanced mitochondrial metabolic and production [24,25] rates, provide compelling evidence that animals with higher rates of basal mitochondrial metabolism have faster rates of ROS production and, as a consequence, shorter lifespans [23,25,26]. Other factors such as mitochondrial and somatic DNA changes as well as an increased shortening of telemetric DNA [27] are also associated with aging and could be linked with elevated mitochondrial ROS production.

Various studies imply that a combination of reduced ATP and elevated oxidative stress is associated with aging of the retina. Figure 1 shows a summary of electroretinogram (ERG) recordings from a number of young (3-month-old) and aged (20-month-old) brown hooded rats showing that following identical ischemic/reperfusion insults the amplitudes of the a- and b-waves were greater in young rats. These studies can be interpreted to suggest that with aging less ATP is available to generate the ERG. This is consistent with a report showing that the inner retina andoptic nerve head (ONH) in younger mice were less vulnerable than those in older mice to insults due to ischemia [28] or reduced ATP through oxygen and/or glucose deprivation [29]. Moreover, Tezel *et al.* [30] demonstrated that the functional state of ganglion cells *in situ* decreases in rats with progressive aging.

Even though uncoupling proteins that buffer excess ROS are known to exist within mitochondria [31], it is clear that optimum mitochondrial function decreases with age and this is accompanied by an elevation in ROS and oxidative stress. A decline in the efficiency of the electron transport chain in mitochondria is likely to be a major cause of this being the case. Good evidence suggests that protons generated from electrons donated from NADH and $FADH_2$ for the production of ATP become inefficient during the aging process, with the result being that more ROS are formed leading to oxidative stress [32,33].

## Age-related changes and neurodegeneration

All neuronal degeneration is associated with mitochondrial dysfunction [33,34] and generally develops as aging progresses. Mitochondrial oxidative stress increases with aging because of a gradual increase in ROS, and as a consequence they become more prone to insults that they would otherwise have tolerated. Thus, specific mitochondrial insults, including genetic defects that might have little or no influence on young mitochondria, gradually become apparent in aging mitochondria. For example, Huntington's disease is an autosomal dominantly inherited neurodegenerative disorder caused by a CAG repeat expansion in the gene encoding huntingtin that manifests as a progressive increase in chorea and dementia [35], with symptoms usually beginning between 30 and 44 years of age. By contrast, in Parkinson's disease oxidative stress and impaired dopamine metabolism are thought to have crucial roles in causing specific neurons to be affected, although certain gene mutations associated with mitochondrial proteins (G399S and OMI/HtrA2) might play a part in specific cases [36,37]. Importantly, regardless of whether a defined neurodegeneration is clearly associated with a genetic mutation or not, mitochondrial dysfunction appears to be a major cause of

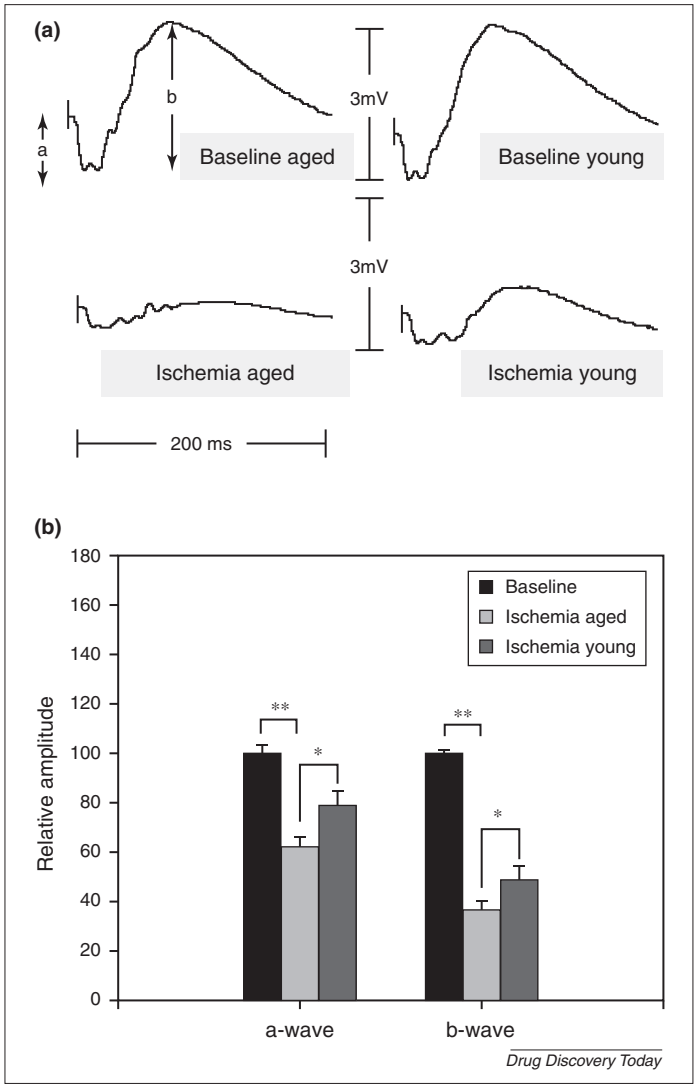


**FIGURE 1**

Results of some studies carried out on young (3-month-old) and old (20-month-old) male brown hooded rats. Electroretinograms (ERGs) were recorded from both eyes of all animals before (baseline recordings) and after ischemia/reperfusion. Ischemia (raised intraocular pressure 115 mmHg for 60 min) was induced in one eye from all animals. After a reperfusion period of 12 days ERGs in both eyes were recorded (ischemia). The a- and b-wave amplitudes were determined from the ERG tracings as shown **(a)**. **(b)** Shows that compared with baseline recordings the a- and b-wave amplitudes of the young rats are significantly less reduced than those from the older rats, suggesting that younger rats were more resistant to ischemia/reperfusion than the old rats. Results are ±SEM where $n = 10$, $^*P < 0.05$ by Student's unpaired *t*-test.

