Experimental Eye Research xxx (2016) 1–9

Contents lists available at ScienceDirect

Experimental Eye Research

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

Review

Lens glutathione homeostasis: Discrepancies and gaps in knowledge standing in the way of novel therapeutic approaches

Xingjun Fan [a, *], Vincent M. Monnier [a, b], Jeremy Whitson [a]

[a] Department of Pathology, Case Western Reserve University, USA
[b] Department of Biochemistry, Case Western Reserve University, USA

ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form
10 March 2016
Accepted in revised form 25 June 2016
Available online xxx

Keywords:
Lens
Cataract
Glutathione
Homeostasis
Aging
Transporter

ABSTRACT

Cataract is the major cause of blindness worldwide. The WHO has estimated around 20 million people have bilateral blindness from cataract, and that number is expected to reach 50 million in 2050. The cataract surgery is currently the main treatment approach, though often associated with complications, such as Posterior Capsule Opacification (PCO)-also known as secondary cataract. The lens is an avascular ocular structure equipped with an unusually high level of glutathione (GSH), which plays a vital role in maintaining lens transparency by regulating lenticular redox state. The lens epithelium and outer cortex are thought to be responsible for providing the majority of lens GSH via GSH *de novo* synthesis, assisted by a continuous supply of constituent amino acids from the aqueous humor, as well as extracellular GSH recycling from the gamma-glutamyl cycle. However, when *de novo* synthesis is impaired, in the presence of low GSH levels, as in the aging human lens, compensatory mechanisms exist, suggesting that the lens is able to uptake GSH from the surrounding ocular tissues. However, these uptake mechanisms, and the GSH source and its origin, are largely unknown. The lens nucleus does not have the ability to synthesize its own GSH and fully relies on transport from the outer cortex by yet unknown mechanisms. Understanding how aging reduces GSH levels, particularly in the lens nucleus, how it is associated with age-related nuclear cataract (ARNC), and how the lens compensates for GSH loss via external uptake should be a major research priority. The intent of this review, which is dedicated to the memory of David C. Beebe, is to summarize our current understanding of lens GSH homeostasis and highlight discrepancies and gaps in knowledge that stand in the way of pharmacologically minimizing the impact of declining GSH content in the prevention of age-related cataract.

Contents

* Corresponding author. Department of Pathology, Case Western Reserve University, Cleveland, OH, USA.
E-mail address: xxf3@case.edu (X. Fan).

http://dx.doi.org/10.1016/j.exer.2016.06.018
0014-4835/

1. Introduction

The lens has evolved as an anaerobic biological system with millimolar concentrations of glutathione (GSH). The critical role of GSH in maintaining lens redox status and transparency is well recognized and has been, over the years, the subject of several excellent reviews (Giblin, 2000; Lou, 2000; Reddy, 1990; Truscott, 2005). While one could argue that nothing new was to be expected concerning the protective role of GSH in the lens, our interest in GSH homeostasis in the lens was rekindled with the unexpected finding that lenticular GSH levels were not completely suppressed in the LEGSKO mouse in spite of complete absence of γ-glutamylcysteine ligase (Fan et al., 2012). This issue, which is the subject of intense investigation in our laboratory, is closely linked to lenticular ascorbate metabolism and cataractogenesis and the work of David Beebe who has pioneered the importance of the vitreous as a source of oxidative stress to the lens (Beebe et al., 2014; Holekamp et al., 2005; Li et al., 2013b; Shui et al., 2009). These paradigm shifting studies inspired us to study lens biology in connection not only to the aqueous humor but also to the vitreous humor. Additionally, Dr. Beebe's pioneer studies provide the mechanistic framework for a potential therapeutic treatment of high risk (>90%) and rapid (within two years) nuclear cataract formation after vitrectomy surgery (Petrash, 2013).

In order to provide a complete coverage of lens GSH homeostasis, we have to discuss the lenticular GSH dynamics from the perspective of both protein conjugated GSH and free GSH/oxidized GSH (GSSG). Since several excellent reviews have covered the protein glutathionylation (Lou, 2000, 2003; Lou and Dickerson, 1992; Lou et al., 1990, 1995), we will mainly focus on the roles of latter. Below we review the established mechanisms and pathways that are involved in lens GSH homeostasis. We also provide a brief summary of recent progress regarding lens nucleus GSH homeostasis, as well as the impact of aging on lens GSH homeostasis, since age-related nuclear cataract (ARNC) is often believed to be, in part, associated with declining nuclear GSH levels in the aging human lens (Giblin, 2000).

