©2004, Acta Pharmacologica Sinica
Chinese Pharmacological Society
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Causative and preventive action of calcium in cataractogenesis[1]

Purshottam Das GUPTA[2], Kaid JOHAR, Abhay VASAVADA

Iladevi Cataract and IOL Research Centre, Gurukul Road, Memnagar, Ahmedabad 380 052, India

KEY WORDS calcium; lens; cataract

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-

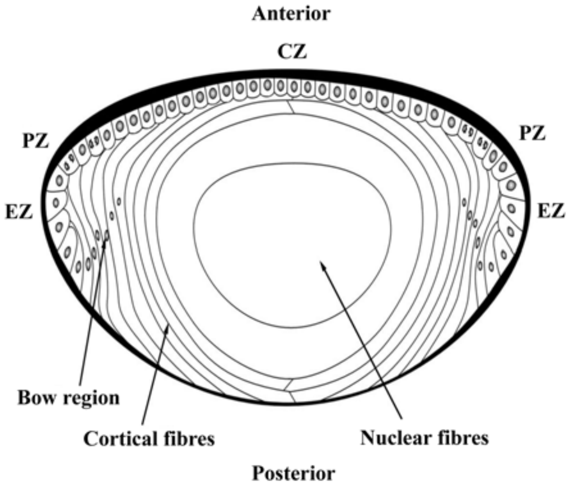

Fig 1. Schematic drawing of adult human lens. The anterior surface of the lens is lined by single layer of lens epithelial cells (LEC). Based on the location the lens epithelium is divided into central (CZ), pre-equatorial (PZ) and equatorial zone (EZ). Generally the cells of CZ are mitotically quiescent while cells of PZ are proliferative and produce new cells that migrate towards the EZ where they terminally differentiate to form fiber cells. Fiber cells constitute central major mass of the lens and they do not possess cell organelles and nucleus. The lens is enclosed in thick basement membrane called lens capsule.

---

[1] Supported by Council of Scientific and Industrial Research (CSIR), New Delhi, India. No 27(0114)/02/EMR-II.

[2] Correspondence to Purshottam Das GUPTA, PhD.
Phn 91-79-2749-0909/2749 2303. Fax 91-79-2741-1200.
E-mail pdg2000@hotmail.com

Received 2004-02-23   Accepted 2004-06-22

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].

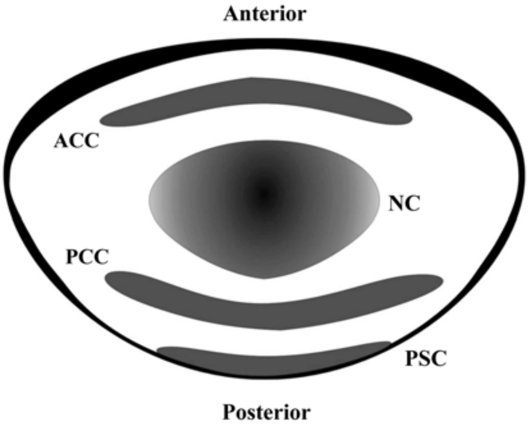

Fig 2. Schematic drawing of human lens showing various types of cataracts. Nuclear cataract (NC) is located in the centre of the lens and takes place due to slow oxidative changes which may be related with aging. Anterior and posterior cortical cataracts (ACC and PCC) are located in anterior and posterior cortical region of the lens and develop due to degeneration and liquefaction of cortical lens fibers. Posterior subcapsular cataract (PSC) is located just beneath the posterior capsule and takes place due to abnormal differentiation and migration of LEC.

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[3,4]. 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[5-7]. 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 ($InsP_3$) 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+} [5]. Influence of these receptor systems by their antagonists or agonists releases Ca^{2+} from ER through $InsP_3$ 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 Ca^{2+} sensing receptors (CaR) are identified which has opened up the possibility that Ca^{2+} 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 Ca^{2+} in the lens when the Ca^{2+} levels in the blood falls below normal and causes hypocalcaemic cataract[16].

