Mitochondrion 36 (2017) 103–113

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Mitochondrial dysfunction and oxidative stress in corneal disease☆

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ARTICLE INFO

Keywords:
Cornea
Mitochondria
Antioxidants
Oxidative stress
Keratoconus
Fuchs endothelial dystrophy
Kearns Sayre Syndrome

ABSTRACT

The cornea is the anterior transparent surface and the main refracting structure of the eye. Mitochondrial dysfunction and oxidative stress are implicated in the pathogenesis of inherited (e.g. Kearns Sayre Syndrome) and acquired corneal diseases (e.g. keratoconus and Fuchs endothelial corneal dystrophy). Both antioxidants and reactive oxygen species are found in the healthy cornea. There is increasing evidence of imbalance in the oxidative balance and mitochondrial function in the cornea in disease states. The cornea is vulnerable to mitochondrial dysfunction and oxidative stress due to its highly exposed position to ultraviolet radiation and high oxygen tension. The corneal endothelium is vulnerable to accumulating mitochondrial DNA (mtDNA) damage due to the post- mitotic nature of endothelial cells, yet their mitochondrial genome is continually replicating and mtDNA mutations can develop and accumulate with age. The unique physiology of the cornea predisposes this structure to oxidative damage, and there is interplay between inherited and acquired mitochondrial dysfunction, oxidative damage and a number of corneal diseases. By targeting mitochondrial dysfunction in corneal disease, emerging treatments may prevent or reduce visual loss.

1. Introduction

The eye is a highly specialized organ of photoreception, an optical system able to focus the light energy from the environment on to the retina, which is the receptor of the visual pathway. The cornea, the anterior transparent window of the eye, is a crucial part of this optical system, creating 80% of the refractive power of the eye. The cornea is a dome-shaped transparent structure, and its shape and clarity, are the main characteristics enabling such great refractive power. The cornea, being avascular, obtains its nutrients from the tear film, the aqueous humour and blood vessels at the peripheral edge of the cornea. Human corneal transparency is the result of a number of related factors: avascularity, structural regularity of the covering epithelium, regular arrangement of the extracellular and cellular components in the stroma and functionality of the endothelium to regulate corneal hydration (Nita and Grzybowski, 2016; Zierhut et al., 2008). The normal corneal structure comprises of five well-defined layers from the external to internal corneal surface: the epithelium (multilayer), Bowman's layer, stroma (interlaced with keratocytes), the Descemet membrane and the endothelium (monolayer) (Fig. 1). Even small malfunctions or malformations in any of these components and/or an impaired communication between them can compromise their function. The unique physiology of the cornea predisposes this structure to oxidative damage, and there is interplay between inherited and acquired mitochondrial dysfunction, oxidative damage and a number of corneal diseases.

Mitochondrial mutations and dysfunction has been implicated in other ocular conditions. The most common mitochondrial disease is Leber's hereditary optic neuropathy (LHON) (Chinnery et al., 2001). This results in the degeneration of retinal ganglion cells and a progressive degeneration of the optic nerve (Jankauskaitė et al., 2016). About 70% of all LHON cases are caused by the 1178G > A mutation of the mitochondrial deoxyribonucleic acid (mtDNA) (Cwerman-Thibault et al., 2014). There is an increasing body of evidence from genetic studies that mtDNA mutations and subsequent dysfunction may contribute to the pathogenesis of other debilitating ocular conditions including glaucoma (Lascaratos et al., 2012; Sundaresan et al., 2014) and age related macular degeneration (AMD) (Terluk et al., 2015). Also rarely oncocytomas (or oncocytic adenomas) may arise in the simple or glandular epithelia of the ocular adnexa (Jones et al., 2016). In this

Abbreviations: BCL-2, B-cell lymphoma protein; TEM, transmission electron microscopy; CEC, corneal endothelial cells; ROS, reactive oxygen species; RNS, reactive nitrogen species; UV, ultraviolet; Nrf-2, nuclear erythroid factor 2; H_2O_2, hydrogen peroxide; mtDNA, mitochondrial DNA; SOD, superoxide dismutase; NADPH, nicotinamide adenine dinucleotide phosphate; ALDH3A1, aldehyde dehydrogenase 3; MT3, metallothionein 3; TXNRD1, thioredoxin reductase 1; FECD, Fuchs endothelial corneal dystrophy; OCT, optical coherence tomography; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; MMP-2, matrix metalloproteinase2; TIMP-1, tissue inhibitors of matrix metalloproteinase

