Anti-cataract therapies: is there a need for a new approach based on targeting of aquaporins?

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Introduction

A quote attributed to Leonardo da Vinci that "*Simplicity is the ultimate sophistication"* can be applied to the elegant yet relatively simple structure of the eye lens which, devoid of blood vessels and nerves, retains its most important function of transparency for decades, allowing light to traverse and reflect image carrying information to the retina at the back of the eye. The lens is a lamellar structure in which fibre cells grow over existing layers. The whole structure is enveloped in a semi-elastic capsule that maintains the lens shape and is thought to mediate the forces from the ciliary muscle that act to alter lens shape to allow for changes in focus of the eye. The ability to alter focus decreases with age as does the amount and wavelength of light transmitted and the causal factors involved in these losses of function, whilst not conclusively found to be related, have been linked to changes in the lens proteins.

From the biological perspective the lens is essentially a sac of protein and water. Yet, it has one of the most sophisticated optical forms in nature: that of a gradient refractive index which provides a very accurate optical focus and the high level of image quality needed by the eye [1]. This image quality is disrupted when cataracts develop. The type and manifestation of these opacifications varies. The most common type of cataract, which develops with age, can appear in the central layers, or nucleus, of the lens, in the peripheral (cortical) regions or under the capsule of the lens (posterior sub-capsular cataract). Cataracts also form with chronic, systemic disease such as diabetes. There is no effective anti-cataract drug or medication; the only treatment available is surgical removal of the eye lens and replacement with an intraocular implant. No implant has, as yet, been created with the sophisticated gradient of refractive index nor with the same ability to alter the focus of the eye that can compare with the biological lens.

Current treatment and approaches

The quest for anti-cataract formulations has been somewhat subdued over decades since surgical advances have made it possible to restore sight in an eye with cataract. Notwithstanding the comparative simplicity of the surgery and the high success rate, surgery is not without risks. Furthermore, it is not readily available in many parts of the world, with waiting lists growing in the developed world as the

population ages and as prevalence of chronic diseases that can cause cataract, rises. The need for an effective anti-cataract medication is gaining renewed interest.

The biochemical explanation for cataract is that various changes in the cytoplasmic proteins of the lens, the crystallins, that lead to alteration in protein folding, their structural form and their relationship with water, cause protein aggregation and consequent lacunae filled with water as protein bound water is released. The difference in density and refractive index between protein aggregates and water causes scatter of light rather than refracting light to the retina (as in a transparent lens), and this is manifested as an opacification or cataract. The changes to crystallins that are thought to contribute to cataract formation are multifactorial and include deamidation, phosphorylation, glycation and oxidation [2,3].

Given that crystallins constitute over 90% of the total protein in the human lens, the search for anti-cataract medications has unsurprisingly been focussed on these cytoplasmic proteins and means of preventing, halting and ultimately reversing structural changes to these proteins that are detrimental to sight. Given the many different causal factors associated with cataract formation, genetic, epigenetic and environmental, approaches to development of anti-cataract formulations have been varied. The most common type of cataract, senile cataract, has attracted a number of approaches including aldose reductase inhibitors which have some reported limited success in animal models, enzymatic and non-enzymatic anti-oxidants [4], aspiring and non-steroidal anti-inflammatory drugs [5].

Vitamin D deficiency has been identified as a risk factor for posterior subcapsular cataract prompting the suggestion that this may be a potential remedy for cataract. Indeed, increasing vitamin D levels in early stages of posterior subcapsular cataract was found to lead to a clearance of the opacification [6]. Promising findings in animals using lanosterol were reported to dissolve protein aggregates and hence restore transparency [7,8]. The mechanism of action of lanosterol (25-hydroxycholesterol) was thought to increase chaperone activity leading to dissolution of crystallin aggregates. However, this has not been reproduced in *in vitro* human lenses [9]. Recent research on cerium oxide has shown multiple actions of ceria nanoparticles on cytoplasmic proteins in lens epithelial cells including as anti-oxidants, anti-glycating agents and catalase mimetics, indicating potential for treating different types of cataract [10].