The total Ca^{2+} in the LEC is in mmol/L range (0.1 mmol/L); however the free cytosolic Ca^{2+} is very low in μmol/L range (100-300 nm)[10]. Such a wide difference in the concentration of free and bound Ca^{2+} is maintained either by sequestration of Ca^{2+} in the ER including nuclear envelop, Golgi complex and mitochondria or by preferential binding of Ca^{2+} to complex protein molecules[17]. Major proteins of the lens, β, γ-crystallins act as a potential binding site of Ca^{2+} [18]. During the formation of cataract total lenticular Ca^{2+} increases beyond 20 mmol/L, however free cytosolic Ca^{2+} equilibrate with the aqueous humour (1 mmol/L). Therefore during the formation of cataract increasing Ca^{2+} must be converted into some non-diffusible or bound form[4]. This binding site of Ca^{2+} in cataractous lenses is quite specific and is different from those in the normal lenses. Most of Ca^{2+} in cataractous lenses is bound to water insoluble protein and such binding is very strong; Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} initiate cascade of events that lead to increase in light scattering[20].

ROLE OF CALCIUM IN CATARACTOGENESIS

Activation of Ca-dependent enzymes Calpains, the Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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[2,33]. 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]. Ca^{2+} also regulates the gap junction coupling in the LEC[6]. The observed cell death and uncoupling of LEC even in small areas leads to cell