1.1. GSH de novo synthesis and its constituent amino acids transport

Intracellular GSH is synthesized by two ATP-dependent enzymes: γ-glutamylcysteine ligase (GCL) and glutathione synthase (GS) to produce γ-glutamylcysteine and GSH, respectively. The mammalian GCL is a heterodimer enzyme consisting of a 73-kDa catalytic subunit, Gclc, and a 28-kDa modulatory subunit, Gclm. The catalytic subunit, Gclc has the enzymatic activity and is regulated via a GSH feedback inhibition mechanism (Richman and Meister, 1975). Gclm has no enzymatic activity, but heterodimer formation of Gclm and Gclc significantly decreases the Km value for glutamate and increases the Ki value for the feedback inhibition by GSH (Chen et al., 2005).

Like other tissue systems, the lens has a functional GSH *de novo* synthesis machinery, which mostly lies in the epithelial and cortical layers, while mature fibers cells sit in inner layers of the lens that have lost cell organelles such as nucleus and mitochondria (Bassnett and Beebe, 1992). However, due to the avascularity of the lens, for GSH biosynthesis to take place, the constituent amino acids, glutamic acid, glycine and cysteine have to be transported to the epithelial and outer cortical fibers cells. Pioneering work from Reddy et al. has demonstrated that these amino acids are delivered to the lens from the plasma via the aqueous humor and the lens epithelium, from which they are delivered to the rest of the lenticular system (Reddy, 1973, 1979) (Fig. 1). For GSH synthesis, the Km value of GCL for cysteine is ~0.15 mM, while that for glutamate is ~1.7 mM, and that of GS for glycine is ~0.8 mM (McBean, 2012). In order for GSH biosynthesis to take place, the required intracellular concentration of these amino acids is thought to be close to their Km value. However, various studies suggest species-specific results regarding these amino acids levels in the lens epithelium and outer cortex. For example, Lim et al. (Lim et al., 2007) find that the concentration of the three amino acids in the rat lens cortex is lower than that required for the GCL and GS Km value of its proper constituent amino acids. In contrast, other studies in human, rabbit and bovine lens demonstrate much higher values than these required for the Km (Barber, 1968; Kern and Ho, 1973; Reddy, 1973). It has to be pointed out that these studies measured the total GSH from the homogenate of the lens tissue, and that this does not exclude the possibility that intracellular amino acid level might be much higher to fulfill the needs of GSH *de novo* synthesis. Nevertheless, similar to other body systems, such as the central nervous system (CNS) (Aoyama et al., 2012), cysteine level is relatively lower than glutamic acid and glycine. It is, therefore, the rate-limiting substance in lenticular *de novo* GSH synthesis and an adequate cysteine supply is essential wherever GSH *de novo* synthesis is taking place.

1.1.1. Cysteine/cystine transport

From the above considerations, it is clear that cellular cysteine and GSH synthesis are tightly linked. Multiple mechanisms have been postulated in terms of intracellular cysteine homeostasis in studies of the central nervous system (CNS) or hepatocytes (Lu, 1999; McBean and Flynn, 2001). The sodium independent cystine/glutamate exchanger (Xc^-) has been shown to take up cystine into cells, which is subsequently reduced into cysteine for GSH synthesis (Lewerenz et al., 2013; Lim and Donaldson, 2011). In one set of studies, the Xc^- exchanger was found present in the entire rat lens, predominantly in the cytoplasm in the outer cortex cells, while it was more membranous in the inner cortex region (Lim et al., 2005). In the human lens, Xc^- is present in the entire lens region at a young age, but no immunoreactivity is found in the central lens region of aged human lenses (Lim et al., 2013). In contrast, in other studies, Xc^- was reported to be predominately present in the membranes of outer cortex cells, and no detection was observed in the nuclear region of the dog lens(Lall et al., 2008). These studies provide evidence for the presence of Xc^-, but whether this exchanger is important for lens cysteine homeostasis is still not very clear. Several findings point to quite different research directions. In a vascular eye perfusion study in guinea pigs, radiolabeled cysteine, cystine and methionine were injected through the common carotid artery (Mackic et al., 1997). In this study, cysteine, but not cystine, was readily taken up by the lens epithelial and cortical fiber layers, while infused cystine failed to incorporate into GSH synthesis. Other evidence in support of cysteine rather than cystine uptake is that high levels of free

Please cite this article in press as: Fan, X., et al., Lens glutathione homeostasis: Discrepancies and gaps in knowledge standing in the way of novel therapeutic approaches, Experimental Eye Research (2016), http://dx.doi.org/10.1016/j.exer.2016.06.018