☆ This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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http://dx.doi.org/10.1016/j.mito.2017.05.009
Received 8 August 2016; Received in revised form 23 January 2017; Accepted 18 May 2017
Available online 23 May 2017

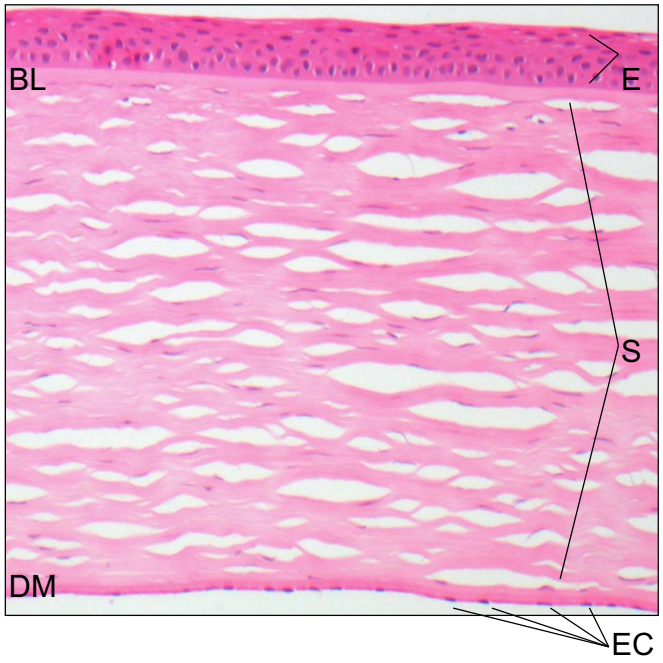

Fig. 1. Histological section of the cornea stained with haematoxylin and eosin stain demonstrating the five layers of a normal human cornea (E - epithelium, BL - Bowman's layer, S - stroma, DM - Descemet's membrane, EC - endothelial cells).

condition these cells demonstrate an eosinophilic appearance and excessive and abnormal mitochondria content (Østergaard et al., 2011).

2. Mitochondrial function in the healthy cornea

Mitochondria have a functional genome separate from that of nuclear DNA (Leonard and Schapira, 2000). The human mitochondrial genome is 16,569 bp long and forms a closed circular molecule (Anderson et al., 1981). Human mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function. Thirteen of these genes encode enzymes, which are crucial for the oxidative phosphorylation pathway required for the production of the majority of cellular adenosine triphosphate (ATP)·(Lascaratos et al., 2012).

There are three main cell types in the cornea with have specific structural and functional roles: corneal epithelium cells, keratocytes in the corneal stroma and a highly-specialized corneal endothelium. The corneal epithelium is stratified (consisting of five or seven cell layers) and its central thickness is approximately 50–52 μm (Hogan et al., 1971). The corneal epithelium is metabolically highly active, especially the deepest basal epithelial cells, which support the complete turnover of the epithelium over 5 to 7 days (Hanna and O'Brien, 1960). Despite this high turnover rate, the epithelium must maintain the same thickness profile over time to maintain corneal power and refraction. The epithelial thickness profile can affect the total corneal power because it determines the shape of the air-tear film interface, but also because of the difference in refractive index between the epithelium and the stroma (Patel et al., 1995). Mitochondria provide energy for cellular activities and play an essential role in apoptotic signal transduction in the corneal epithelium. The normal function and energy production in the corneal epithelium depends on the action of both pro- and anti-apoptotic B-cell lymphoma (BCL-2) family proteins and their interaction at the mitochondrial membrane (Hazlett, 2007; Lim et al., 2009; Niswander and Dokas, 2007; Szegezdi et al., 2009). Mitochondrial damage has been implicated in corneal epithelial cell death in dry eye disease, where hyperosmolarity (a state seen in dry eye disease) induces apoptosis of human corneal epithelial cells through cytochrome *c* mediated death pathway (Luo et al., 2004).