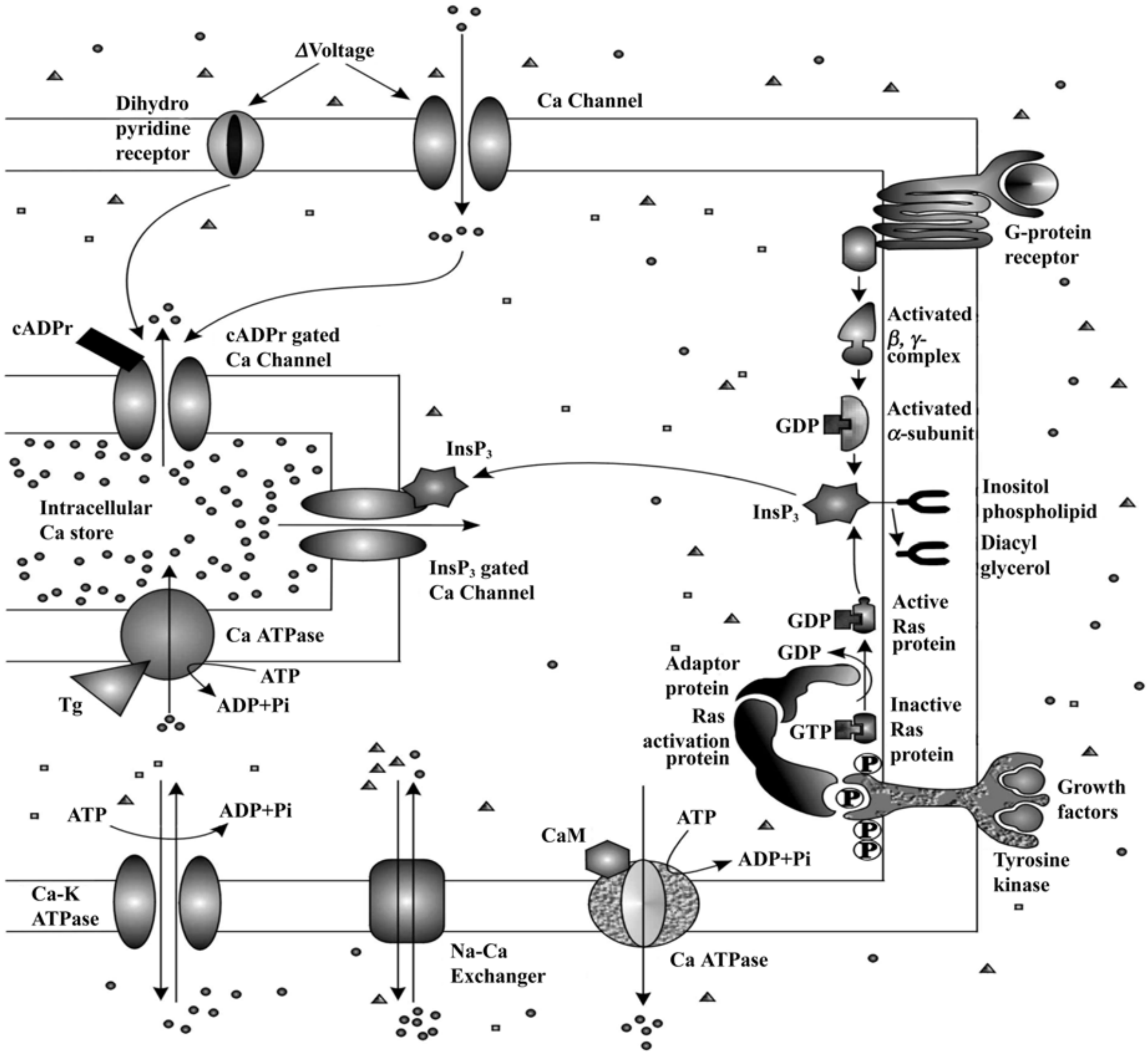

Fig 3. Regulation of free cytosolic calcium in the lens epithelial cells. Chief sources of cytosolic Ca^{2+} in the LEC are extracellular, aqueous humour and intracellular stores such as endoplasmic reticulum (ER) and mitochondria. Release of Ca^{2+} from the ER is an important event and takes place through $InsP_3$ and/or cADPr gated channels. $InsP_3$ gated Ca-channel releases Ca^{2+} upon the activation of G-protein or tyrosine kinase coupled receptor system while cADPr g; ated Ca-channel releases Ca^{2+} upon activation by voltage dependent system or dihydropyridine receptor system. (○: Ca^{2+}; △: Na^+; □: K^+; Tg: thapsigargin; CaM: calmodulin).

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 Ca^{2+} 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 Ca^{2+} in LEC leads to catastrophic events, which may terminate in to the cell death since decrease

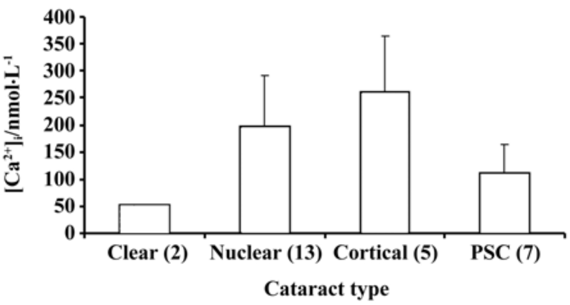

Fig 4. Total calcium in the central zone of lens epithelium obtained from different types of human cataracts. The central zone of lens epithelium from nuclear (NC), cortical (CC) and posterior subcapsular (PSC) cataract patients were obtained after cataract surgery. The central zone lens epithelium of clear lenses was obtained from cadaver eyes obtained from eye bank. After weighing, the samples were dried, digested using mixture of HNO_3 and $HClO_4$ and diluted with deionized distilled water. The total calcium in the solution was measured by inductive coupled plasma atomic emission spectrometry (ICP-AES). The data were expressed as mean±SD and number of samples of each cataract type was given in parenthesis.

in the cell density of lens epithelium in various types 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 aminoguanidine (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*[50] 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

1 Gupta PD, Kaid Johar SR, Rajkumar S, Dave M, Patel D, Raj S, Vasavada AR. Cataract: an old age disease. Ind J Gerontol 2003; 17: 306-24.

2 Kaid Johar SR, Rawal UM, Jain NK, Vasavada AR. Sequential effects of ultraviolet radiation on the histomorphology, cell density and antioxidative status of the lens epithelium – an *in vivo* study. Photochem Photobiol 2003; 78: 306-11.

3 Maraini G, Mangili R. Differences in proteins and in the water balance of the lens in nuclear and cortical types of senile cataract. Ciba Found Symp 1973; 19; 79-94.

4 Duncan G, Williams MR, Riach RA. Calcium, cell signalling and cataract. Prog Ret Eye Res 1994; 13: 623-52.

5 Duncan G, Wormstone IM. Calcium cell signalling and cataract: role of the endoplasmic reticulum. Eye 1999; 13: 480-3.

6 Churchill GC, Lurtz MM, Louis CF. Ca(2+) regulation of gap junctional coupling in lens epithelial cells. Am J Physiol Cell Phusiol 2001; 281: 972-81.

7 Yawata K, Nagata M, Narita A, Kawai Y. Effects of long-term acidification of extracellular pH on ATP-induced calcium mobilization in rabbit lens epithelial cells. Jpn J Physiol 2001; 51: 81-7.

