ELSEVIER

Seminars in Cell & Developmental Biology 19 (2008) 100–112

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

www.elsevier.com/locate/semcdb

Review

The role of corneal crystallins in the cellular defense mechanisms against oxidative stress

Natalie Lassen [a,1], William J. Black [a,1], Tia Estey [b,1], Vasilis Vasiliou [a,b,*]

[a] *Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO 80262, USA*
[b] *Center for Pharmaceutical Biotechnology, Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO 80262, USA*

Available online 10 October 2007

Abstract

The refracton hypothesis describes the lens and cornea together as a functional unit that provides the proper ocular transparent and refractive properties for the basis of normal vision. Similarities between the lens and corneal crystallins also suggest that both elements of the refracton may also contribute to the antioxidant defenses of the entire eye. The cornea is the primary physical barrier against environmental assault to the eye and functions as a dominant filter of UV radiation. It is routinely exposed to reactive oxygen species (ROS)-generating UV light and molecular O_2 making it a target vulnerable to UV-induced damage. The cornea is equipped with several defensive mechanisms to counteract the deleterious effects of UV-induced oxidative damage. These comprise both non-enzymatic elements that include proteins and low molecular weight compounds (ferritin, glutathione, NAD(P)H, ascorbate and α-tocopherol) as well as various enzymes (catalase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, glutathione reductase, and superoxide dismutase). Several proteins accumulate in the cornea at unusually high concentrations and have been classified as corneal crystallins based on the analogy of these proteins with the abundant taxon-specific lens crystallins. In addition to performing a structural role related to ocular transparency, corneal crystallins may also contribute to the corneal antioxidant systems through a variety of mechanisms including the direct scavenging of free radicals, the production of NAD(P)H, the metabolism and/or detoxification of toxic compounds (i.e. reactive aldehydes), and the direct absorption of UV radiation. In this review, we extend the discussion of the antioxidant defenses of the cornea to include these highly expressed corneal crystallins and address their specific capacities to minimize oxidative damage.

Keywords: Cornea; Crystallins; Ultraviolet radiation; Reactive oxygen species; Oxidative stress; ALDH3A1; ALDH1A1; Transketolase; Serum albumin; α-Enolase; Cyclophilin; Isocytrate dehydrogenase

Contents

* Corresponding author at: Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Denver, CO 80262, USA. Tel.: +1 303 315 6153; fax: +1 303 315 6281.
E-mail address: vasilis.vasiliou@uchsc.edu (V. Vasiliou).
[1] These authors contributed equally to this work and should both considered first authors.

1084-9521/$ – see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.semcdb.2007.10.004

1. Introduction

The cornea is a unique avascular and transparent tissue covering the anterior portion of the eye and serves two vital functions: it functions as a protective barrier for the internal ocular structures (iris, pupil, and anterior chamber) against environmental stresses while also providing the majority of the optical focusing power of the eye. The cornea and lens may function together as a single unit, termed the "refracton", to provide the proper refractive properties so that an accurate image is cast upon the retina [1].

As the cornea is under persistent exposure to both physical and chemical insults from the environment, it has evolved a repertoire of systems to protect its viability and function. Ultraviolet radiation (UVR) is one of the dominant environmental stresses on the cornea. The majority of UVR is absorbed by the cornea, specifically in the wavelengths of UVC (190–290 nm) and UVB (290–320 nm) light. Wavelengths of 320–400 nm (UVA) are transmitted through the cornea and absorbed primarily by the lens whereas visible light (400–700 nm) penetrates completely through these structures to the retina [2]. The shorter and higher energy wavelengths of UVR (UVC and UVB) are potent initiators of photo-chemical reactions that induce oxidative stress through the generation of reactive oxygen species (ROS). ROS result from the incomplete reduction of oxygen and can be produced by both exogenous, such as solar radiation, and endogenous sources such as the mitochondrial electron transport chain and the endoplasmic reticulum [3] as well as through the activity of enzymes *viz* cytochrome P450, xanthine oxidase, urate oxidase, and D-amino acid oxidase [4]. Due to their electrophilic nature, ROS are tightly regulated by inter/intracellular systems to prevent oxidative damage to cellular nucleophiles, such as lipids, proteins, and DNA. Oxidative stress arises when the rate of ROS formation is greater than the rate of their removal. If left unchecked, these species can lead to the degenerative autocatalytic process of lipid peroxidation that can result in the production of a number of reactive aldehydic products that act to further modify cellular nucleophiles. The ability of the cornea to absorb these shorter wavelengths of UVR is indicative of its indispensable role in the protection of internal ocular tissues from UVR-induced damage. In doing so, however, the cornea itself is vulnerable to such damage due to its near continual exposure to these wavelengths. Molecular oxidation, lipid peroxidation, protein modification, and extensive DNA damage are potential sequelae of UVR-induced ROS in the corneal tissue. These changes have the potential to diminish the integrity of the cornea, decrease its viability and lead to corneal cell death by both apoptotic and necrotic pathways as will be described in more detail below.

2. UV-induced oxidative damage in the cornea

Chronic UVR-induced ROS formation is believed to be responsible for various degenerative diseases in the eye including cataract formation as supported by increased incidences at high elevations (Tibet and Bolivia) and at lower latitudes (approaching the equator) [5] as well as macular degeneration [6]. ROS have the capacity to damage all biomolecules including proteins, nucleic acids, and lipids. They can initiate lipid membrane peroxidation (LPO) which, in turn, generates highly toxic aldehydes such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) [7]. Additionally, the accumulated action of these toxic and reactive compounds is one of the dominant theories describing the molecular mechanisms behind the process of aging [8]. A number of *in vivo* and *in vitro* studies have shown that UVR-induced oxidative stress can cause an array of compromising pathologies in the corneal tissue layers. Microscopically, the epithelial layer appears loosened and disruption of tight junction integrity between the corneal epithelial cells have been observed [9]. Changes are also noted in the thickness of the epithelial layer [10,11] and in the proliferation rates of the epithelial cells around the conjunctiva [12]. UVR exposure decreases the metabolic capacity of a number of enzymes [13]. Specifically, activities of alcohol dehydrogenase [14], aldehyde dehydrogenase [15], catalase and glutathione peroxidase [16] are all decreased in cornea after UV exposure. Nuclear fragmentation [9] and alterations in gene expression including those of p53 and p21 [17] as well as the activation of a number of cell signaling pathways has been reported [18].

Corneal clouding is often observed following high levels of UV exposure [14]. Proteoglycans, which consist of glycosaminoglycans (GAGs) covalently bound to a protein core, surround and envelop the collagen fibrils at specific locations in the corneal stromal layer. These proteoglycans are vital for maintaining regularity in the parallel spacing of the collagen fibers and the uniformity of their diameter, which allows for the transmittance of light through the stromal matrix [19]. It has been suggested that ROS may cause the direct cleavage of these elements and alter their physiological properties, resulting in an increased susceptibility of the proteoglycans to enzymatic degradation [20]. Loss of these stromal proteoglycans can result in collagen fibril aggregation in edematous corneas [21] and may be correlated to corneal clouding induced by UV radiation (W. Black and V. Vasiliou, manuscript in preparation).

3. Non-enzymatic and enzymatic antioxidants in the cornea

Due to the cornea's structure and function, a number of antioxidant defense mechanisms are present to minimize