neuronal cell death and, also important, the diseases are age-related. Thus, the potential use of methodologies directed at minimising mitochondrial dysfunction might enable the treatment of many types of neurodegeneration irrespective of whether they have a strong link to a genetic dysfunction or not.

Primary open angle glaucoma (POAG) is a neurodegenerative disease associated with the retina. POAG is a type of optic neuropathy, characterised by a variable loss of RGCs and by specific changes to the ONH [1,7]. POAG develops in older adults [38–40], in whom the risk of developing the disorder can be affected by a variety of medical conditions that include hypertension and

raised intraocular pressure. The aetiology for the cause of POAG is multifactorial [2,4–6] and the disease is family associated. Support for the hypothesis that specific gene defects are strongly associated with POAG [41–45] is unimpressive. By contrast, more persuasive evidence exists for mutations in genes like myocilin (MYOC) and cytochrome P450 (CYP)1B1 being important in early-onset glaucoma, which occurs infrequently when compared with POAG and develops in late childhood or early adulthood [42,46].

## RGC mitochondrial oxidative stress in POAG

The rationale for believing that ischemia plays a major part in the initiation of POAG is overwhelming [2–6], although definitive proof remains to be demonstrated [1,7–9]. We have proposed [5,6] that a constant or intermittent change in the normal dynamics of blood delivery to the ONH might cause ischemia of a specific nature to initiate POAG. This should be contrasted with situations where blood flow to the ONH is completely blocked causing ischemic optic neuropathy. It should be noted that ischemia is a pathological situation where cellular demands are compromised owing to some degree of inadequacy in blood delivery [47]. The type of ischemia to the ONH necessary to initiate POAG is suggested to be of a specific characteristic yet variable in nature. Ischemia by definition involves an inadequacy (not necessarily a complete absence) of appropriate blood delivery to meet cellular energy demands and, as such, has a component of hypoxia (hypoxia by definition is a complete lack of oxygen) and this might vary in different cases. Figure 2 summarises the hypothesis where RGC mitochondria and glial cells (astrocytes and microglial) are

