Delivery of Glutathione to the Lens Nucleus

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Glutathione (GSH), an anionic tripeptide, is the principal antioxidant in the lens where it functions to maintain protein thiols in a reduced state.[1-4] Levels of GSH are high in both the epithelium and the outer cortex of the lens where the anti-oxidant is synthesized from the amino acids cysteine, glutamate and glycine by g-glutamylcysteinesynthetase and glutathione synthetase. A reducing environment in the outer fiber cells is also maintained by regeneration of GSH from GSSG by the enzyme glutathione reductase which utilizes NADPH produced via the hexokinase shunt pathway as a cofactor.[1-4] In contrast, levels of GSH are 80-90% lower in the nucleus of the lens owing to the low specific activity of enzymes involved in its synthesis and regeneration. This regional difference in GSH levels worsens with age. The age-dependent reduction in GSH levels in the nucleus and the resulting increase in GSSG lead to oxidation of cysteine and methionine residues in proteins and formation of protein mixed disulfides (PSSG) and eventually, loss of transparency.[1-4] This decline in GSH levels is believed to be the key initiating factor in the formation of age-related nuclear cataracts, the leading cause of blindness in the world.

How GSH is transported to the lens nucleus and why this process is disrupted with age is not clear. According to one view, GSH is transported by an extracellular pathway driven by the lens microcirculation.[5] All vertebrate lenses studied to date have a circulating ionic current that enters at the poles and exits at the equator of the lens.[6] This internal circulating current is primarily carried by sodium ions, and enters the lens along the extracellular spaces between cells. After crossing the fiber cell membranes in the lens interior, it flows from cell to cell towards the surface through gap junction mediated pathways. The high concentration of gap junction channels at the equator allows the intracellular current to be directed to surface cells where Na^+/K^+ pumps are located in epithelial cells transporting sodium out of the lens.[6] The circulating ionic current generates fluid flow through the lens, as indicated by recent measurements of intracellular hydrostatic pressure in vertebrate lenses.[7] Glucose and other nutrients are convected into extracellular spaces surrounding fiber cells, where uptake transporters allow their delivery into fiber cells. Anti-oxidants such as GSH (and ascorbic acid) are believed to be carried to the lens core in a similar fashion.[5] However, no uptake transporters specific for GSH in the nucleus have yet been identified. In addition, it is not established whether the ability of the circulation system to deliver sufficient antioxidants is reduced with age.

An alternate view advanced by Truscott and others is that the transport of GSH to the nucleus occurs by diffusion of the metabolite from the outer cortex via gap junctions.[8] A barrier to diffusion of GSH from the periphery to the center was shown to develop with age, leading to a reduction in the delivery of GSH to the lens nucleus.[8] This view was based on studies wherein lenses were incubated with 35S cysteine and movement of the label within the lens was followed over time. The movement of cysteine label occurred along the length of the fiber cells in the equatorial plane where gap junctions are distributed.[8] Diffusion of GSH via gap junctions is favored by the large concentration gradient from the outer cortex to the nucleus. Another factor favoring diffusion of anionic GSH to the core is that the resting membrane potential of fiber cells in the nucleus is more positive as compared to that of cells in the cortex.

Our recent studies using electrophysiological methods indicate that gap junctions in fiber cells, which are formed by two connexin isoforms, Cx46 and Cx50, are permeable to large anions including GSH (PNa:PGSH ~12:1, unpublished data), suggesting that the contribution of this intracellular pathway is likely to be significant. The development of a barrier to GSH diffusion that occurs with age might be explained by the age-dependent reduction in coupling conductance.

Additional studies are necessary to assess the *in vivo* significance of permeability of lens gap junctions to GSH. Measurement of levels of the anti-oxidant in the nucleus in the absence of connexin mediated coupling, e.g. Cx46 and Cx50 in knockout lenses, might clarify whether maintenance of adequate levels of the anti-oxidant in the lens core depends on gap junctional coupling. The contribution of the extracellular pathway also warrants additional study. The presence of a large hydrostatic pressure gradient due to the lens microcirculation would tend to oppose the concentration- and voltage-gradients favoring diffusion. Thus, examining the spatial distribution of GSH in the lens in the presence of inhibitors of the Na^+/K^+ pump, which ultimately drives the lens microcirculation, is necessary to define the role of hydrostatic pressure gradient and the extracellular pathway. Understanding the underlying mechanisms of GSH transport will aid in the development of therapeutic agents that increase delivery of the vital antioxidant to the lens nucleus and possibly delay the progression of age-related nuclear cataracts.

Conflicts of Interest

None.

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