COMMENTARY

Light Effects on Mitochondrial Photosensitizers in Relation to Retinal Degeneration

N. N. Osborne · T. A. Kamalden · A. S. A. Majid ·
S. del Olmo-Aguado · A. G. Manso ·
D. Ji

Abstract The retina captures and converts light between 400–760 nm into electrical signals that are sent to the brain by way of the optic nerve and in the process helps to translate these electrical signals into what is known as vision. The same light that allows vision to occur is nevertheless also potentially toxic to retinal cells in certain situations. The shorter wavelengths of light are known to interact with chromophores in photoreceptors and pigment epithelial cells to cause oxidative stress and severe damage. Indeed it is generally accepted that short wavelength light effects is one cause for loss of photoreceptor function in age-related macular degeneration. Recent studies have demonstrated that light may be a contributing factor for the death of retinal ganglion cells in certain situations. Light as impinging on the retina, especially the short wavelength form, affect mitochondrial chromophores and can result in neurone death. Importantly ganglion cell axons within the eye are laden with mitochondria and unlike the outer retina are not protected from short wavelength light by macular pigments. It has therefore been proposed that when ganglion cell function is already compromised, as in glaucoma, then light impinging on

N. N. Osborne (✉) · T. A. Kamalden · A. S. A. Majid · D. Ji
Nuffield Laboratory of Ophthalmology, University of Oxford,
John Radcliffe Hospital, Level 6, West Wing, Headley Way,
Oxford OX3 9DU, UK
e-mail: Neville.osborne@eye.ox.ac.uk

N. N. Osborne · S. del Olmo-Aguado · A. G. Manso
Fundación de Investigación Oftalmológica, Instituto
Oftalmológico Fernández-Vega, Oviedo, Spain

their mitochondria might be a contributor to their eventual demise.

Introduction

The region of the electromagnetic spectrum termed "visible light" is between 400 and 760 nm. Light with wavelengths below this region are generally harmful to humans (ultraviolet, X-rays) and are filtered out by the earth's atmosphere. Wavelengths above this region are the infrared (heat) forms and those wavelengths used in various communication and detection technologies (radio, radar, TV and microwave).

Historically, the detrimental influences of light to the eye beyond the lens have been associated with photoreceptors and retinal pigment epithelial (RPE) cells in the outer retina. The wavelengths of light reaching the retina is between 400–760 nm. The more reactive shorter wavelengths are prevented from reaching the retina by the cornea and lens [1]. The cornea absorbs wavelengths below 295 nm while the lens strongly absorbs wavelengths of light between 300–400 nm [2]. Both cornea and the lens also absorb part of infrared radiation (980–1,430 nm) and the vitreous absorbs light above 1,400 nm [3]. Absorption of the shorter wavelengths of visible radiation by the lens rises exponentially with age, due to yellowing caused by a gradual elevation of chromophores in the lens [4]. The greatest exposure of the retina to blue light therefore probably occurs in the first two decades of life [5] with individuals above 60 years of age being exposed to significantly lower fluxes of short-wavelength visible

radiation than younger individuals. Blue light has been shown in numerous studies to be particularly toxic to the retina and significantly its intensity impinging on the retina is substantially reduced during the ageing process because of the yellowing of the lens. It has been suggested that the gradual yellowing of the lens is a protective physiological process for the ageing retina from blue light toxicity. This has been recognised clinically and as a consequence modern intraocular lens inserted after cataract removal filter out most of the blue wavelength light.

Photosensitizers in the Retina

Retinal photoreceptors are uniquely adapted to function over a wide range of ambient conditions. However, in most species prolonged intense visible light exposure can lead to photoreceptor damage and a generation of reactive oxygen species (ROS). In nocturnal animals, the light intensity to cause visual cell damage needs only to be two or three times above room lighting for this to occur [6–8]. Such findings have historically been related to the aetiology of debilitating ocular conditions where photoreceptor dysfunction takes place as occurs in age-related macular degeneration (AMD). It is generally acknowledged that oxidative damage that results from ROS is involved in the development of AMD [9, 10] and that ROS is generated by light interaction with photosensitizers or chromophores associated with the outer retina.

