Journal of Clinical Gerontology & Geriatrics 1 (2010) 17–21

Contents lists available at ScienceDirect

Journal of Clinical Gerontology & Geriatrics

journal homepage: www.e-jcgg.com

Review article

Senile cataracts and oxidative stress

Min-Chieh Ho, MD [a], Yi-Jie Peng, MD [a], Shih-Jen Chen, MD, PhD [b,c], Shih-Hwa Chiou, MD, PhD [b,c,d,*]

[a] Department of Ophthalmology, Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, Taiwan
[b] Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan
[c] Institute of Clinical Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan
[d] Department of Medical Research and Education, Taipei Veterans General Hospital, No. 201, Section 2, Shih-Pai Road, Taipei 11217, Taiwan

ARTICLE INFO

Article history:
Received 5 February 2010
Received in revised form
27 July 2010
Accepted 29 July 2010

Keywords:
Antioxidant enzymes
Cataracts
Glutathione
Oxidative stress

ABSTRACT

In numerous epidemiological and animal models, it can be inferred that oxidative stress is a key factor in cataract formation. Production of reactive oxygen species and reduction of endogenous antioxidants both contribute to cataract formation. In the cataractogenous process, lens proteins lose sulfhydryl groups and become thiolated or cross-linked by disulfide bonds. The resultant high molecular weight aggregates become insoluble and affect lens transparency. All these are consequences of changes in the redox state. A mixed protein-thiol and protein-protein disulfide bond precedes the morphological changes of cataract. Normally, sustained high levels of reduced glutathione provide a protective effect, while depletion of glutathione causes damage to epithelial cells and fiber cells. UV rays in the ambient environment evoke reactive oxygen species formation and also contribute to cataracts. The reduction in free UV filters and increase in their binding to lens proteins make the lens more predisposed to UV damage and oxidation. In the aqueous humor of cataract lenses, there is a decrease in antioxidant enzymes and increase in nitric oxide, which demonstrates the relationship between oxidative stress and cataracts. Though surgical intervention is the standard treatment for cataracts, experimental medical therapies for cataracts are under extensive investigation. Carnosine, a pro-drug of carnosine-N-acetylcarnosine, bendazac, ascorbic acid, and aldose reductase inhibitors are under therapeutic evaluation, and prevention of cataract formation may be possible in the future.

1. Introduction

Cataract (lens opacification) is a major contributing factor of blindness. An estimate from the World Health Organization is that the current global prevalence of blindness is 0.57% (range, 0.2–1.0%), with more than 82% of all blindness occurring in individuals aged 50 and older. Cataract accounts for 47.8% of the roughly 37 million blind people in the world.[1] While the main treatment for cataract is surgical intervention,[2] it is associated with certain risks and subsequent suboptimal outcomes. Endophthalmitis is the most devastating complication following cataract surgery and usually results in severe vision impairment. Several studies have demonstrated that pseudophakic patients have a fourfold cumulative risk of retinal detachment for up to 20 years after cataract surgery.[3] The incidence of pseudophakic posterior capsular opacification remains high,[4] while laser capsulotomy is associated with a 3.9-fold increased risk of retinal detachment.[5]

Cataractogenesis is influenced by multiple risk factors, such as aging, diabetes mellitus, drugs, trauma, toxins, genetics and other ocular diseases.[6–8] Among the various causes, oxidative stress is considered to play a key role in the molecular mechanism of cataract formation.[9–11] In this article, we review the current understanding of oxidative stress in the process of cataract formation and examine the correlation between the proposed mechanisms and prospective therapeutic agents for cataract prevention.

2. Oxidative stress and cataract formation

The main composition of the lens is protein, also known as crystalline, that is generated from migrating fiber cells from anterior cubodial epithelia during embryogenesis. During migration, nuclei and mitochondria are lost and render the fiber cells susceptible to damage because of the absence of turnover process.[12] There is also simultaneous loss of repair systems of the mitochondria that can restore the oxidative damage by utilizing glutathione (GSH), thioredoxin (Trx), NADPH and FADH2 as electron donors, and maintain the proteins in stable redox status.

* Corresponding author. Department of Medical Research and Education, Taipei Veterans General Hospital, No. 201, Section 2, Shih-Pai Road, Taipei 11217, Taiwan.
E-mail address: shchiou@vghtpe.gov.tw (S.-H. Chiou).

2210-8335
doi:10.1016/j.jcgg.2010.10.006

It is generally accepted that oxidation is a key feature of cataract formation. Free radicals, including numerous reactive oxygen species (ROS) such as superoxide anion radical ($\cdot O_2^-$), H_2O_2, and hydroxyl free radical ($\cdot OH$), may lead to structural damage of the crystalline lens and contribute to cataract formation. ROS may be generated exogenously after UV light and ionizing radiation exposure, or endogenously as a result of normal metabolism through the enzymatic reaction of lipoxygenases, cytochrome P450, NADPH, and mitochondrial electron transport in various cellular compartments (mitochondria, peroxisomes, cytoplasm). Oxidative stress occurs when the level of pro-oxidants surpass the level of antioxidants. Thus, the level of ROS should be finely regulated, otherwise damage to the mitochondria and overproduction of ROS may occur.

