Causative and preventive action of calcium in cataractogenesis

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ABSTRACT

Calcium and Ca-dependent enzymes play specific role in the development of human cataracts. Entry of Ca$^{2+}$ into the lens epithelial cells (LEC) is highly regulated by quantum of receptors. The Ca$^{2+}$ level controls homeostasis and growth of entire lens. Intracellular overload of Ca$^{2+}$ in the LEC trigger a series of events such as activation of Ca-dependent enzymes, irreversible breakdown of important structural proteins and cell death. Proper maintenance of Ca$^{2+}$ levels by regulating activity of Ca-pumps and Ca-channels and inhibition of Ca-dependent enzymes can help in prevention of cataract. Induction of cell death in the LEC by increase in the intracellular Ca$^{2+}$ may be utilized for the prevention of posterior capsular opacification.

INTRODUCTION

The human ocular lens is transparent, biconvex, elliptical organ located in the visual axis of the eye between anterior aqueous humour and posterior vitreous humour. The anterior surface of the lens is lined by a single layer of the lens epithelial cells (LEC) (Fig 1). In the equatorial region of lens, these LEC terminally differentiate to form lens fibres which do not possess any nucleus and cell organelle. The absence of nucleus and cell organelles, on one side, mean crystal clear transparency of the lens but, on other side the lens fibres lose machinery that keeps them metabolically active. The opacification of the lens fibres in any region of lens is called cataract which is a leading cause of visual im...
pairment throughout the world. Based on the region of opacification cataracts are mainly of three types; nuclear, cortical and posterior subcapsular cataracts (PSC)\(^1\) (Fig 2). Being the most anterior portion of the lens, the lens epithelium (LE) is the first target site exposed to any sort of insult coming through the aqueous humour which may result in cataract. Although the LEC has machinery to combat with cataractogenic insults, any alteration in the LE precede further in the remaining lens and may lead to cataract\(^2\).

Ca\(^{2+}\) is a versatile intracellular signal that regulates many different cellular functions. Understanding the role of Ca\(^{2+}\) in the intracellular signalling and regulation of cellular processes has been worked out in many systems. Ca\(^{2+}\) has a direct role in controlling the expression patterns of its signalling systems that are constantly being remodelled in both health and diseases. Alteration in Ca\(^{2+}\) homeostasis is associated with various types of human and experimental cataracts\(^1,4\). Extensive reviews were written to delineate the role of Ca\(^{2+}\) in cataractogenesis. However, very little stress is given on the role of LEC in controlling lenticular Ca\(^{2+}\) and its role in the development of various types of human cataracts. The role of LEC in controlling the lenticular Ca\(^{2+}\) is interesting since other components of lens do not possess intracellular Ca-store such as endoplasmic reticulum (ER) and mitochondria. Lately focus has shifted to LEC, on the role of LEC in the altered Ca-signalling and its subsequent effects which finally lead to cataract\(^5\). Therefore the present review concentrates on the recent advances in the mechanism of Ca\(^{2+}\) uptake by the LEC and possible role of Ca\(^{2+}\) in the development of various types of human cataracts. Certain other related aspects such as the role of Ca-dependent enzymes, cell death and loss of cellular integrity in the LEC are also reviewed. We have also described recent advancements in preventing cytoplasmic uptake of Ca\(^{2+}\) and inhibiting activity of Ca-dependent enzymes for reducing incidence of cataracts. Recently advocated hypothesis for the prevention of posterior capsular opacification (PCO) by increasing levels of Ca\(^{2+}\) in the residual LEC are also taken into consideration with the help of published literature.

**CYTOSOLIC FREE CALCIUM IN THE LEC**

**Transport of Ca\(^{2+}\)** The anterior surface of the lens lined by LEC is bathed by aqueous humour, which is an important source of nutrients, growth factors and mineral ions including Ca\(^{2+}\) to the lens. The total Ca\(^{2+}\) concentration in the lens is 0.1 mmol/L while in the aqueous humour it is approximately 1 mmol/L (0.45-2.0 mmol/L)\(^8,9\). Therefore, a large gradient of Ca\(^{2+}\) exists on both the sides of LE that constantly drives Ca\(^{2+}\) into the lens. Recent studies on cultured LEC indicate various types of Ca-channels and pumps located in the plasma membrane and endoplasmic reticulum (ER) responsible for the regulation of cytosolic free Ca\(^{2+}\) (Fig 1). The existing literature hypothesize that the rise of cytosolic Ca\(^{2+}\) in the LEC mainly takes place in two phases and both aqueous humour and intracellular stores take part in this process. In the first phase, release of Ca\(^{2+}\) take place from intracellular stores such as ER through the channels gated by inositol-1,4,5-trisphosphate (Ins\(_P_3\)) or ryanodine (cyclic ADPribose, cADPr) either under the influence of initial moderate increase of Ca\(^{2+}\) from aqueous humour or may be mediated by the receptor systems\(^10,11\). In the second phase it takes place from the aqueous humour through plasma membrane Ca\(^{2+}\) channels under the influence of depleted intracellular store\(^7\).