Light damage to the retina is probably multi-factorial with several different mechanisms being involved depending on the chromophore being activated for the initiation of photodamage. The retina contains a large number of chromophores of which the most obvious are the visual pigments. These are 11-cis-retinal-protein complexes, which collectively absorbs light across the whole of the visible spectrum. Selectivity is achieved by the different light absorption characteristics of rods and the three different types of cones in the human retina. Individual cone pigments vary and are maximally sensitive to long (red light), medium (green light) and short (blue light) wavelengths of light. Rods in contrast are all sensitive to blue-green light.

Rhodopsin is undoubtedly the chromophore responsible for photoreceptor damage in nocturnal rodents but this might not be entirely the case in primates [11]. It seems probable that in some species photo-bleaching of rhodopsin and/or other visual pigments is more the cause for the formation of phototoxic products to cause photodamage to the retina. The chromophore all-trans-retinal (vitamin A aldehyde), is one of the products of photo-bleached rhodopsin and is abundantly present in the retina and shown to be particularly sensitive to blue-light absorbance to cause cell toxicity [12].

A number of retinal chromophores exist in addition to rhodopsin and all-trans-retinal that when activated could lead to visual cell damage. These include the broad band absorbers of melanin complexes and lipofuscin that are present in RPE cells [13] and increase in content with age [14]. It has been demonstrated that white light irradiation of melanin or lipofuscin results in increased production of various types of ROS [15–17] to cause RPE death.

Haemoglobin and other proteins that contain porphyrin, such as mitochondrial enzymes like cytochrome oxidase (COX), have absorption maxima around 400 nm [18]. The porphyrin triplets formed by absorption of photons of suitable wavelengths can result in the formation of singlet oxygen and ROS [19] which may damage various cellular constituents. It is known that COX absorbs in the blue part of the spectrum in both its oxidised and reduced forms [20]. Another mitochondrial enzyme, cytochrome P450 is also known to absorb light of wavelengths near 450 nm when bound to carbon monoxide [21].

Flavins such as riboflavin (vitamin B_2) and flavoprotein nucleotides are essential components of numerous cytosolic and mitochondrial enzyme systems. Absorption spectra of flavins are around 450–520 nm and when activated induce oxidation of several substances as well as generation of hydrogen peroxide [22, 23].

Mitochondrial Homeostasis Related to Ageing and Neurone Susceptibility

Neurones in particular have a heavy dependence on their mitochondria [24] and good evidence suggests that optimum mitochondrial homeostasis decreases with age [25, 26]. A decline in neuronal mitochondrial function is now recognised to occur in various neurodegenerative diseases and in ageing generally [27–29]. Indeed, the incidence of glaucoma in which loss of vision is caused by ganglion cells dying increases exponentially with age [30]. Unfortunately, the precise molecular basis underlying this detrimental loss in mitochondrial function in ageing remains a mystery. Despite the presence of uncoupling proteins within mitochondria to buffer the production of excessive ROS [31] optimum mitochondrial function still decreases with ageing. Altered regulation of mitochondrial fission and fusion might play a part as such processes control mitochondrial numbers, morphology, and functions [32, 33]. A decline in the efficiency of the electron transport chain function of mitochondria clearly appears to occur during ageing. For example, mitochondrial ATP production decreases and ROS increases in mitochondria from aged rodents [34, 35]. Moreover, good evidence exists to show that an increase in cellular ROS affects mtDNA function as well as induce a number of other biochemical processes,

loosely termed as oxidative stress [25, 36, 37]. It is of interest to note that Tezel et al. [38] found ganglion cells from aged (24 months old) rats to be more susceptible to an insult of elevated intraocular pressure than younger (3 months old) animals showing that the functional state of neurones in situ decreases as age progresses.