Novel therapies: focus on aquaporins

The search for cataract medications, focussed on the preventing aggregation of cytoplasmic proteins, has not yielded significant success. The other major component of the lens is water. It constitutes around two-thirds of the lens material content and maintenance of the correct relationship between proteins and water is essential for transparency [11]. Water exists in a free state or is protein-bound and the ratio of free to protein-bound water increases with age [12,13]. The link between this change in water state with age and opacification is not yet clear.

Given that the lens has no blood supply, the only means of providing necessary nutrients to lens cells and removing metabolic waste material is via water, an elegant model of microcirculation has been proposed [11,14] and has experimental support [15-18]. The microcirculation of water is postulated to be caused by a Na^+ flux that flows into the extracellular spaces between lens cells at the polar surface of the lens (along the line of the optic axis), traverses the cell membranes and, following an intercellular pathway, flows to the lens surface at the equator. The equator is the furthest part of the lens from the optic axis and the region in which differentiation of lens epithelial cells to lens fibre cells occurs. Here the abundance of Na^+/K^+ pumps is higher than at the poles and Na^+ ions undergo active transport out of the lens [14,19]. Gap junctions linking inner layer cells to their counterparts in surface layers create a negative membrane potential in these inner layers (as there is a paucity of Na^+/K^+ pumps and K^+ channels) to maintain the microcirculation [19]. This microcirculation of current causes water, carrying nutrients, to enter the lens through an extracellular route and water, transporting waste material, to flow out through intracellular passage [19]. Recent research has modelled the relationship between fluid flow and ion fluxes emphasising the importance of extracellular space and pressure differences between intracellular and extracellular spaces [20].

An important class of lens proteins, which are far lower in proportion that the crystallins, but which play a vital role in water transport, microcirculation and homeostasis in protein/water balance in the lens are the aquaporins [21]. Aquaporins are small membrane proteins found in a number of tissues in humans and animals. The three main aquaporins that are expressed in the human lens are aquaporin 0, 1 and 5 [21]. These aquaporins are water transporting proteins [22]. Aquaporin 0 (also known as Membrane Intrinsic Protein) is found in the fibre cells throughout the lens, constituting over half of the protein concentration of the cell membranes [22]. Aquaporin 1 is found in the epithelial cells [22] and aquaporin 5 has been detected in all parts of the lens [23] and is the second most prevalent of the aquaporins in mature lens fibre cells [23]. Aquaporin 7, an aquaglyceroporin which facilitates transport of small molecules as well as water and may be active in transport of nutrients, has also been detected in the eye lens epithelium [22]; it has a significantly lower water permeability than aquaporin 1 [24].

Whilst aquaporin 0 is the most abundant of the membrane proteins and the major water channel in lens fibre cells, its permeability is relatively low and this has been proposed as critical for effective microcirculation and transparency [25] and required to permit changes in water flow when needed [22]. It has been suggested that modulations of aquaporin 0 in the older cells located in the central layers of the lens may change the function of this protein from that of water transportation to a junctional protein [22] and that aquaporin 0 may have a function in pore gating [26]. However, experimental work has shown that, age-related modifications to aquaporin 0 such as truncation [27] do not affect water transporting ability of the protein [28,29]. It has been speculated that in older lens cells, which are found

in central regions of the lens where aquaporin 0 permeability is lower, aquaporin 5 may provide some compensation [22].

Impaired aquaporin function

Aquaporin 0 has also been shown to have cell-to-cell adhesion function [30, 31] which, when impaired lead to cataract formation [32]. Congenital autosomal cataract in five generations of a family caused by a missense mutation of arginine to cysteine at amino acid 33 was replicated in the laboratory using site-directed mutagenesis [32]. The results indicated that the mutation did not cause any obstruction in water transport capacity but a significant reduction in the cell adhesion properties of aquaporin 0, indicating that the main function of aquaporin 0 may well be that of cell adhesion [32]. Given the wide distribution of this water channel protein throughout the human lens, mutations of aquaporin 0 that cause a disturbance of cell adhesion can give rise to a range of opacifications [32].