Antioxidant enzymes work with reducing systems and protein repair systems to protect against ROS-induced damage. In the mitochondria matrix, MnSOD (SOD2) converts the superoxide anion, which is generated by the electron transport chain, into hydrogen peroxide. By regulating the level of SOD2, lens epithelial cells can overcome oxidative stress.[13] CuZnSOD (SOD1), present in the mitochondria intermembrane space, also effectively transforms superoxide anion to hydrogen peroxide in a similar manner.[14] Elevated levels of SOD1 protects the lens from H_2O_2-mediated damage.[15]

Hydrogen peroxide is a relatively stable ROS species with moderate destructive potency. Enzymes including catalase (CAT), GSH peroxidase and the peroxiredoxins cooperate to reduce the potential for damage. Among ROS, H_2O_2 is the most stable oxygen species present in the aqueous humor. H_2O_2 can also diffuse into the interior lens. Lenticular epithelial cells have antioxidant defences (including CAT, GSH, and GSH peroxidase), which are capable of removing aqueous-derived H_2O_2. The normal concentration of H_2O_2 in aqueous humor and lens is 25–30 μM, but there can be a threefold increase in cataract patients.[16,17] RO S-induced destruction at different targets within the crystalline lens, such as proteins or lipids, is believed to underlie the pathogenesis of cataracts.[18]

GSH is abundant in the lens and can be synthesized by the lens.[19] The GSH repair system consists of reduced GSH and its oxidized form (GSSG). GSH is maintained in its reduced form rather than its oxidized form by GSH reductase enzymes in the presence of NADPH, and the formation of mixed disulfide is prevented. Reduced GSH acts as an electron donor for GSH peroxidase while reducing H_2O_2 to H_2O and O_2.[20] The level of oxidized GSH rises significantly once cataract develops. In experimentally-induced cataract, decrease in GSH level is also a typical finding. Furthermore, studies have shown that reduced GSH prevents protein sulfhydryl groups from intramolecular and intermolecular disulfide cross-linking. Therefore, GSH can reduce high molecular weight protein aggregation in the lens and prevent subsequent light scattering and cataract deterioration. Hence, GSH is considered to be a crucial factor involved in cataract formation. The protective effect of GSH is considered to be concentration-dependent. GSH above 1 mM effectively inhibits hydroxyl radical formation, whereas a concentration below 1 mM accelerates its production.[21,22]

Trx, another electron donor, is a small thiol protein with active-site dithiol, which reduces protein disulfide actively. Oxidized Trx is further reduced by Trx reductase in a NADPH-dependent manner. The production of NADPH increases under oxidative stress, making it important in redox control.[23]

To correlate these biochemical facts to lens morphology, the lens barrier may be of most importance. The barrier at the cortex/nucleus interface impedes the flow of molecules such as antioxidants into the nucleus and thus predisposes the lens center to oxidative damage. Furthermore, the unstable molecules stay much longer in the central zone and cause more structural oxidative damage in the central lens.[24,25]

Many antioxidants, such as vitamins C and E and the carotenoids, are considered to work as ROS scavengers. In animal models, antioxidant levels have been found to correlate with cataract progression. However, studies on the protective effects of antioxidant supplementation in human lens have been limited by few participants, high dropout, coeffectiveness of previous nutritional supplements, and dietary differences.

3. Photooxidative stress

The lens that works together with the cornea to focus radiation on the retina is constantly exposed to ambient radiation including UVA, UVB, and visible light, which lead to photochemical insult to the eye. As already mentioned, there is no turnover of proteins in the lens throughout life, so the lens is more vulnerable to photochemical insult. The differences in cataract risk in epidemiological assessments, particularly in geographic studies, indicate an association between cataract and UV light exposure.

3-Hydroxykynurenine (3OHKyn), present as a UV filter in the human lens, reacts with lens proteins and affects lens coloration.[26] After continual exposure to 300–400 nm UV light, loss of 3-hydroxykynurenine glucoside (3HKG, glucosided form of 3OHKyn) and subsequent yellowing of the lens is noted. Other compounds of 3OHKyn, such as oxidized xanthurenic acid and xanthommatin, are also potentially cataractogenic molecules. In the lens compartment where the antioxidant (e.g., GSH) is depleted, 3OHKyn can act alternatively as an antioxidant.[27] Since the attachment of 3OHKyn to lens proteins results in lens coloration, increased photon and UV light absorption may be a potential causative cofactor in cataract development.