Retinal Ganglion Cell Mitochondria

Retinal ganglion cells are unusual because they have quite long unmyelinated axons in the eye and only become mylinated when in the optic nerve [39, 40]. The unmyelinated parts of ganglion cell axons are laden with mitochondria [24, 41]. In contrast mitochondria are few in number in the mylinated parts of ganglion cell axons being restricted to areas of the Nodes of Ranvier [40, 42]. It is generally assumed that the large amount of mitochondria in the unmyelinated parts of ganglion cell axons is to provide the necessary energy to propagate the action potential out of the eye globe which does not occur in the optic nerve where propagation is relayed from one Node Ranvier to another.

It is well known that the redox balance within mitochondria is critical for cellular homeostasis since the mitochondria house the energy producing systems of a cell and mitochondria plays a key role in ageing and age-related disorders [24, 37]. Production of ROS such as hydroxyl radical, singlet oxygen, hydrogen peroxide and peroxynitrite is finely balanced with sophisticated antioxidant and repair systems located in this complex organelle. Loss of these systems leads to protein oxidations that are the hall marks of many ocular diseases including retinal degenerations associated with ganglion cell loss as in glaucoma.

Significantly, the mitochondria associated with ganglion cell axons lacking myelin are unique in that they are directly exposed to light impinging on the retina. Other neurones in the retina have relatively short unmyelinated axons and therefore less heavily dependent on mitochondria for normal functions in which transference of information is clearly of importance. Photoreceptor inner segments have significant numbers of mitochondria required primarily for protein synthesis as the photoreceptor outer segments are being continuously phagocytosed and renewed. Significantly, photoreceptor mitochondria are shielded from light by the macular pigments but this is not the case for ganglion cell axonal mitochondria [43, 44].

Light Affects on Mitochondrial Photosynthesizers

As already discussed light of defined wavelengths has the potential to be absorbed by mitochondrial constituents such
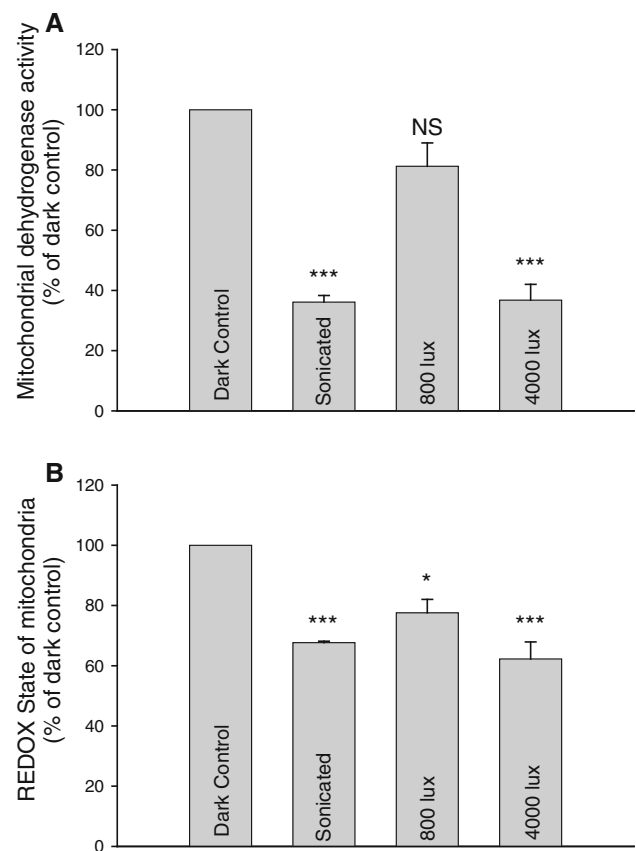
Fig. 1 The influence of light on isolated mitochondria from rat liver. Mitochondria in physiological medium were placed in dark or exposed to white light (800 or 4,000 lux) for 12 h. In some cases mitochondria were first sonicated and then placed in the dark. Redox potential (MTT assay) and mitochondrial dehydrogenase (WST-1 assay) associated with the samples were then determined. Sonication disrupts mitochondrial function indicated by a reduction in mitochondrial dehydrogenase and the redox state. Moreover, mitochondrial function is less efficient in the light than dark. (From Osborne et al. [44])