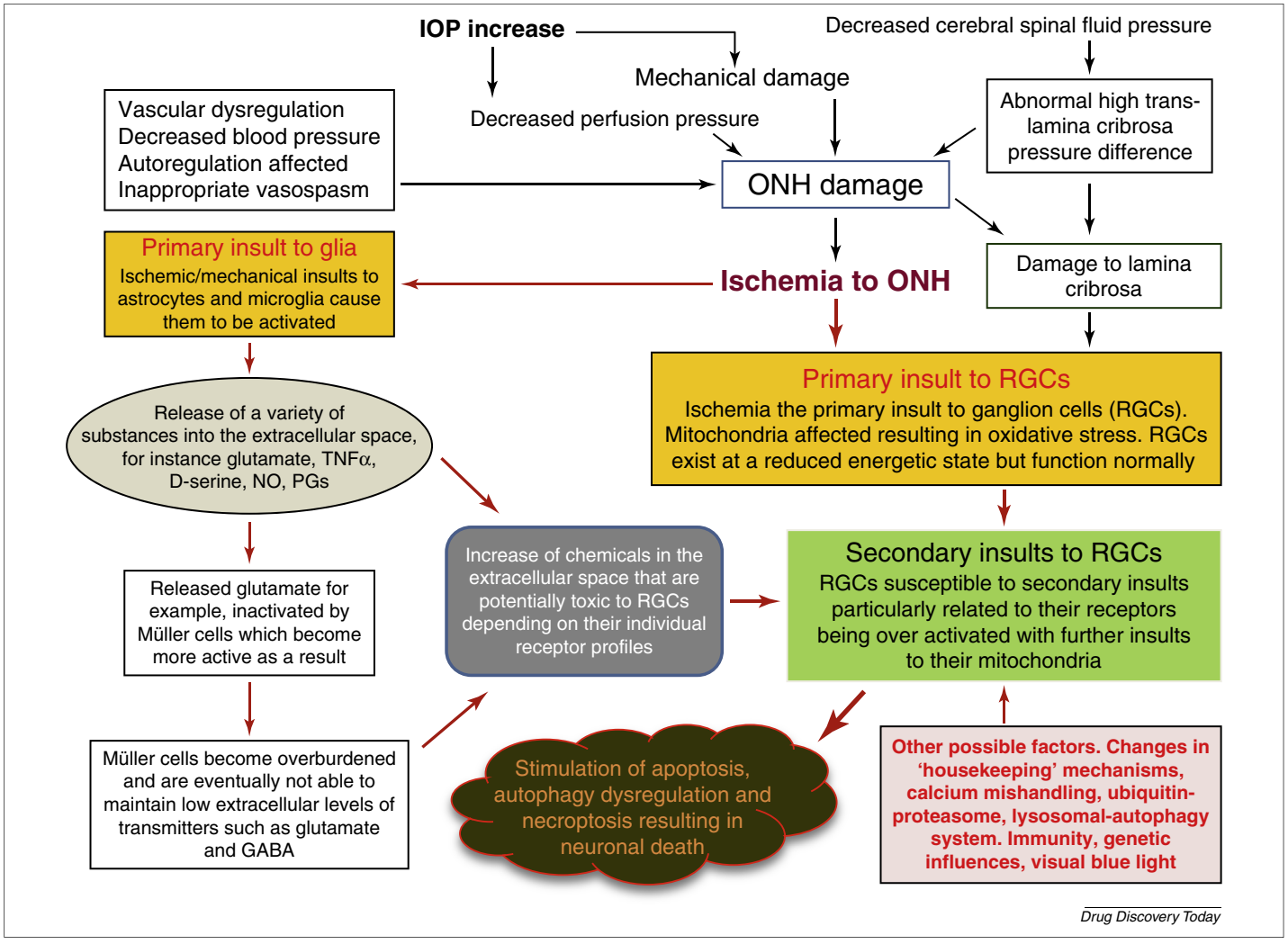


**FIGURE 2**

It is hypothesised that glaucoma is caused by various factors that influence particularly the blood supply in the optic nerve head (ONH) to cause ischemia of a defined nature. As a consequence, retinal ganglion cells (RGCs) are subjected to oxidative stress but initially still maintain function. At the same time ONH astrocytes and microglia cells are activated causing them to release specific chemicals with the initial goal being to maintain retinal homeostasis. However, as time progresses these chemicals [including nitric oxide (NO), prostaglandins (PGs), tumour necrosis factors such as TNFα, glutamate, D-serine] gradually accumulate in the extracellular space and become potential toxins to the retina. This will occur in particular when Müller cells are unable cope with the demand of maintaining a homeostatic extracellular environment. RGCs as opposed to other retinal neurons will then be primarily affected because they are already subjected to oxidative stress. A negative effect of the different extracellular chemicals on individual RGCs is envisaged to occur primarily because of over stimulation of their receptors. However, this will occur at very different times to specific RGCs depending on their individual receptor profiles. Over stimulation of neurons is known to cause mitochondrial dysfunction primarily because of calcium influx and subsequent death. As a consequence, RGC death occurs at different times in glaucoma primarily because of oxidative stress and mitochondrial energy depletion. However, other factors cannot be excluded to play a part to account for the variable loss of RGCs, as indicated.

affected by altered blood dynamics to initiate POAG. Importantly, the hypothesis suggests that, in initiating POAG, RGC mitochondria are affected so as to cause oxidative stress (a reduced energetic state) but the neurons remain functionally normal although more prone to insults than they would be otherwise. This is therefore not unlike what might occur in aging neurons that function normally despite a gradual increase in oxidative stress. Moreover, in the initiation of POAG, glial cells in the ONH are activated and respond unlike neurons (because they are less dependent on mitochondria) to release chemicals that have the immediate goal of maintaining retinal homeostasis and preventing neuronal dysfunction. However, the continuous release of these chemicals from activated glial cells will eventually negatively impact retinal neurons when they become sufficiently concentrated to stimulate their receptors excessively and cause mitochondrial oxidative stress. This will, in particular, negatively impact RGCs in POAG because they are already subjected to a degree of oxidative stress, unlike other retinal neurons. The prediction therefore is that a major reason why individual RGCs die at different times in POAG is that they become excessively depolarised by selected chemicals released from activated glial cells. The receptor profile of different RGCs (numbers and types of receptors that have an affinity for various chemicals released from activated glial cells) and perhaps also their number of mitochondria might offer the means to determine when individual neurons will die in POAG. This hypothesis provides a rationale for why RGC death occurs at different times in POAG and suggests that in the initiation of POAG all RGCs are subjected to oxidative stress, thus mimicking the situation in aging neurons. Moreover, it suggests that the therapeutic targeting of RGC mitochondria to enhance their function provides a realistic approach to prolong RGC survival and vision in POAG.

## Pharmacological targeting of RGC mitochondria for POAG

Delivery of pharmacological agents directly to influence only RGCs or their mitochondria remains a theoretical possibility. There is a need to try and achieve this goal, primarily to deliver effective concentrations of drugs to the site of action and to reduce their side effects. A number of potential ways of delivering agents to the retina with various efficiencies is shown in Fig. 3; they include topical, periocular, sclera, systemic and intravitreal routes. In the case of the intravitreal route polymeric colloidal systems (microparticles, nanoparticles) and biodegradable encapsulated