In comparison to the epithelium, the remainder of the corneal cells have a slow turnover rate therefore increasing its susceptibility to damage by oxidative processes (Zierhut et al., 2008). The stroma, the major component (about 90%) of the cornea, is a collagenous tissue composed of multiple lamellae of tightly packed parallel collagen fibrils and keratocytes. Uniform stromal collagen diameter and orderly packing is essential for tissue transparency. The keratocytes (3% of the stromal volume) remain quiescent throughout adult life (Pinnamaneni and Funderburgh, 2012). Transmission electron microscopy (TEM) has shown that anterior stromal keratocytes contain twice the number of mitochondria as the posterior two-thirds of the stroma, which correlates with the higher oxygen tension and cell density of the anterior stroma (Kaufman et al., 2011; Muller et al., 1995; Snyder et al., 1998).

The corneal endothelium is a monolayer of hexagonal cells, which has a critical role in maintaining corneal hydration and thus transparency. Corneal endothelial cells (CECs) are highly inter-digitated and possess apical junctional complexes that, together with abundant cytoplasmic organelles including mitochondria, are indicative of their crucial role in active fluid transport (Zavala et al., 2013). In order to maintain the corneal transparency, active transport of water out of the corneal stroma and endothelium into the anterior chamber is required. The corneal endothelium has a high rate of metabolic activity to regulate corneal hydration by active transport in the corneal endothelium (Nita and Grzybowski, 2016). Histologically, the corneal endothelium cells contain a large nucleus, a prominent endoplasmic reticulum and a large number of mitochondria, which provide the high amount of ATP necessary for the endothelial Na^+/K^+ ATPase active transport pump (Laing et al., 1992; Yu et al., 2011).

The corneal endothelial cell count declines throughout life with an estimated rate of decline of 0.6% per year (Bourne et al., 1997); the endothelial cell density of 3 to 6 year old children is 4000 to 3500 cells per mm^2 (McCarey, 1979), whereas middle aged adults (30 years of age) can have a range between 2700 and 2900 cells per mm^2, and adults > 75 years of age can have a range of endothelial cell densities between 2400 and 2600 cells per mm^2 (Hoffer, 1979; McCarey, 1979; Yee et al., 1997). The endothelium is the corneal cell layer with the lowest mitotic activity and there is no evidence that human endothelial cells divide under normal circumstances (Bourne, 2003). Damage to the endothelium is therefore clinically and functionally more significant than damage to other corneal layers, as this can result in irreversible cell loss and subsequent loss of visual and corneal function (Zavala et al., 2013). Oxidative stress has been shown to cause corneal endothelial cell death by apoptosis or necrosis (Cho et al., 1999; Hull and Green, 1989). The corneal endothelium can be subjected to a number of different stressors which can accelerate the normal enlargement and loss of cells with age e.g. intraocular surgery (Glasser et al., 1985; Matsuda et al., 1984; Olsen, 1979), endothelial wounds (Landshman et al., 1989; Yee et al., 1987), ocular (Brooks and Gillies, 2016; Olsen, 1979; Setala, 1979) and systemic diseases e.g. diabetes (Schultz et al., 1984). Reactive oxygen species (ROS) generated from ultrasonic energy (Takahashi, 2005) (specifically from phacoemulsification in cataract surgery) may be a major mechanism causing the subsequent endothelial cell damage and failure.

3. Oxidative stress and the cornea

The cornea, given its highly exposed position, receives a significant amount of high-tension atmospheric oxygen and sunlight, including the ultraviolet range. These factors result in the generation of ROS and subsequent oxidative stress in the cornea (Wenk et al., 2001). Oxidative stress in the cornea is a consequence of an imbalance between Reactive oxygen species (ROS) production and the antioxidant capacity of the corneal cells (Choi et al., 2011). Reactive nitrogen species (RNS), especially those containing oxygen, may also contribute to oxidative stress. ROS are important in cellular homeostasis (Knapp and Klann, 2002) and cells require a specific balance of ROS and RNS for normal cellular physiological functions such as cell growth, proliferation,