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Photostimulation of mitochondria as a treatment for retinal neurodegeneration

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ABSTRACT

Absorption of photon energy by neuronal mitochondria leads to numerous downstream neuroprotective effects. Red and near infrared (NIR) light are associated with significantly less safety concerns than light of shorter wavelengths and they are therefore, the optimal choice for irradiating the retina. Potent neuroprotective effects have been demonstrated in various models of retinal damage, by red/NIR light, with limited data from human studies showing its ability to improve visual function. Improved neuronal mitochondrial function, increased blood flow to neural tissue, upregulation of cell survival mediators and restoration of normal microglial function have all been proposed as potential underlying mechanisms of red/NIR light.

1. Introduction

The therapeutic properties of light have been known since antiquity, as far back as 1400 BCE, where it was used by Hindus to treat skin disorders (Roelandts, 2002). The ancient Egyptians, Greeks and Romans were also reportedly aware of the beneficial effects of sunlight which they used to treat various ailments (McDonagh, 2001). The evidence for the use of phototherapy in those time, however, is purely anecdotal. It was not until 1903 that the therapeutic power of light gained scientific recognition, when Niels Finsen was awarded the Nobel Prize in medicine for the discovery of UV light as a treatment for skin tuberculosis (*lupus vulgaris*) (Finsen, 1901).

Red light was, later, found to have biostimulatory effects; an unintentional discovery made by Endre Mester, in 1967, who wanted to assess the ability of 694 nm lasers to cause carcinogenesis in mice (Mester et al., 1971). The mice in both the light-treated and untreated groups were shaved prior to laser exposure. The results found that the light-treated group did not develop cancer, but more intriguingly, the hair grew back on the laser treated mice at a faster rate than the untreated group.

In more recent times, there has been a surge in the use of red and near infrared (NIR) lasers and LEDs in clinical and preclinical research (Desmet et al., 2006). As red and NIR light have relatively long wavelengths, they have the advantage of a greater penetration depth over shorter wavelengths, making them an ideal choice for the treatment of neural tissue (Hartwig and Van Veen, 1979). In addition to light being able to penetrate into the tissue of interest, another requirement is that the photon energy corresponds to the absorption characteristics of the chromophores responsible for triggering the beneficial effects upon photoexcitation. It appears that red and NIR light correspond to the absorption maxima of such chromophores as will be discussed later. For various reasons, LEDs are most commonly used as the light source in these studies. Most importantly, red/NIR LED therapy has been approved for use in humans and has been deemed as a non-significant risk by the U.S. Food and Drug Administration. Although shorter wavelengths of visible light and UV light are also employed for therapeutic purposes, their safety for use in humans, especially for the eye, is less clear (Rozanowska et al., 2009; Rozanowska, 2012). With the ultimate objective of exploring the efficacy of phototherapy as a treatment for neurodegeneration in the human retina, this review will focus only on the use of wavelengths that are least likely to cause adverse effects, that is, red and NIR light (Barolet, 2008).

Abbreviations: NIR, near infrared; LED, light emitting diode; FDA, the U.S. Food and Drug Administration; COX, cytochrome *c* oxidase; RGC, retinal ganglion cell; ERG, electroretinogram; NO, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; NF, nuclear factor; IL, interleukin

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2. The potential of red/NIR light as a treatment for neurodegeneration

2.1. Evidence from in vitro studies

Red and NIR light have been shown to provide protection against the deleterious effects of mitochondrial electron transport chain inhibitors and excitotoxic cell death in neurons *in vitro* (Wong-Riley et al., 2005; Ying et al., 2008; Huang et al., 2014). Since impaired mitochondrial function and excitotoxicity are common causes of cell death in neurodegenerative conditions, the ability of red/NIR light to protect against these challenges *in vitro* has emphasized the potential of this therapy in various neurodegenerative conditions.

2.2. The effects of red/NIR light in models of neurodegeneration

Red/NIR light therapy has shown great potential in the treatment of acute neurodegenerative conditions, showing neuroprotective effects in rodent models of spinal cord injury, traumatic brain injury and stroke (Byrnes et al., 2005; Wu et al., 2012; Xuan et al., 2015; Dong et al., 2015; Oron et al., 2006; Giacci et al., 2014).

Furthermore, red light has been shown to have beneficial effects in animal models of some of the most prevalent neurodegenerative diseases. A reduction in cell loss and other markers of disease severity was seen with red/NIR light treatment, in rodent models of multiple sclerosis, Alzheimer's and Parkinson's disease (Muili et al., 2012, 2013; Purushothuman et al., 2013, 2015; Oueslati et al., 2015; Johnstone et al., 2014; Peoples et al., 2012; Shaw et al., 2010).

2.3. The potential of red/NIR light as a successful treatment for neurodegeneration in humans

While transcranial red/NIR light therapy is yielding remarkable results in numerous rodent models of neurodegeneration, the real question is how well these results will translate when applying this therapy to human patients.