8 Duncan G, Bushell AR. Ion analysis of human cataractous lenses. Exp Eye Res 1975; 20: 223-30.

9 Duncan G, Jacob TJC. Calcium and the physiology of cataract. In: Human Cataract Formation. Ciba Foundation Symposium 106. London: Academic Press; 1984. p 132- 52.

10 Duncan G, Webb SF, Dawson AP, Bootman MD, Elliott AJ. Calcium regulation in tissue-cultured human and bovine lens epithelial cells. Invest Ophthalmol Vis Sci 1993; 34: 2835-42.

11 Churchill GC, Louis CF. Imaging of intracellular calcium stores in single permeabilized lens cells. Am J Physiol 1999; 276: C426-34.

12 Reid TW. Growth control of cornea and lens epithelial cells. Prog Ret Eye Res 1994; 13: 507-54.

13 Candia OA, Zamudio AC, Polikoff LA, Alvarez LJ. Distribution of acetylcholine-sensitive currents around the rabbit crystalline lens. Exp Eye Res 2002; 74: 769-76.

14 Duncan G, Collison DJ. Role of the non-neuronal cholinergic system in the eye: a review. Life Sci 2003; 72: 2013-9.

15 Hofer AM, Brown EM. Extracellular calcium sensing and signalling. Nat Rev Mol Cell Biol 2003; 4: 530-8.

16 Brown EM, Chattopadhyay N, Vassilev PM, Hebert SC. The calcium-sensing receptor (CaR) permits Ca^{2+} to function as a versatile extracellular first messenger. Recent Prog Horm Res 1998; 53: 257-81.

17 Vrensen GFJM, de Wolf A. Calcium distribution in the human eye lens. Ophthalmic Res 1996; 28: 78-85.

18 Rajini B, Shridas P, Sundari CS, Muralidhar D, Chandani S, Thomas F, *et al.* Calcium binding properties of γ-crystallin. J Biol Chem 2001; 276: 38464-71.

19 Duncan G, van Heyningen R. Differences in the calcium binding capacity of normal and cataractous lenses. Doc Ophthalmol Proc Ser 1976; 9: 229-32.

20 Tang D, Borchman D, Yappert MC, Vrensen GF, Rasi V. Influence of age, diabetes and cataract on calcium, lipid-calcium and protein-calcium relationships in human lenses. Invest Ophthalmol Vis Sci 2003; 44: 2059-66.

21 Yoshida H, Murachi T, Tsukahara I. Distribution of calpain I, calpain II, and Calpastatin in bovine lens. Invest Ophthalmol Vis Sci 1985; 26: 953-95.

22 Ma H, Fukiage C, Kim YH, Duncan MK, Reed NA, Shih M, *et al*. Characterization and expression of calpain 10. J Biol Chem 2001; 276: 28525-31.

23 Sanderson J, Marcantonio JM, Duncan G. A human lens model of cortical cataract: Ca^{2+} induced protein loss, vimentin cleavage and opacification. Invest Ophthalmol Vis Sci 2000; 41: 2255-61.

24 Karlson J, Anderson M, Peterson A, Sjostrand J. Proteolysis in human lens epithelium determined by a cell-permeable substrate. Invest Ophthalmol Vis Sci 1999; 40: 261-4.

25 Qian W, Shichi H. Cataract formation by a semiquinone metabolite of acetaminophen in mice: possible involvement of Ca^{2+} and calpain activation. Exp Eye Res 2000; 71: 567-74.

26 Vijyalakshmi V, Gupta PD. Role of transglutaminase in keratinization of vaginal epithelial cells in oestrous cycling rats. Biochem Mol Biol Int 1997; 43: 1041-9.

27 Hidasi V, Muszbek L. Transglutaminase activity in normal human lenses and in senile cataracts. Ann Clin Lab Sci 1995; 25: 236-40.

28 Hidasi V, Adany R, Muszbek L. Localization of transglutaminase in human lenses. J Histochem Cytochem 1995; 43: 1173-7.

29 Shridas P, Sharma Y, Balasubramanian D. Transglutaminase-mediated cross-linking of α-crystallin: structural and functional consequences. FEBS Lett 2001; 499: 245-50.