as COX, cytochrome P45 isoenzymes, and flavin protein nucleotides. Indeed a variety of studies have suggested that photosynthesizers located to mitochondria generate ROS as a result of interaction with light [44–51]. Good evidence for this being the case was obtained by studies where freshly isolated mitochondria from the rat liver was exposed to light (400–760 nm) as impinging on the retina. Fig. 1 shows data where freshly isolated mitochondria or disrupted mitochondria caused by sonication were maintained in the dark or exposed to light at two different intensities (800 lux or 4,000 lux) for 12 h. Thereafter, the mitochondrial dehydrogenase (WST-1 assay) and redox potential (MTT assay) of the samples were assessed. WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) is a tetrazolium dye containing an electron-coupling agent that is cleaved by mitochondrial dehydrogenase enzyme to a formazan dye

with an absorbance at 490 nm. MTT is reduced to an insoluble, blue formazan product because of acceptance of electrons from cellular reducing equivalents such as NADH, NADPH or succinate thus providing an assay for the redox state of a sample. The results show that light (4,000 lux) decreases mitochondrial dehydrogenase and the redox state of mitochondria as compared with the dark state. Moreover, where mitochondrial function was disrupted by sonication light had no affect on mitochondrial dehydrogenase or redox state. These studies demonstrate proof of principle for the idea that light (400–760 nm) can decrease the metabolic state of mitochondria by reducing mitochondrial dehydrogenase and altering the redox state which implies a production of ROS. Moreover, this process only occurs in functional mitochondria which suggest that the cause of the light affect is by an action on the electron transport system [44].

Figure 2 and 3 show studies that have been conducted both on a transformed retinal ganglion cell line (RGC-5 cells) and primary cultures of rat neurones [52, 53]. Here it was demonstrated that exposure of such cultures to white light (400–760 nm) for defined periods resulted in a generation of ROS accompanied by cell death. Cell death was measured in a variety of ways which included assessing the numbers of GABAergic neurones in primary culture, counting the number of TUNEL positive cells and in viability assays where the WST-1 and MTT assays were employed. The negative effect of light on cell survival in

these cell culture studies is blunted by a variety of agents that include α-lipoic acid, trolox and the flavonoid epigalicatechin gallate (ECCG) (Fig. 4). Immunohistochemical evidence to suggest that light-induced RGC-5 cell death is elicited by an effect on their mitochondria is shown in Fig. 5. The protein OPA-1 is exclusively associated with mitochondria identified by use of a mitochondrial stain (mitotracker) in RGC-5 cells kept in the dark but when exposed to light OPA-1 now does not co-localise with mitotracker in all instances. More persuasive evidence for light inducing cell death by affecting their mitochondria was demonstrated in cultures of fibroblasts [54]. In these studies fibroblasts were exposed to ethedium bromide for 50 days so impairing mitochondrial DNA and affecting their respiratory chain activity. These fibroblasts with impaired mitochondria were unaffected by a defined light insult. In contrast the same light insult caused a generation of ROS and cell death to fibrobalsts with functional mitochondria [54].

Expose of RGC-5 cells in culture to white (400–760 nm), blue (400–450 nm) or red (600–700 nm) light show that blue light affects cell survival significantly more than white light (Fig. 6). In contrast red light had no affect on RGC-5 cell survival which is consistent with studies conducted by others. For example near-infrared light via light-emitting diode treatment is known to promote wound healing in humans and animals [55] and to upregulate cytochrome oxidase activity and ATP content in