Interestingly, in a neurotoxin-induced monkey model of Parkinson's disease, 670 nm light was delivered directly to the macaque midbrain using an implanted optical fibre which was activated over the period of time of 5–7 days when the neurotoxin precursor, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was injected (Darlot et al., 2016). The study found a reduction in clinically-assessed behavioural impairment with this method of red light delivery in this primate model as well as neuroprotection to the dopaminergic neurons of the *substantia nigra*. Although a more invasive method of delivery than transcranial red light treatment, no major adverse effects were observed following surgical implantation of the optical fibre. However, it would have been of great interest if the effects of transcranial light delivery were also tested in this model, for comparison.

3. Red light treatment in retinal degenerative diseases

Since the retina is an extension of the CNS, the neuroprotective effects of red/NIR light, as discussed above, should also be observed in this tissue. In fact, irradiating the retina with red or NIR light seems more likely to be successful as a non-invasive treatment for human patients as the issue of tissue penetration is avoided.

3.1. The safety of light treatment on the retina

The greatest concern arising when aspiring to use red light therapy to treat retinal degeneration, is the potential retinal damage that may occur upon direct exposure of the retina to light with high levels of irradiance. The dangers of high levels of irradiance on the retina is highlighted in a study on anesthetised monkeys (Friedman and Kuwabara, 1968). It was found that white light with a retinal irradiance of 270 mW/cm^2 caused irreversible damage to the photoreceptors and retinal pigment epithelium. White light is made up of light of all wavelengths in the visible light spectrum, with light of shorter wavelengths and higher frequencies having a greater damaging effect on photoreceptors. Blue light, with a relatively short wavelength, was found to cause irreversible damage to S cones (Harwerth and Sperling, 1975). While exposure to green and red light caused damage to M and L cones, respectively, the damage to these cones was reversible, with a full recovery of function seen after a few weeks. More recent studies on macaque monkey, however, have demonstrated that yellow light of 568 nm wavelength can cause retinal damage manifested as disruption of the retinal pigment epithelium at the dose below the Maximal Permissible Exposure established by the American National Standard Institute's (ANSI) as a standard for the safe use of lasers (Hunter et al., 2012).

Albeit transient and less severe than light of shorter wavelengths, damage to L cones upon exposures to high levels of red light would be a cause for concern when considering red light as a treatment for retinal degeneration. This concern has been addressed with numerous *in vivo* studies. These studies have shown that therapeutic effects were achieved, in the absence of retinal damage, when rodent retinas were exposed to 670 nm light with a therapeutically effective irradiance and exposure times (Albarracin et al., 2011, 2013; Giacci et al., 2014). Further, this included irradiance of 60 mW/cm^2 which is the highest irradiance level found in studies of *in vivo* models of retinal degeneration where positive results were achieved using 670 nm light. This demonstrates the safety of using 670 nm light as a treatment for retinal degeneration.

In addition to photoreceptor damage, the possibility of photothermal damage to the retina and surrounding ocular structures evokes further concern when considering using light to treat retinal degeneration (Youssef et al., 2011). Comparing the effects of green, red and NIR laser light exposure on the temperature rise in the human choroid, it was found that the longer wavelengths led to a smaller degree of choroidal heating, due to the decrease in absorption by melanin with increasing wavelength (Vogel and Birngruber, 1992). The variation in choroidal heating between green and red wavelengths was minor compared with the difference between green and NIR wavelengths.

Still the question remains as to which would be the optimal wavelength for use as a neuroprotective agent in the retina. Addressing this, the efficacy of red and NIR light was compared in a model of partial optic nerve transection (Giacci et al., 2014). It was found that although protective effects were seen in retinas treated with both red and NIR light, 670 nm light was more effective in improving visual function compared with 830 nm light. However, in a rat model of light induced retinal degeneration protective effects were observed with 670 nm light treatment, but no protection was seen with 830 nm light (Giacci et al., 2014). It is therefore not surprising that most studies testing the effectiveness of phototherapy on neurodegeneration in the retina use red light at 670 nm.

3.2. 670 nm light therapy in models of photoreceptor damage

As discussed above, exposing the retina to bright light can cause photoreceptor damage, an event that can occur with excessive sunlight exposure or accidental exposure to high intensity artificial light sources. Models of light induced photoreceptor damage are also used to simulate retinal degenerative diseases, involving photoreceptor specific death. Emphasizing the vast and diverse effects of light on biological tissue, irradiating the retina with red light provided protection against the structural damage to the outer retina and loss in photoreceptor function in a rat model of light induced photoreceptor degeneration (Albarracin et al., 2011). Methanol can also induce damaging effects on the retina causing photoreceptor toxicity due to the ability of its metabolite, formic acid, to inhibit cytochrome *c* oxidase, the terminal enzyme of the electron transport chain. In a rat model of methanol induced retinal