30 Gupta PD, Pushkala K. Importance of the role of calcium in programmed cell death: a review. Cytobios 1999; 99: 83- 95.

31 Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, *et al*. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. J Cell Biol 1995; 130: 169-81.

32 Horocopos GJ, Alvares KM, Kolker AE, Beebe DC. Human age related cataract and lens epithelial cell death. Invest

Ophthalmol Vis Sci 1998; 39: 2696-706.

33 Vasavada AR, Cherian M, Yadav S, Rawal UM. Lens epithelial cell density and histomorphological study in cataractous lenses. J Cataract Refract Surg 1991; 17: 798-804.

34 Marcantonio JM. Calcium-induced disruption of the lens cytoskeleton. Ophthalmic Res 1996; 28 Suppl: 48-50.

35 Dahm R. Lens fibre differentiation-a link with apoptosis? Ophthalmic Res 1999; 31: 163-83.

36 Rao KS, Zanotti S, Reddy AG, Rauch F, Mannherz HG, Gupta PD. Oestradiol regulated programmed cell death in rat vagina: terminal differentiation or apoptosis? Cell Biol Int 1998; 105-13.

37 Wride MA. Cellular and molecular features of lens differentiation: a review of recent advances. Differentiation 1996; 61: 77-93.

38 Singh S, Gupta PD. Induction of phosphoinositide-mediated signal transduction pathway by 17-β-oestradiol in rat vaginal epithelial cells. J Mol Endocrinol 1997; 19: 249-57.

39 Walsh SP, Patterson JW. Effects of hydrogen peroxide oxidation and calcium channel blockers on the equatorial potassium current of frog lens. Exp Eye Res 1994; 58: 257-65.

40 Ettl A, Daxer A, Gottinger W, Schmid E. Inhibition of experimental diabetic cataract by topical administration of RS-verapamil hydrochloride. Br J Ophthalmol 2004; 88: 44-77.

41 Mathur P, Gupta SK, Wegener AR, Breipohl W, Ahrend MH, Sharma YD, *et al*. Comparison of various calpain inhibitors in reduction of light scattering, protein precipitation and nuclear cataract *in vitro*. Curr Eye Res 2000; 21: 926-33.

42. Lorand L, Stern AM, Velasco PT. Novel inhibitors against the transglutaminase-catalysed crosslinking of lens proteins. Exp Eye Res 1998; 66: 531-6.

43 Nabekura T, Koizumi Y, Nakao M, Tomohiro M, Inomata M, Ito Y. Delay of cataract development in hereditary cataract UPL rats by disulfiram and aminoguanidine. Exp Eye Res 2003; 76: 169-74.

44 Wejde G, Kugelberg M, Zetterstorm C. Posterior capsule opacification: comparision of 3 intraocular lenses of different materials and design. J Cataract Refract Surg 2003; 29: 1556-9.

45 Meacock WR, Spalton DJ, Royce J, Marshall J. The effect of posterior capsule opacification on visual function. Invest Ophthalmol Vis Sci 2003; 44: 4665-9.

46 Duncan G, Wormstone M, Liu CS, Marcantonio JM, Davies PD. Thapsigargin-coated intracellular lenses inhibit human lens cell growth. Nat Med 1997; 3: 1026-8.

47 Collison DJ, Wang L, Wormstone IM, Duncan G. Spatial characteristics of receptor-induced calcium signalling in human lens capsular bags. Invest Ophthalmol Vis Sci 2004; 45: 200-5.

48 Geissler FT, Li DW, James ER. Inhibition of lens epithelial cell growth by induction of apoptosis: potential for prevention of posterior capsule opacification. J Ocul Pharmacol Ther 2001; 17: 587-96.

49 Beck R, Nebe B, Guthoff R, Rychly J. Inhibition of lens epithelial cell adhesion by the calcium antagonist Mibefradil correlates with impaired integrin distribution and organization of the cytoskeleton. Graefes Arch Clin Exp Opthalmol 2001; 239: 452-8.

50 Maloof A, Neilson G, Milverton EJ, Pandey SK. Selective and specific targeting of lens epithelial cells during cataract surgery using sealed-capsule irrigation. J Cataract Refract Surg 2003; 29: 1566-8.