**Homeostasis of Ca\(^{2+}\)** Various authors have suggested G-protein and tyrosine kinase (TK) coupled receptor system for the initial increase in the cytosolic Ca\(^{2+}\)\(^3\). Influence of these receptor systems by their
antagonists or agonists releasesCa2+ from ER through InsP3 gated channel (Fig 1). Growth factors such as FGF, PDGF, etc present in aqueous humour act as agonists for TK receptor and play important role in the normal lens development and homeostasis[12]. G-protein receptor includes molecular species such as acetylcholine (ACh), adrenaline, histamine and ATP[4]. Among them ACh is important since near by tissues of the lens such as iris are the sources of ACh. The lens epithelium has receptors for ACh and highest level of acetylcholine esterase (AChE) activity compare to any other mammalian tissues to hydrolyse ACh[13]. Exposure to inhibitors of the AChE is associated with increased risk of cataract[14].

Recently the extracellular Ca2+ sensing receptors (CaR) are identified which has opened up the possibility that Ca2+ might also function as an extracellular messenger[15]. This CaR is expressed in varying amounts on the surface of many cell types including the LEC[16]. This receptor signals to the interior of many cell types through unique G-protein coupled receptor systems. The presence of CaR on the LEC may explain the entry of Ca2+ in the lens when the Ca2+ levels in the blood falls below normal and causes hypocalcaemic cataract[16]. The total Ca2+ in the LEC is in mmol/L range (0.1 mmol/L); however the free cytosolic Ca2+ is very low in μmol/L range (100-300 nm)[10]. Such a wide difference in the concentration of free and bound Ca2+ is maintained either by sequestration of Ca2+ in the ER including nuclear envelop, Golgi complex and mitochondria or by preferential binding of Ca2+ to complex protein molecules[17]. Major proteins of the lens, β, γ-crystallins act as a potential binding site of Ca2+[18]. During the formation of cataract total lenticular Ca2+ increases beyond 20 mmol/L, however free cytosolic Ca2+ equilibrate with the aqueous humour (1 mmol/L). Therefore during the formation of cataract increasing Ca2+ must be converted into some non-diffusible or bound form[4]. This binding site of Ca2+ in cataractous lenses is quite specific and is different from those in the normal lenses. Most of Ca2+ in cataractous lenses is bound to water insoluble protein and such binding is very strong; Ca2+ binds even in the presence of strong chelating agents like EGTA. Duncan and van Heyningen[19] showed that the normal lens proteins did not have this ability. In the normal lens most of the diffusible Ca2+ is found in the intracellular spaces between the lens fibres and it is bound to the lipid molecules of the outer leaflet of the lipid bilayer. Diminished capacity of these lipids to bind Ca2+ initiate cascade of events that lead to increase in light scattering[20].

**ROLE OF CALCIUM IN CATARACTOGENESIS**

**Activation of Ca-dependent enzymes** Calpains, the Ca2+ dependent cysteine proteases were also detected in the lens of many animals including human. Calpain II, LP82, LP85, and calpain 10 show their highest activity in the lens epithelium[21,22]. Physiologically important substrates for calpain in the lens are not known with certainly, however indirect evidence suggests that cytoskeletal and membrane proteins, crystallins, ion channels, etc[23]. Many authors have suggested that uncontrolled calpain activation due to increased Ca2+ leads to increased proteolytic activity in LEC that results in the digestion of cytoskeletal and junctional proteins and it may initiate cortical opacity[24,25]. Transglutaminase (TGase) is another Ca2+ dependent enzyme responsible for the cross-linking of peptide chains[26] and it is also implicated in cataractogenesis[27]. It is synthesized and secreted from the LEC into virtual space between the capsule and peripheral cortex[28]. Several proteins including crystallins[29] also act as endogenous substrates for TGase in the lens. TGase may also be involved in the cross-linking of proteolytically degraded proteins that may be responsible for the formation of high molecular weight proteins associated with light scattering in the cataractous lens.

**Cell death and loss of cellular integrity** Ca2+ plays a very important role in programmed cell death (PCD) for the embryonic development and tissue homeostasis[30]. Both the above processes in PCD are brought about by subtle changes in Ca2+ distribution within the intracellular compartments. Li et al[31] have shown involvement of LEC apoptosis in non-congenital cataract development. However conflicting observations also exists in the age related cataractogenesis[32]. We have observed decrease in the cell density of EC in the human and experimental cataracts which may be explained by death of LEC[22,33]. Our observations suggest that the time required for the opacification of the lens is related to the time required for significant decrease in the cell density of the LEC. The death of LEC leads to rearrangement of LEC, which may lead to uncoupling. However, proper cellular coupling of the LEC is considered to be important for the maintenance of lens transparency[4]. Ca2+ also regulates the gap junction coupling in the LEC[30]. The observed cell death and uncoupling of LEC even in small areas leads to cell
heterogeneity, which may lead to abnormal functioning of LEC including osmotic stress and leads to cataractogenesis[34]. As it is well known that LEC differentiation is regulated by positional effect (special signals), the space created by apoptosis of LEC impart altered signals for migration of cells from proliferative to central and equatorial zones. It may lead to superimposition and multilayering of the LEC in the central and equatorial zones, which are normally single layered[2,33].