Fig. 2 This figure shows dissociates of rat retinal cells maintained in culture (dark) for 7 days. The cultures in the *upper panels* were "stained" for GABA and are twice the magnification of the cultures shown in the *lower panels*, processed for reactive oxygen species (ROS). It can be seen in **b** that the processes of GABA neurones are shorter following a white light insult (1,000 lux for the last 2 days) when compared with cells maintained in the dark for all seven days. Moreover, such a light insult **d** causes a stimulation of ROS (appears as white dots) which is virtually absent from cultures kept in the dark **c**. (From Lascaratos et al. [52])
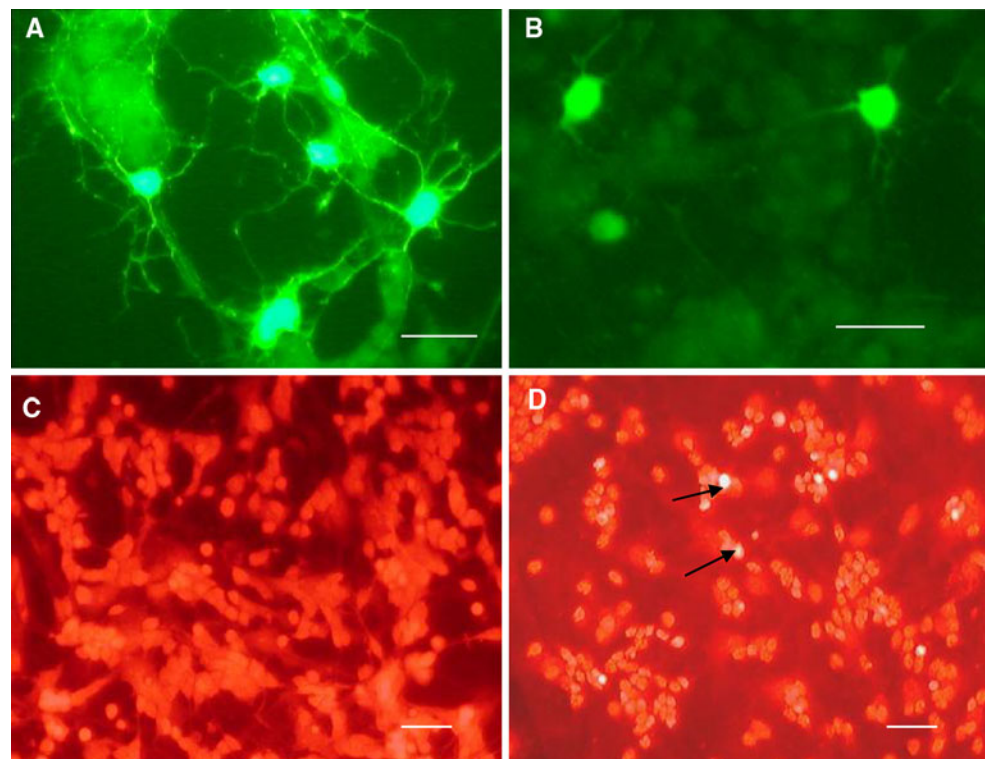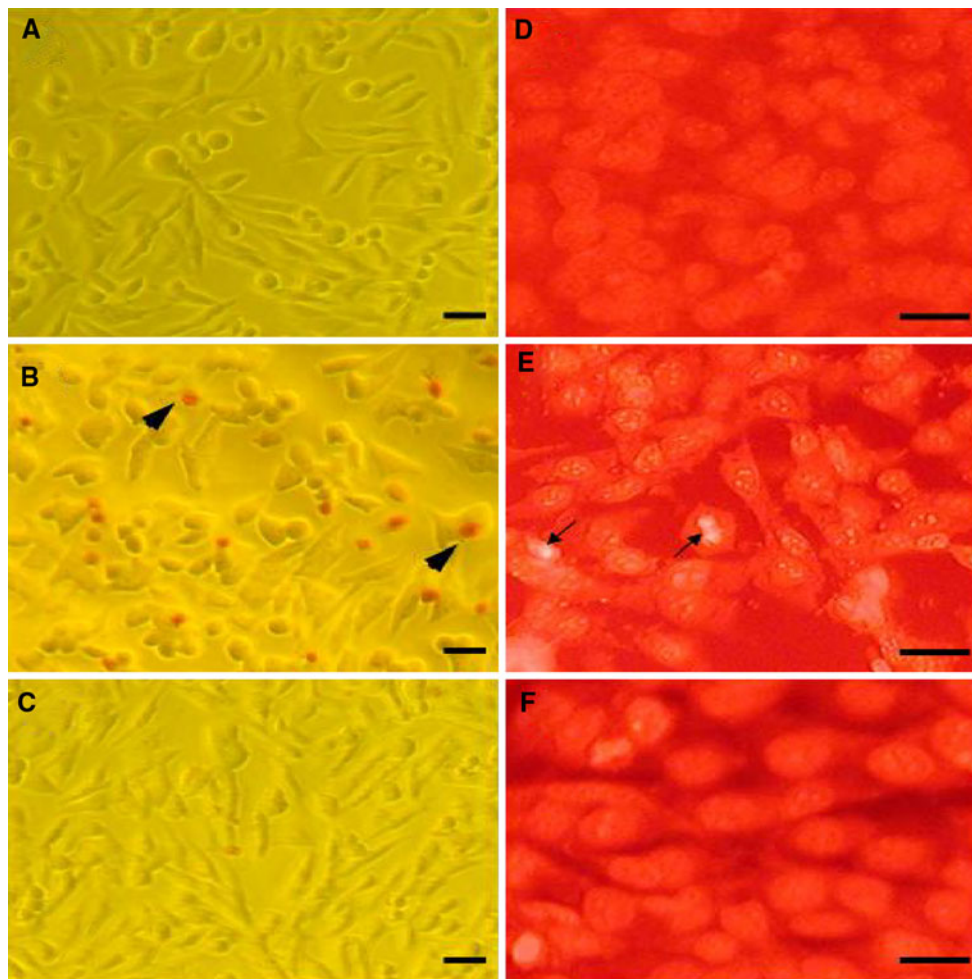
Fig. 3 This figure shows cultured RGC-5 cells stained for phosphatidylserine to indicate apoptosis (**a–c**) and ROS (**d–f**). **a** and **d** are dark maintained cultures. Cultures exposed to white light (1,000 lux, 48 h) show some cells to exhibit apoptosis (arrow heads, Fig. **b**) and the presence of ROS (*small arrows*, Fig. **e**). When 10 μM epigallocatechin gallate (EGCG) was present in the culture medium (**c** and **f**) during the light insult little sign of apoptosis or ROS production were apparent. (From Zhang et al. [65])
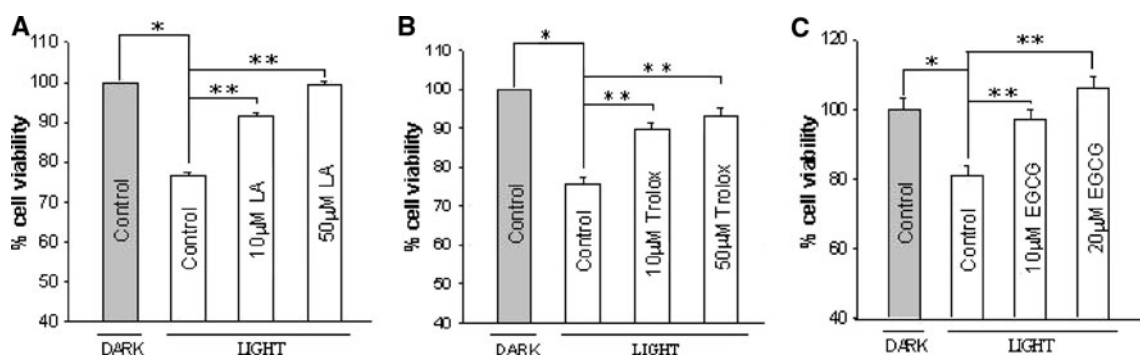

