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Retinal degeneration and protection

# Glaucoma: Focus on mitochondria in relation to pathogenesis and neuroprotection

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ABSTRACT

Primary open-angle glaucoma (POAG) is a common form of glaucoma in which retinal ganglion cells (RGCs) die at varying intervals. Primary open-angle glaucoma is often associated with an increased intraocular pressure (IOP), which when reduced, can slow down the progression of the disease. However, it is essential to develop better modes of treatments for glaucoma patients. In this overview, we discuss the hypothesis that RGC mitochondria are affected during the initiation of POAG, by becoming gradually weakened, but at different rates because of their specific receptor profiles. With this in mind, we argue that neuroprotection in the context of glaucoma should focus on preserving RGC mitochondrial function and suggest a number of ways by which this can theoretically be achieved. Since POAG is a chronic disease, any neuroprotective treatment strategy must be tolerated over many years. Theoretically, topically applied substances should have the fewest side effects, but it is questionable whether sufficient compounds can reach RGC mitochondria to be effective. Therefore, other delivery procedures that might result in greater concentrations of neuroprotectants reaching RGC mitochondria are being developed. Red-light therapy represents another therapeutic alternative for enhancing RGC mitochondrial functions and has the advantage of being both non-toxic and non-invasive.



## 1. Introduction

A variety of different types of glaucoma exist, with primary open-angle glaucoma (POAG) being the most common. Glaucoma is the second-leading cause for blindness worldwide and is caused by optic nerve damage at the point where it leaves the eye, known as the optic nerve head (ONH). Glaucoma is an optic neuropathy that manifests by optic nerve cupping and atrophy similar to that observed in a variety of primary mitochondrial optic neuropathies (Lee et al., 2011). The actual pathogenesis of POAG remains unknown and a variety of studies suggest that mitochondrial dysfunction plays a role in glaucoma pathogenesis.

## 2. Mitochondrial functions

Mitochondria are cytoplasmic organelles that regulate both metabolic and apoptotic signaling pathways. Their major functions include energy generation in the form of adenosine triphosphate (ATP), the regulation of cellular calcium homeostasis, balancing reactive oxygen species (ROS) production and detoxification, the mediation of apoptosis cell death, and the synthesis and metabolism of various key molecules such as fatty acids (Chan, 2006; Schon and Manfredi, 2003; Osborne et al., 2014b). Mitochondrial dysfunctions lead to diverse pathologies in a multitude of human diseases (Liang et al., 2014).

Mitochondrial numbers vary between cells, depending on the cells' metabolic demands. In particular, central nervous system (CNS) neurones require an abundance of ATP; approximately 90% of mitochondrially generated ATP is used to maintain the membrane dynamics that are essential to sustain action potentials and neuronal survival. In the retina, particularly large numbers of mitochondria are present in the retinal ganglion cell (RGC) unmyelinated axons (Bristow et al., 2002; Wang et al., 2003, Carelli et al., 2011) and in the inner segments of photoreceptors (Stone et al., 2008). Significantly, most diseases that cause blindness are associated with mitochondrial dysfunctions due to the death of RGCs or photoreceptors (Schrier and Falk, 2011; Yu-Wai-Man et al., 2011).

Mitochondria contain their own genome — mitochondrial DNA (mtDNA) — which encodes essential subunits of the respiratory chain, in which electrons are combined with oxygen to enable the flow of energy through the mitochondria. Energized mitochondria can then synthesize ATP to fuel energy-dependent intracellular reactions (such as endocytosis, ion transport, and

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neurotransmitter biosynthesis) and to sustain other critical mitochondrial functions ($Ca^{2+}$ transport, ROS production, among others) that contribute to intracellular signaling (Wallace et al., 2010). Equally important is the relatively recent discovery that mitochondria dynamically undergo shape changes through regulated processes of fusion and fission (to form larger or smaller organelles, respectively) and actively traffic between cell compartments such as the soma, axon, and presynaptic boutons (Chan, 2012).

Extracellular signals directly affect mitochondria; for example, glucocorticoid receptors translocate into mitochondria and regulate $Ca^{2+}$ re-uptake and ROS production (Du et al., 2009). Mitochondria also respond acutely to the metabolic environment by undergoing morphological and functional changes that can influence the cellular aging process (Picard and Turnbull, 2013). Interestingly, although mitochondrial dysfunction resulting from mtDNA mutations often causes severe multi-systemic disease, the CNS appears to be extremely vulnerable to mitochondrial defects, suggesting that neurons are particularly sensitive to bio-energetic fluctuations, and consequently, that mitochondria regulate fundamental aspects of CNS function.

Oxidative stress is a common symptom of mitochondrial dysfunction and is strongly implicated in the pathogenesis of all neurodegenerative diseases, including glaucoma (Tezel, 2006; Osborne, 2010). The free radical superoxide anion, lipid radicals, hydroxyl radical, nitric oxide and hydrogen peroxide are major contributors to oxidative stress. Reactive oxygen species are primarily produced by the mitochondrial respiratory chain (oxidative phosphorylation), which is composed of five multi-enzyme complexes (Fig. 1). Complexes I, II, III and IV form the electron transport chain (ETC), whereas complex V or ATP synthase produces