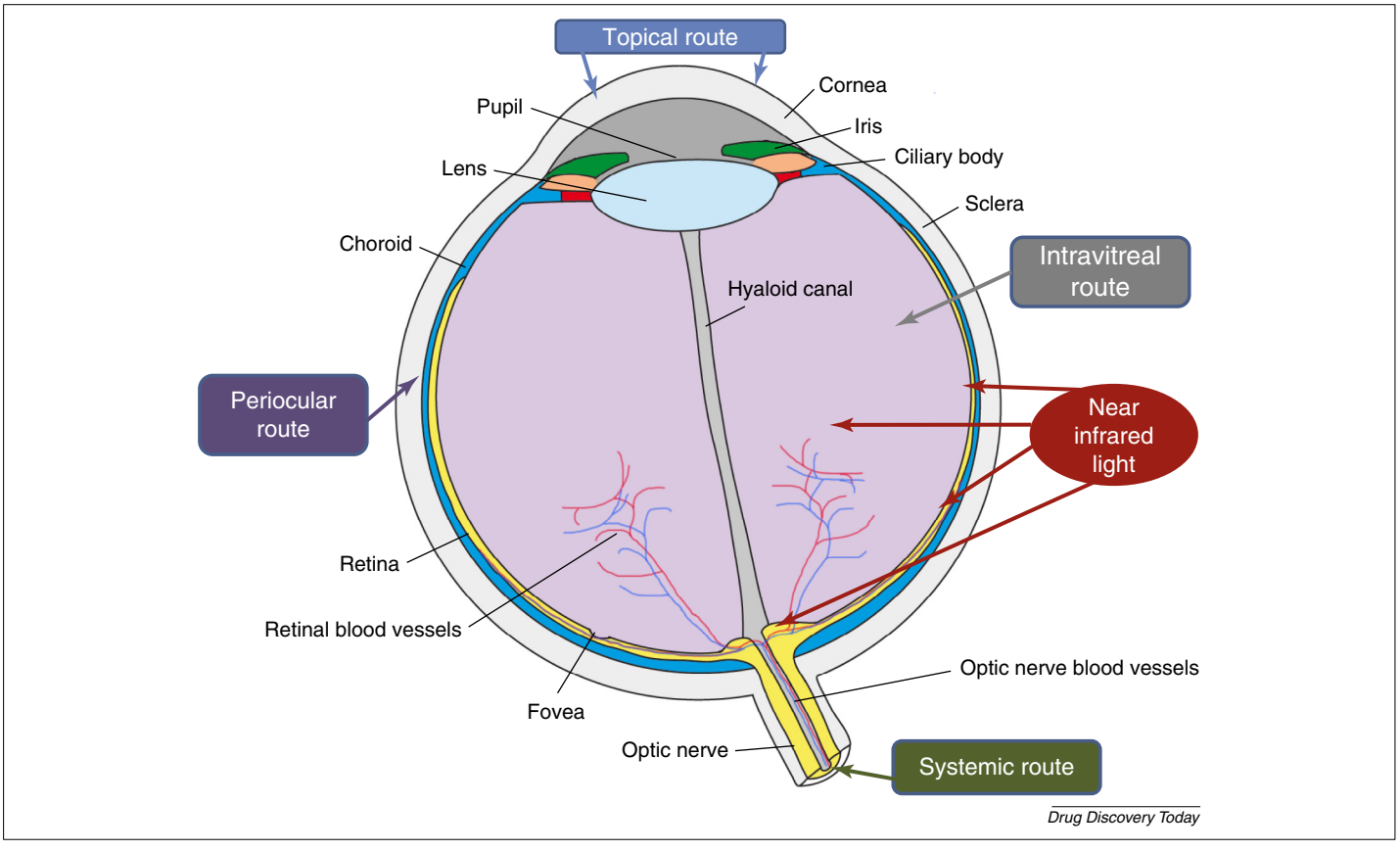


**FIGURE 3**

Diagrammatic representation of the eye showing the location of the main structures. As illustrated, it is possible to administer various substances to the eye via different routes, to reach the retina with different degrees of efficiency. Administration of substances via the intravitreal route provides the greatest efficiency for substances to reach the retina and will result in the fewest side effects. Moreover, encapsulated preparations of, for example, micro- or nano-particles enable a slow and continuous release of defined substance over long periods of time to affect the retina. The use of intravitreal implants to release substances continuously to attenuate retinal ganglion cell dysfunction in glaucoma is therefore an attractive possibility. An alternative exciting non-invasive way of treating the retina is by use of near-infrared light (600–900 nm) because it can penetrate the surrounding tissues of the eye. Significantly, red light has been shown to attenuate neuronal death *in situ* in various animal models and also in traumatic brain injury. The attractiveness of using near-infrared light to treat glaucoma by direction on retinal ganglion cells is self-evident.

substances for delivery over varying times and at precise concentrations are possible. For example, Vitrasert® (Bausch & Lomb), Retisert™ (Bausch & Lomb) and Ozurdex® (Allergan) are three implants already approved for intravitreal administration of specific substances to treat, respectively, cytomegalovirus retinitis, chronic noninfectious uveitis and macular oedema. Delivery of antioxidants directly to the retina such as vitamin E, vitamin C, *N*-acetylcysteine, glutathione, riboflavin (vitamin $B_2$), α-lipoic acid and β-carotene are therefore possible because all have been shown to protect rodent RGCs from various insults such as can occur in glaucoma [48–52]. However, it is worth noting that no clear beneficial or adverse effects have been demonstrated in POAG patients through the use of orally administered antioxidants [53,54], although a prospective study in a cohort of 3500 glaucoma patients, lasting some ten years, showed an association between low intake of antioxidant nutrients and a greater risk of open angle glaucoma [55]. Many antioxidants proposed for use in POAG might never be effective for specific reasons. For example, vitamins E and C do not appear capable of crossing the blood–retinal or blood–brain barriers and so will not reach the site (mitochondria) where ROS are generated [56]. There is also no evidence to suggest that specific antioxidants administered to Alzheimer's and Parkinson's disease patients are elevated in their mitochondria [57]. It is also possible that certain antioxidants might only have beneficial effects when administered at the very early stages of POAG.