Further mutations have been identified in two other types of human inherited cataracts [33]. A missense mutation that resulted in a threonine to arginine substitution at codon 138 in aquaporin 0 manifested, in one affected family, as progressive punctate opacities in the middle and outer lens layers or in the polar regions of the lens [33]. The phenotypic appearance in a second family, where the other mutation caused a substitution of glutamic acid for glycine at codon 134, was of minute opacities that were non-progressive, lamellar and located in the sutures of the lens [33]. These mutations may have prevented formation of a water channel or impeded transportation of water through the channel [33]. A doubling in amount of aquaporin 1 was found in the lens epithelium of patients with cataract compared to healthy lenses and was proposed to be a means of increasing water permeability in the lens epithelium to protect from further opacification [34].

Studies using animal models to investigate the function of aquaporin 0, have found variations in cataract severity depending on whether the aquaporin 0 knockout was a mouse strain with or without beaded filaments [35]. Lenses from aquaporin 0 knockout mice with beaded filaments exhibited increased water circulation and less opacification than aquaporin 0 knockout mice without beaded filaments pointing to a modulation of gap junctions by aquaporin 0 in the presence of beaded filaments [35]. Lenses from aquaporin 5 knock out mice, subjected to hyperglycemic conditions, exhibited increased water content and swelling caused by osmotic stress suggesting a critical role for aquaporin 5 in regulation of efflux and cell volume [36].

Aquaporins in drug development

Aquaporins have been highlighted as drug development targets for a range of conditions [37,38]. A limiting factor is the lack of effective and safe pharmacological modulators [37]. One class of compounds that hold promise as therapeutic agents by modulating aquaporin function are the polyphenols: phytochemicals that are abundant in wide variety of foods such as fruits, vegetables and edible oils [39, 40]. Polyphenols such as curcuminoids and flavonoids modify the function of aquaporins that are found in the lens: curcumin has been shown to downregulate aquaporin 1 in cell cultures [41] and was also found to regulate aquaporin gating [42].

The proportion of investigations on aquaporins as drug targets that has been dedicated to the eye has been relatively small and that devoted to the lens even smaller. Yet the lens, with its high water content, may provide the perfect model for investigation of aquaporin drug therapies. Advanced optical measurements now offer the prospect of detecting subtle changes in transparency [43].

The significance of vitamins and a healthy food intake on maintaining a transparent lens cannot be underestimated and has been suggested previously [44]. As the lens has no blood supply it is entirely reliant on nutrition and an effective circulation which in turns depends on the aquaporins. It is time the importance of nutrition was revisited and coupled with an emphasis on investigating the water channel proteins as potential targets for anti-cataract therapy.

Expert Opinion

One may ask why there has been such a paucity of progress on developing therapeutic anti-cataract treatments. The relatively simple structure of the lens should render it an ideal candidate organ for investigating new therapies and the most common type of cataract is the slowly progressing age-related cataract that allows plenty of time for a therapeutic modality to take effect. Yet, the very reasons that should lead to interest in development of anti-cataract drugs are conversely the same reasons why they may not have been. Simplicity in structure, with no direct connections to the circulatory or nervous systems has allowed for an uncomplicated surgical procedure in the majority of cases. The most common type of cataract which manifests with age, is like most age-related conditions, multifactorial and likely to vary with individuals.