Fig. 4 This figure shows that exposure of RGC-5 cell cultures to white light (1,000 lux for 48 h) reduced cell viability by approximately 25% measured by the MTT assay. This effect is attenuated in a concentration-dependent manner by antioxidants such as α-lipoic acid (LA), trolox and EGCG. (From Osborne et al. [54])

primary cultures of rat visual cortical neurons functionally inactivated by tetrodotoxin, potassium cyanide, sodium azide or rotenone [56–58].

The hazards of blue light to the retina has been recognised particular by its action on RPE/photoreceptors involving chromophores formed by rhodopsin
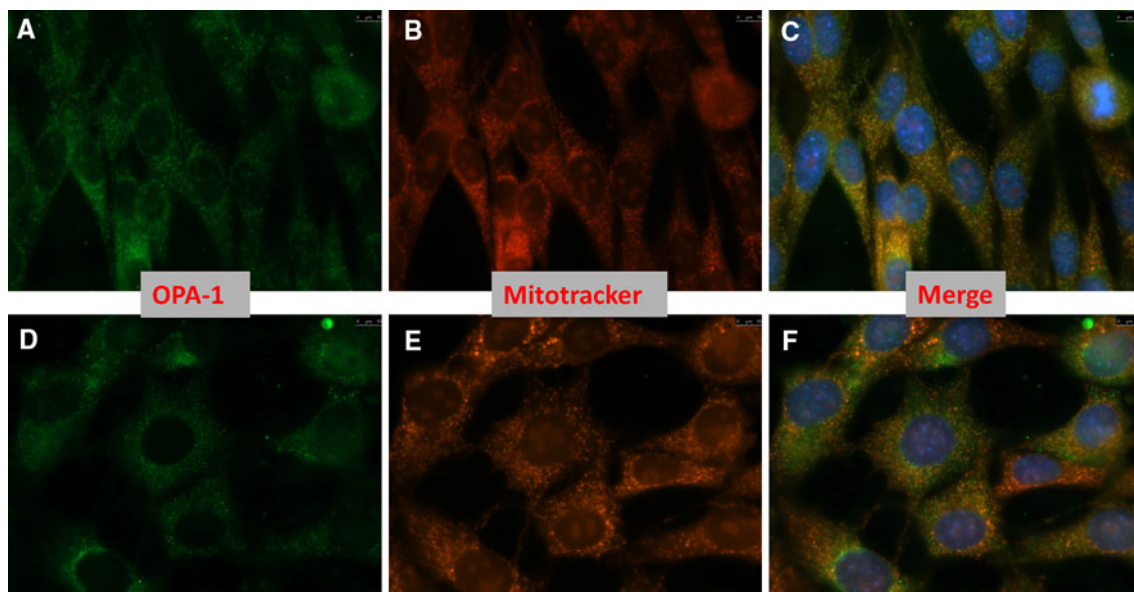
Fig. 5 This figure shows RGC-5 cell cultures stained for the localisation of mitochondrial OPA-1 immunoreactivity (**a**, **d**) and for mitochondria by use ofmitotracker (**b**, **e**). The *upper panels* are cultures maintained in the dark. The *lower panels* show cultures that have been exposed to white light (1,000 lux for 48 h). In the dark state OPA-1 immunoreactivity and mitotracker co-localise as shown in C. However, light (**f**) appears to cause a disruption of mitochondria because OPA-1 and mitotracker do not co-localise in many instances (**f**). The RGC-5 cell nuclei in **c** and **f** were stained blue by use of DAPI
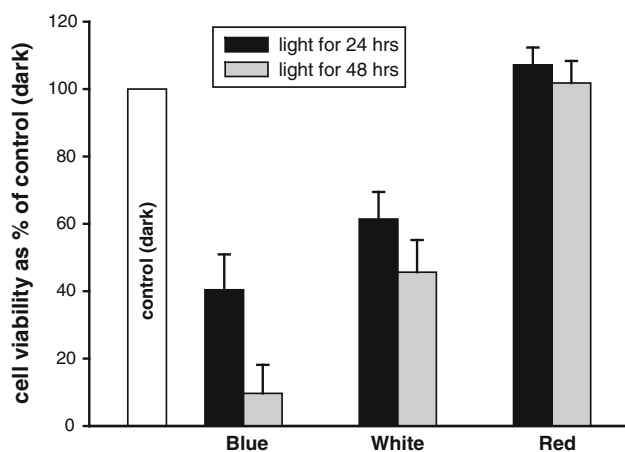
Fig. 6 This figure compares the effect of blue (465–470nm, 400 lux), white (400–600nm, 1,000 lux) and red (625–635nm, 1,000 lux) light on the survival of RGC-5 cells in culture over a period of 24 and 48 h. It can be seen that blue light is much more effective than white light at killing cells and that red light is practically ineffective. Results are means of two experiments