However, it should be recognised that considerable efforts are being made to produce antioxidants to target mitochondria specifically, in order to combat neurodegenerative diseases. For example, hydrogen peroxide formation caused by mitochondrial superoxide leakage perpetuates oxidative stress, and the use of catalase, a hydrogen peroxide degrading enzyme, is an important theoretical antioxidant therapy. Unfortunately, direct use of catalase is restricted by its labile nature and inadequate delivery. A recent study suggests a nanotechnology approach is possible whereby catalase-loaded, poly(lactic c-glycolic acid) nanoparticles could be used. Such nanoparticles have been shown to retain catalase enzyme activity and, when delivered to neurons in culture, attenuate oxidative stress [58].

Other types of mitochondrially targeted antioxidants include the cell-permeable small-peptide-based antioxidants SS-02, SS-31, SS-19 and SS-20, the tripenylphosphonium-based antioxidants like MitoQ®, MitoVitE® and MitoPBN®, and the choline esters of glutathione and *N*-acetyl-1-cysteine [50,59,60]. Of these substances, MitoQ® in particular shows some promise, having been formulated for oral delivery to humans and shown to have a high antioxidant efficacy *in vivo*. Human studies indicate that MitoQ® can be safely delivered to patients for up to a year to decrease liver damage [61]. Thus, the potential use of MitoQ® or any other mitochondrially targeted antioxidant for the effective treatment of POAG is a realistic possibility. Significantly, the synthetic antioxidant idebenone, an analogue of enzyme $Q_{10}$, has recently been shown to be beneficial in the treatment of Leber's hereditary optic neuropathy [62], in which an elevated degree of oxidative stress is associated with RGCs.

The future use of substances that indirectly affect mitochondrial function to reduce oxidative stress might also be useful for the treatment of POAG, but these are likely to have greater side effects than mitochondrially targeted agents. For example, the endogenous antioxidant pathways of mitochondria are tightly regulated at the transcriptional level. One important transcriptional factor in this respect is nuclear transcriptional factor E2-related factor 2 (Nrf2), which influences the expression of a diverse array of antioxidant pathways like that of glutathione as well as cytoprotective genes. Another important regulator of the antioxidant defence system is peroxisome proliferator-activated receptor-γ-coactivator-1α (PGC-1α), which modulates a number of antioxidant proteins that are not regulated by Nrf2 [63]. Substances that stimulate the Nrf2 (e.g. organosulphors, curcuminoids) and/or PGC-1α transcriptional pathways probably reduce mitochondrial ROS indirectly and therefore could also be potentially useful for the treatment of POAG. This also applies to many other substances (e.g. Ginko biloba, flavonoids, polyphenols, catechins, organosulphors, melatonin, curcuminoids, hydrogen sulphide, porphyrins, astaxanthin and flupirtine) shown to protect against RGC dysfunction and to reduce ROS in animal studies [48–52,64–67].

Targeting mitochondrial energy depletion in particular might also be useful for the treatment of POAG. Substances like lipoic acid, creatine, *N*-acetyl cysteine and nicotinamide all achieve this goal in different ways and have been shown to blunt RGC dysfunction in laboratory studies [10,51,68]. Moreover, feeding patients with preparations of glucose and malate to bypass the inhibition of metabolic enzymes in combination with an antioxidant should enhance mitochondrial function and therefore theoretically benefit POAG patients. Another approach is to target proteins that might be modified or dysregulated in POAG, such as transcriptional regulators like peroxisome proliferator-activated receptor-γ (PPARγ) and PGC-1α [69,70].

Another potential way to treat POAG is to use iron chelators such as deferasirox, deferiprone, deferoxamine and clioquinol linked to ROS accumulation. Iron-dependent ROS-producing enzymes and labile iron are thought to contribute to ROS-dependent cell damage and death but exactly how this occurs remains to be established [71]. Iron and iron derivatives are incorporated into, and are essential for the function of, ROS-producing enzymes such as NADPH oxidases (NOXs), xanthine oxidase, lipoxygenases, CYP enzymes and subunits of the mitochondrial electron transport chain. Iron is also found at the active site of the $H_2O_2$-destroying enzyme catalase, found in the peroxisome and in the cytosol and the mitochondrial matrix. These redox-active iron pools are capable of directly catalyzing damaging free radical formation via Fenton chemistry.

## Manipulating mammalian target of rapamycin protein to enhance mitochondrial function in POAG

Mammalian target of rapamycin (mTOR), the key component in the protein complexes mTORC1 and mTORC2, plays a crucial part in, for example, cellular development, tissue regeneration and tissue repair [72–74]. In the initiation of POAG it is hypothesised that the blood supply to the ONH is compromised (Fig. 2) so that overall energy demands to maintain RGC mitochondria in optimum condition cannot be met. One important adaptive response to low oxygen tension or hypoxia involves the suppression of energy-sensitive cellular processes and is mediated in part through the inhibition of mTORC1 [73,75–77]. This involves the low oxygen/hypoxia inducible protein regulated in development and DNA damage 1 (REDD1; also known as RTP801, DDIT4, Dig2) to cause inhibition of mTORC1 activity [78,79].