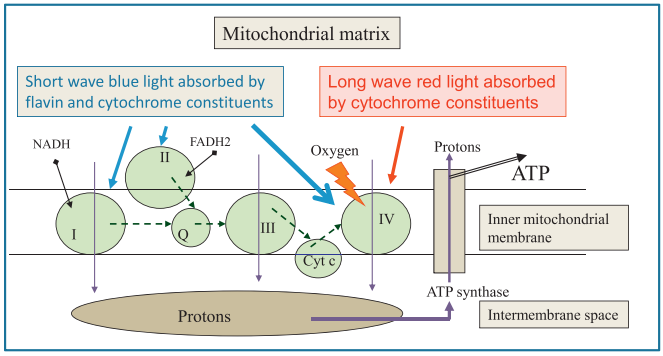


**Fig. 1.** The respiratory chain or electron transport chain is a metabolic pathway that takes place within the inner mitochondrial membrane, using integral membrane proteins. These proteins form four huge trans-membrane complexes called complexes I, II, II and IV. Each complex contain up to 40 individual polypeptide chains, which perform many different functions including enzymes and transmembrane pumps. The electron transport chain generates optimum levels of ATP and free radicals such as nitric oxide when sufficient oxygen is available to bind to complex IV or cytochrome c oxidase. This process requires the substrates NADH and FADH2 to donate electrons (black arrows) to the electron transport chain at complexes I and II and transfer them along a chain of other carriers (ubiquinone or Q, complex III, cytochrome c or Cyt c and complex IV or cytochrome c oxidase) also situated in the inner mitochondrial membrane. At complexes I, III and IV the electrons are used to translocate protons from the mitochondrial matrix to the intermembrane space (purple thin arrows). This process generates a proton-electrochemical potential gradient across the inner mitochondrial membrane, which is known as the proton-motive force. The proton-motive force (thick purple arrows) is used to drive gradient dissipating activities, including the generation of ATP by ATP synthase, which is the main pathway for the return of protons to the matrix. Flavin and cytochrome constituents associated particularly with complex I, II and IV of the electron transport chain are able to absorb different wavelengths of light. Short wave length blue light (400-480nm) is absorbed by both flavin and cytochrome containing constituents (blue arrows). Long wave length red light (800-1000nm) is absorbed particularly by cytochrome-containing constituents (red arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ATP energy for cellular requirements. Excess ROS generated as a toxic by-product occurs via the inhibition of oxidative phosphorylation, which can, for example, be caused by limited oxygen, as occurs in ischemia. Although optimum levels of generated ROS play a positive role in cell signaling, their uncontrolled production results in oxidative stress. Oxidative stress leads gradually to cell damage and eventually death caused by the oxidation of cellular proteins, lipids and nuclear/mitochondrial DNA (Butterfield et al., 1997; Cakatay et al., 2001; Osborne et al., 2004a, 2004b, Sohal et al., 2000; Lascaratos et al., 2012). Cells are known to protect themselves against the negative effect of excess ROS by a number of mechanisms that involve enzymes such as superoxide dismutase, catalase, metallothioneins and glutathione transferase, as well as certain small molecules such as glutathione.

Mitochondria contain several chromophores that absorb light of various wavelengths. This is important for retinal function, as no other central nervous tissue is exposed to light. The wavelength of light that reaches the retina is between 390 and 1000 nm. Proteins such as haemoglobin and the mitochondrial enzyme cytochrome oxidase (COX) or complex VI, contain porphyrin, which has an absorption maximum between 400 and 410 nm (Mellerio, 1994). It is also known that COX absorbs light in the blue and red parts of the spectrum in both its oxidised and reduced forms (Bell and Hall, 1981). Another mitochondrial enzyme, cytochrome P450, absorbs light at wavelengths of about 450 nm when bound to carbon monoxide (Ortiz de Montellano, 1995). Light is also absorbed by flavins such as riboflavin (vitamin B2) and flavoprotein nucleotides, which are components of numerous cytosolic and mitochondrial enzyme systems. The absorption spectra of flavins are between 450 and 520 nm and when activated, these can induce the oxidation of several substances, as well as generate hydrogen peroxide (García and Silva, 1997 and Hockberger et al., 1999). Porphyrins are also targets of blue light and are present in mitochondrial inner membranes (Gorgidze et al., 1998 and Wataha et al., 2004).

Some evidence suggests that mitochondrial genetic dysfunctions occur in POAG and normal-tension glaucoma (NTG) (Lascaratos et al., 2012). For example, a maternal family history of POAG is more likely than a paternal one, as would be expected from mitochondrial genetics: mothers and sisters of glaucoma patients are more likely to have glaucoma than fathers and brothers (Mitchell et al., 2002). Lymphocytes of POAG patients have been reported to possess significantly lower mean mitochondrial respiratory activity, and POAG lymphoblast cell lines have a complex-I ATP synthesis defect (Abu-Amero et al., 2006; Lee et al., 2012; Yu-Wai-Man, 2012). Approximately 52% of Saudi POAG cases were found to harbor novel potentially pathogenic nonsynonymous changes in mtDNA from blood, and 63% of POAG cases involved novel and unusual transversional (purine to pyrimidine) nucleotide substitutions (Abu-Amero et al., 2006). It has been hypothesised that somatic mtDNA transversions are generated in response to oxidative stress in early development or throughout life, and contribute to optic nerve injury in POAG (Abu-Amero et al., 2006). Mutations in genes encoding mitochondrial proteins have also been reported to be associated with NTG (Mabuchi et al., 2007; Wolf et al., 2009).

## 3. Pathogenesis of POAG – the involvement of mitochondria

Studies undertaken worldwide have not produced a consensus explanation for the cause(s) of POAG or why RGCs die at differential rates. It is generally agreed, however, that the two major risk factors in glaucoma are an increase in IOP and vascular dysregulation. Ischemia to components in the ONH can be induced by vascular dysregulation and raised IOP. Therefore, ischemia and