intermediates such as protein A2E a major component of lipofuscin [59, 60]. However, relatively recently it has been shown that blue light still affect RPE cells where the lipofucsin has been removed so suggesting a direct influence on mitochondrial function [51, 61]. Studies on RGC-5 cells show that this is indeed the case because such cells are free from chromophores normally associated with RPE/photoreceptors.

Light Affects on Retinal Ganglion cell Mitochondrial Homeostasis

The only central nervous neurones directly exposed to visible light are associated with the retina. The outer retinal neurones are to some extent protected from light by the macular pigments but this is not the case for retinal ganglion cells. Moreover, ganglion cell axons within the retina are laden with mitochondria. Since laboratory studies show that light can affect mitochondrial function the potential for this occurring to ganglion cells in situ cannot be ignored. We have therefore proposed that light entering the eye will directly affect ganglion cell mitochondria resulting in a generation of ROS but this is kept in a balanced state by normal scavenging mechanisms associated with the neurones. As a consequence in the normal healthy ganglion cell light does not have a detrimental influence on the neurone's mitochondria or function. However, should ganglion cell homeostasis be compromised for one reason or another then light, particularly the blue component, might become a hazard [44]. For example, Leber optic atrophy is a maternally transmitted disorder, affecting males more frequently than females, due to mutations in mitochondrial genes encoding complex I sub-unit proteins [42, 62]. Clinically, there is an acute onset of visual loss caused by ganglion cell dysfunction, first in one eye, and then a few weeks to months later in the other. Since all mitochondria are affected by mutations it remains a mystery why ganglion cells show loss of function before other

central nervous system neurones. One possibility is that it is because of light having a secondary negative affect on ganglion cell mitochondria.

It is also known from experimental studies that mitochondrial function becomes less efficient as aging progresses suggesting that optimum neuronal homeostasis decreases with age [24, 62, 63]. Moreover, the numbers of retinal ganglion cells in retinas from young individuals are known to be significantly greater than in old individuals [64]. It is therefore tempting to speculate that light might be a risk factor to ganglion cells as aging proceeds.

Finally in a disease like glaucoma ganglion cell homeostasis might be initially affected by, for example raised intraocular pressure so causing a decrease blood flow and ischemia to the optic nerve axons. Light might now become one cause for the ganglion cells to subsequently die as the disease progresses [24, 44].

Conclusions

Historically light, particularly blue light has been considered to be a hazard to the retina by affecting RPE/photoreceptors and to be a major factor for the cause for age-related macular degeneration. Our recent studies have focussed on how light affects mitochondrial function and related to the fact that ganglion cell axons within the retina are laden with mitochondria. We therefore suggest that light is a risk factor for the survival of retinal ganglion cells in certain situations where the neurones homeostasis is compromised for one reason or another. This might be the case in glaucoma; certain diseases associated with mitochondrial mutations, ageing and even diabetes. In such cases a longer preservation of vision might be obtained by simply use of appropriate spectacles aimed at filtering out blue light.

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