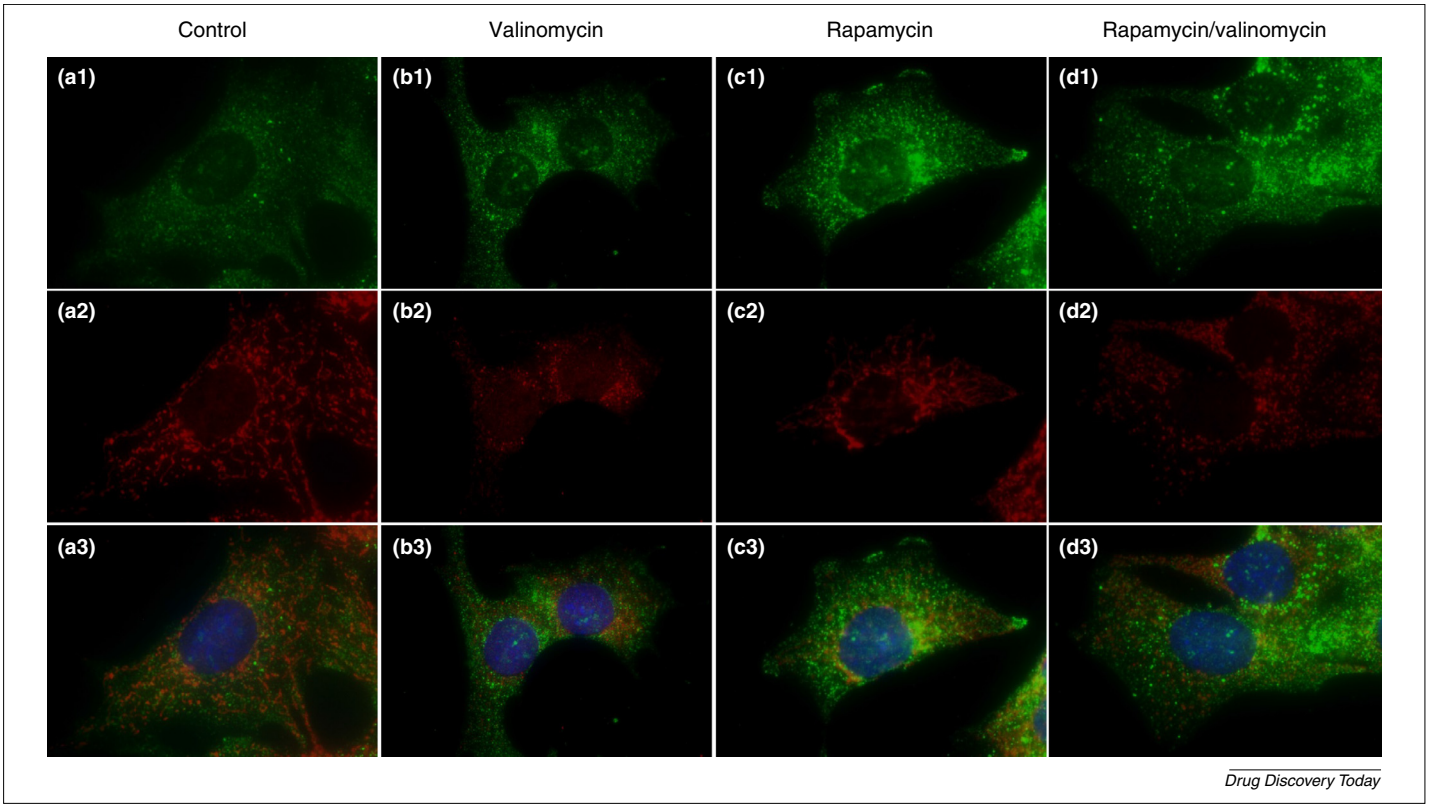


**FIGURE 4**

Cells in culture [retinal ganglion cell (RGC)-5] processed for the localisation of regulated in development and DNA damage 1 (REDD1) (green; **a1**) and oxidative phosphorylation (OXPHOS) (within mitochondria red; **a2**) immunoreactivities and after exposure to the mitochondrial depolarisation agent valinomycin (**b1**, **b2**), rapamycin (**c1**, **c2**) or a combination of valinomycin and rapamycin (**d1**, **d2**). The results show that REDD1 immunoreactivity appears dotted in pattern in the cytoplasm (a1) and does not match up with the elongated location of OXPHOS immunoreactivty present in mitochondria (a2). Support for this is shown in **a3** where a clear mismatch occurs between REDD1 and OXPHOS immunoreactivities and where nuclei are stained blue with diamidino-2-phenylindole (DAPI). Following exposure to valinomycin, mitochondria are disrupted indicated by OXPHOS immunoreativity being reduced and no longer elongated in form (b2). By contrast, REDD1 immunoreactivity is upregulated by valinomycin treatment (b1) and shows no association with OXPHOS (**b3**). Rapamycin causes an upregulation of REDD1 (c1), no change in OXPHOS (c2) and no evidence for their co-localisation (**c3**). REDD1 immunoreactivity is significantly elevated following exposure to valinomycin and rapamycin (d1), whereas OXPHOS immunoreactivity is reduced and disrupted in localisation (d2). This is confirmed by double labelling for OXPHOS and REDD1 (**d3**).