**LEC and various types of human cataracts**

Cataract is a multifactor disease and different types of cataract have different aetiologies. Most of human cataractous lenses have opacification in more than one region and many factors are responsible for their occurrence that adopts different mechanisms. Role of calcium in the development of cortical cataracts is well explained[8,23]. Nuclear cataracts do not involve calcium alteration in the lens[8,9] while role of Ca2+ in PSC is still not understood. Preliminary data from our laboratory on nuclear, cortical and PSC are shown in Fig 4. It clearly indicates that in both nuclear cataract and PSC, the total calcium level is higher than the clear lenses. This increase of Ca2+ in LEC leads to catastrophic events, which may terminate in to the cell death since decrease
in the cell density of lens epithelium in various types of human cataracts have indicated by our group earlier[33]. Role of Ca\(^{2+}\) in PSC is interesting because it is reported that removal of nuclei from the terminally differentiating LEC take place by diffoptosis as coined by Dahm[35]. Diffoptosis means terminally differentiated but not dead cells as indicated by Gupta et al[30,36] where they described that programmed cell death has two subsets terminal differentiation and apoptosis. These two processes follow common pathways up to certain steps but later they adopt different pathways, one lead to terminal differentiation and the other lead to apoptosis. PSC develops due to abnormal differentiation and posterior migration of LEC and loss of activity of Ca-channels and pumps of ER may results in PSC[37]. Steroids are shown to be associated with the mobilization of intracellular Ca\(^{2+}\) pool in other tissues[38]. It is also reported that use of steroids carries high risk of PSC can be explained by above hypothesis.

**Calcium and prevention of cataract** Many compounds are used to delay cataract formation in lens culture system and in various animal models where the development of cataract involves alteration of Ca\(^{2+}\) in the lens. Ca\(^{2+}\) channel blockers (varapamil, D600, etc) reduce extent of opacity in oxidative stress induced cataract[39,40]. Anti-calpain drugs such as E64, AK295, SJA6017, MDL 28170, etc are also shown to delay cataract formation in both *in vivo* and *in vitro* model[41]. TGase inhibitors are shown to be effective in the prevention of dimerisation of crystallins[42]. Many antioxidants such as disulfiram and inhibitors of reactive oxygen species generating enzymes such as amino-guanidine (nitric oxide synthatase inhibitor) works as anti-cataractogenic factors. These factors also indirectly prevent uptake of Ca\(^{2+}\) and subsequently prevents cataract development[43]. However, none of the above drug is effective in the clinical studies. There are many reasons for their failure or not suitable as therapeutic agents for the prevention of human cataracts. The most important reason for their failure is that most of the human cataracts have opacity in more than one region (mixed nature of cataract) and each one may have different etiologies. Above-mentioned drugs can prevent only cataracts, which are caused by the particular aetiology. It is observed that irrespective of type of cataract Ca\(^{2+}\) levels are always found high in the LEC (Fig 4). Therefore it can be hypothesised logically that if Ca\(^{2+}\) levels are monitored less than the threshold levels, no matter the aetiology of cataract the incidence of the disease can be prevented.

Posterior capsular opacification (PCO) is the most common complication of cataract surgery and it depends on the type of intraocular lens (IOL) material, structure and some other parameters[44]. PCO is formed due to extensive proliferation and migration of residual equatorial LEC on the posterior capsule. The occurrence of PCO in early stages causes loss in contrast sensitivity and visual acuity[45]. The way to treat PCO formation is by Nd-YAG laser capsulotomy. This technique has significant adverse medical, social and financial consequences. It is well known that increased level of Ca\(^{2+}\) is responsible for cell apoptosis. Therefore, any alteration in the channels and pumps regulating the intracellular Ca\(^{2+}\) store may lead to selective and effective induction of death in the residual LEC responsible for PCO. Recently the use of Ca\(^{2+}\) signalling aspect for the prevention of PCO is also suggested[46,47]. In experimental models thapsigargin (inhibitor of ER Ca-ATPase) coated intracellular lens was used for the prevention of PCO[46]; however its use in clinical application remains to be tested. Apoptosis induced by Ca\(^{2+}\) ionophore, calcimycin and T-type Ca\(^{2+}\) channel blocker, midefradil is also suggested for the prevention of PCO[48,49]. The improper delivery or diffusion of these agents out side the capsular bag may affect other surrounding tissues of eyes. Therefore the targeted delivery of these agents
inside the capsular bag is important. Recently Maloof et al[30] have invented a device (target drug delivery system), which can selectively apply certain agents to the residual LEC without harming other tissues of the eye.

Thus, there is a need to understand the role of LEC in maintaining the homeostasis of Ca\(^{2+}\) levels in order to prevent cataract, irrespective of its etiology. The published literatures supplemented by our studies are reviewed in this article. PCO, the major setback of cataract surgery can also be regulated very well by keeping Ca\(^{2+}\) levels high to induce apoptosis of residual LEC after the surgery.

REFERENCES