Given the diversity in causation, it is unlikely that any anti-cataract therapy will be universally effective for all types of cataract. Rather, drugs should be targeted at specific types of cataract and therapies varied accordingly depending on where the opacity is located and on cataract maturity. If indeed, microcirculation of water is a means of maintaining lens transparency [11, 14] and its impairment a causal factor in cataract formation [22] therapeutics that specifically target the water permeability properties of aquaporins require further exploration. These need to take into account the fact that the lens continues to grow throughout life and that the refractive index gradient, which is directly related

to the crystallin protein concentration [1] also alters with age [45]. To develop effective therapies for senile cataract, water microcirculation needs to be carefully modelled for different lens age, and hence size, as well as for age-related changes in aquaporin distribution and consideration given to increasing post-translational modifications [27, 46]. Truncation of over half of aquaporin 0 was found in human lenses from the third decade and this rises substantial in older lenses except in very outermost lens layers [27, 46]. Other post-translation modifications of aquaporin 0 with age: acetylation, carbamylation and oleoylation have also been reported [46]. Whilst none of these appear to affect lens transparency [46], they may alter the function of aquaporin 0 and truncation could contribute to an increased hardening of the tissue with age [27]. Such changes in tissue properties need to be considered in drug development given that hardening may be related to increase in localised protein density.

Therapies for senile cataract should ideally be prophylactic but will also be needed when cataracts have started to form. In the earliest stages of cataract formation, when aggregation of crystallins begins, water permeability needs to be optimised to prevent any further formation of protein aggregates and consequent water-filled lacunae forming around them; the large difference in refractive index between protein aggregates and water exacerbates light scatter. Where cataracts are liked to glycation, such as in diabetes, water content may need to be decreased [36]. Therapeutics focussed on aquaporin 5 to improve efflux of water [36] should be investigated. Determination of the optimum water transportation for a given age and lens size, as well as for effective regulation of cell volume requires accurate fluid dynamic modelling. This is needed to inform pharmacokinetics and the extent to which drugs that can modify the function of a particular aquaporin or aquaporins should control permeability and microcirculation.

Success of any aquaporin specific medication needs to take into account effective delivery to the lens. Given the position of the lens, peri-ocular drug injection into the anterior chamber is a possible option but ideally therapeutics should be transported to the lens without invasive methods. Topical administration of controlled delivery via hydrogel contact lenses offers great potential and warrants further research into drug carriers that can be targeted specifically to the lens, that can pass through the cornea and/or sclera and ultimately through the lens capsule.

For effective treatment, anti-cataract drugs should be developed with personalised medicine in mind and not be seen as a full-scale replacement for surgery but rather as a complement to it. To advance this, more research is needed into the many different cataract types, their varied causal factors, how they are linked to diet, lifestyle, systemic diseases and environmental factors and particularly how these factors affect the state of water and the water channel proteins. It is considered that structural changes in crystallin proteins increase the proportion of free water. More research is needed into what happens to the free water, where it is channelled and how the aquaporins redistribute this free water in such a

way as to maintain the transparency of the lens and its refractive index gradient which is needed for high image quality. Technologies that use resonance spectroscopy such as Magnetic Resonance Imaging (MRI) and Nuclear Magnetic Resonance (NMR) have been used to provide information on the amount of free water in the lens [47,48]. Far more research using such techniques is needed and results of these findings linked with studies on aquaporins, how they alter with age and with exposure to agents that could be developed into effective non-surgical therapies. The polyphenols are one family of compounds that offer such hope and pave the way to a greater emphasis on diet in cataract prevention.

The final frontier in diagnosis is the development of effective imaging methods that could measure water distribution and deduce aquaporin effects in the living lens with such accuracy that subtle changes in free water proportion across the lens could be detected. Such developments need more research into optics of the lens, imaging and associated technologies.

The future holds the very viable prospect of surgical and non-surgical treatments for cataract, a vastly improved understanding of different types of cataract that occur with age and the respective causal factors on which appropriate therapies should be based. In the next five years the interest in lens research is anticipated to shift towards a multidisciplinary approach, incorporating more dialogue between research into optics of the lens and investigations of its components including water with a greater emphasis on the role of aquaporins as potential targets for anti-cataract medications.

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