As already discussed, oxidative stress is associated with the death of RGCs in POAG and with aging, and recent evidence shows that inhibition of mTOR attenuates oxidative stress to extend lifespan and activate and enhance mitochondrial function [80–82]. Regulation of mitochondrial function by mTORC1 is complex and appears to involve multiple mechanisms. One possibility is that rapamycin suppresses mTORC1 in the cytoplasm, causing a reduction in hypoxia-inducible factor (HIF)-1α and the glycolytic flux to increase mitochondrial oxygen consumption simultaneously [83]. This is supported by a report showing that mice lacking mTORC1 activity in adipose tissue show enhanced mitochondrial respiration [84]. Another report shows that mTORC1 actively promotes mitochondrial biogenesis and metabolism through PGC-1α and the transcription factor YY-1 [85]. Interestingly, some of the most significant studies for a direct mitochondrial role in longevity come from studies on yeast in which suppression of mTORC1 results in a metabolic shift towards greater mitochondrial respiration [86].

Recent studies show that REDD1 protein and mRNA are present in mammalian retinas and REDD1 immunoreactivity is most strongly associated with RGCs [87]. In addition, transformed retinal cells in culture (RGC-5 cells) die following an insult of either blue light or $CoCl_2$ and, in both cases, REDD1 is upregulated during the process. Significantly, siRNA silencing of REDD1 synthesis as well as rapamycin counteract the negative effects of blue light and $CoCl_2$ to maintain the survival of RGC-5 cells [87]. Such combined studies strongly suggest that rapamycin might be able to rescue dying RGCs *in situ* by possibly enhancing mitochondrial function. However, we were unable to obtain clear evidence to show that REDD1 is located in mitochondria (identified by use of an antibody to recognise OXPHOS or oxidative phosphorylation components) and indeed immunohistochemistry revealed it more likely to exist in the extracellular cytoplasm (Fig. 4). Such data suggest that rapamycin-induced activation of REDD1 located outside mitochondria results in an enhancement of mitochondrial function.

It is important to note that, although REDD1 immunoreactivity is strongly associated with RGCs, it is present to a lesser extent in other retinal cell types (Fig. 5). Significantly, a recent study [88] revealed that intravitreal injection of rapamycin blunts optic nerve degeneration in a rat glaucoma model. Moreover, a report by Kolosova *et al.* [89] showed that rapamycin reduces spontaneous retinopathy and protects RGCs in senescence-accelerated OXYS rats, an animal model of age-related macular degeneration.

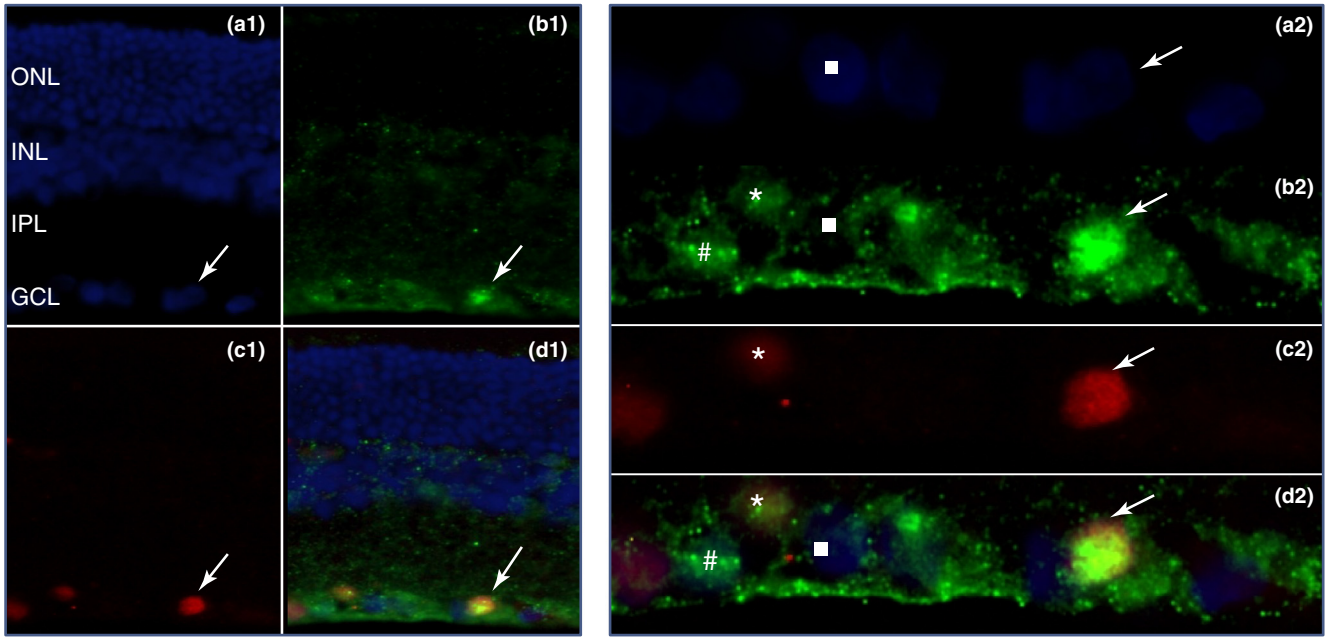


**FIGURE 5**

**(b1)** Shows that low amounts of regulated in development and DNA damage 1 (REDD1) immunoreactivity (green) is present in the inner nuclear (INL) and inner plexiform (IPL) layers of the rat retina but fairly concentrated in the ganglion cell layer (GCL). **(a1)** Shows the same section stained with diamidino-2-phenylindole (DAPI) (blue) to show the various cell body layers of the retina. The same sections stained for Brn3a (red), a specific marker for retinal ganglion cells (RGCs), is shown in **c1**. The co-localisation of Brn3a (red), REDD1 (green) and DAPI (blue) in the section is shown in **d1**. **(a–d2)** show specifically the GCL of the section in greater magnification. It can be seen that some RGCs (indicated by arrows or *) are Brn3a and REDD1 positive showing the association of REDD1 with RGCs. Some cell bodies in the GCL are not RGCs (displaced amacrine cells) because they lack Brn3a and, in the examples shown, can lack (■) or contain (#) REDD1 immunoreactiivity.

These observations raise the possibility that rapamycin might not illicit neuroprotection solely through influencing REDD1/mTORC1. Rapamycin can rescue RGCs through the downregulation of REDD1 while, in the case of photoreceptors and retinal pigment epithelial (RPE) cells, might accomplishing the same by acting on the mTOR/HIF-1 pathway to induce vascular endothelial growth factor (VEGF) production in RPE cells [90,91].

## Potential non-invasive use of red light therapy to target RGC mitochondria

It is known that the human retina is protected from damaging very-short-wavelength radiation by the cornea, which absorbs light at wavelengths below 295 nm, and the lens, which absorbs below 400 nm [92]. RGC mitochondria are therefore directly exposed to the 'visible component' of the electromagnetic spectrum, from 400 to 900 nm, and also to some short-wavelength infrared. Various mitochondrial components are known to have the capacity to absorb such wavelengths of light differentially. These include cytochrome oxidase [93,94], CYP [95] and various forms of flavin proteins that include complex I, complex II and apoptosis-inducing factor (AIF) [96–98]. Moreover, our recent studies have demonstrated unequivocally that blue light as opposed to red light in the visual spectrum has a detrimental effect on isolated mitochondria and that blue light, unlike red light, causes the death of cells in culture [5,10,87].

A large body of evidence actually exists in support of the beneficial effects of tissue exposure to high intensity (more than 10 000 lx) red light at wavelengths between the far-red to near-infrared (NR) spectrum of light (from 600 to 900 nm). This process, which is also known as photobiomodulation or NR phototherapy, has been shown to promote wound healing [99–102], cause complement propagation [103], attenuate cell death in various types of cell cultures [100,104–107] and improve recovery rates of soft tissue injuries and myocardial infarction [108,109]. The underlying mechanism behind the positive effects of NR therapy is that it acts on mitochondrial cytochrome oxidase [101,107,110,111], enhancing mitochondrial function so as to elicit beneficial effects that include reducing oxidative stress, inflammation and cell death (Fig. 6). Most importantly, long-wavelength NR at high

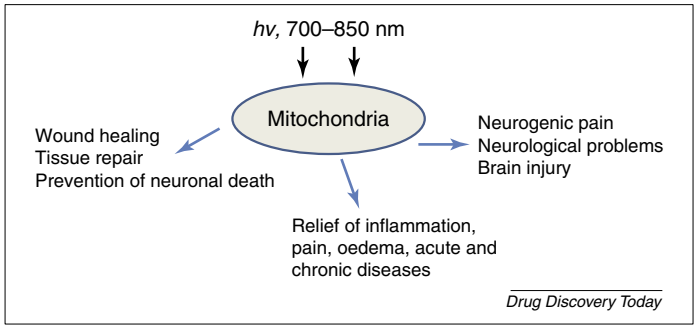


**FIGURE 6**

Summary of the positive effects detected by long-wavelength near-infrared light, delivered by several regimes in laboratory studies and for certain clinical symptoms in man.

intensity has the ability to penetrate deeply through soft tissues, and has even been shown to ameliorate traumatic brain injury [112,113]. In the case of the eye, NR has been shown to protect against photoreceptor death *in situ* [114–116] as well as mitigate oxygen-induced degeneration [117]. Moreover, NR induces nerve generation [118] and brain neuronal damage [119,120] *in situ*. Thus, utilisation of NR therapy to influence RGC mitochondria in the eye *in situ* is not restricted by its entry through the pupil as occurs for blue light. The possible therapeutic use of NR treatment in this way (Fig. 3) through the soft tissues of the external eye therefore provides a viable way to attenuate RGC death in glaucoma and other retinal diseases. Our present animal studies, still in progress, provide support for this notion. For example, low intensity red light (2500 lx) has no negative effect on cells in culture [104] and the same intensity of light delivered through the pupil of a rat while intraocular pressure was being elevated appears to attenuate the negative effects of ischemia to the cornea and retina (unpublished data).

## Concluding remarks

The aim of our future experiments is to substantiate the view that NR light provides a non-invasive way to slow down the progression of glaucoma.

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