α-Crystallin, as a major protein family in the lens, is responsible for the maintenance of lens transparency. It has been demonstrated that exposure to UV induces cross-linking of α-, β- and γ-crystallins, which lead to conformational and solubility change, resulting in light scattering in cataracts. Nevertheless, the α-crystallins themselves can act as chaperones against photodamage by UV irradiation.[28] The lens epithelium also has anti-photooxidative enzymes, which prevent the lens from being damaged by photooxidation induced by UV.[29] The human lens membrane contains high levels of cholesterol, plasmalogen, and dihydrosphingomyelin; UVB irradiation induces peroxidation of membrane lipids, and leads to formation of hydroxyl and hydroperoxyl lipids. Membrane repair can be compromised by reduction of protein synthesis after UV irradiation.[16,30]

4. Non-enzymatic glycation

Non-enzymatic glycation is a condensation reaction between amino groups in proteins and reducing sugars. The glycated product, known as early glycation product, may undergo further reaction by oxidative or non-oxidative pathway. Under oxidative conditions, the early glycation product does not undergo further reaction. Under non-oxidative conditions, the early glycation product can react with many amino groups to give rise to brown and cross-linked products called advanced glycation end products.[31–33]

The lens derives 80% of its metabolic energy from anaerobic glycosis, with the remaining 15% and 5% from the hexose monophosphate shunt and Krebs cycle, respectively. It is hypothesized that increased intracellular glucose may overflow the glycolysis pathway, resulting in activation of aldose reductase. Thereafter, the cellular osmotic pressure increase and influx of water into the cell membrane leads to lens swelling and opacification.[34] In vitro, glycation partially unfolds lens proteins and exposes buried sulfhydryls, leading to disulfide formation and high molecular weight aggregation. Further, cortical proteins are more susceptible to

glycation than are nuclear proteins.[35–37] Another consequence of partial unfolding is exposure of hydrophobic groups, which may promote hydrophobic interaction. This mechanism may also contribute to high molecular weight aggregation and eventual protein insolubilization. In addition to the lens protein, glycation also influences the lenticular membrane. Cross-linking of the intrinsic proteins in the membrane affects membrane rigidity and permeability, and may also lead to membrane opacity.[38]

5. Parameters in aqueous humor

Clinically, epidemiological surveys provide an idea of the potential risk factors of cataract, while animal models and in vitro studies make the hypothesis and mechanism more convincing. Some studies on oxidative stress markers in aqueous humor have been proposed to correlate the aqueous humor micro-condition with cataractogenesis in the human lens. ROS is difficult to detect because of its instability and rapid reactions; hence, measurement of antioxidant enzymes in aqueous humor is a reliable surrogate.

Among these, superoxide dismutase (SOD), a major antioxidant enzyme, which decomposes superoxide into hydrogen peroxide, and CAT, which breaks down hydrogen peroxide into water, are the two major enzymes discussed. These antioxidant enzymes in the aqueous humor protect the lens protein from ROS-induced damage.[39] SOD activities in aqueous humor was typically low in previous reports, but Sawada et al. found that in more advanced cataract, there was a significant increase in the level of SOD activity and total protein in aqueous humor.[40] The increase was proportional to cataract severity but not to patient age. They mainly detected the activities of SOD1 and SOD2 in cells from the cornea, ciliary epithelium and lens, not extracellular SOD. It may be inferred that advanced cataract is associated with molecules leaking through the lens capsule.

Nitric oxide (NO), generated by NO synthases, and known as a detrimental free radical, has been found to regulate cell metabolism and to play an important role in the aging process. Non-enzymatic nitration of α-crystallin in the cataractous lens matrix may be a mechanism in lens protein damage.[41–44] Furthermore, different nitrogen oxide species, including peroxynitrite, are highly reactive species that can oxidize proteins and non-protein sulfhydryl groups. Significantly higher levels of NO in the aqueous humor of patients with mature cataract compared to patients with cortical, nuclear or posterior capsular cataract have been found. In addition, NO levels in the aqueous humor increase with age and in patients with age-related cataract.[45] These oxidative stress markers in the aqueous humor reveal a possible relationship with cataract. However, the precise mechanism is not fully understood. In the future, control of NO formation may be an alternative way to prevent cataract progression.

6. Therapeutic agents

Quinax has been widely used for prophylaxis of cataract formation. Oxidative stress, as the key to cataract formation, is related to a cascade of biochemical reactions as described above. Many enzymes and oxidative agents are involved in cataractogenesis. Once the pathway is blocked, the progression of cataract formation may be controlled. Several medications are under investigation for cataract prevention or treatment.

6.1. Lipid peroxidation and carnosine

Lipid peroxidation (LPO), as an endogenous source of oxidative injury, leads to formation of reactive oxygen radicals and high molecular weight protein aggregation of low solubility.[46,47] A potential treatment strategy is to prevent activation of LPO and accumulation of damaging LPO products by exogenously administered antioxidants with a strong affinity to LPO. Carnosine was found to be the most potent lipid peroxidase mimetic with a powerful antioxidant property that can protect cell membranes from oxidative damage.[48]

The ophthalmic pro-drug carnosine-N-acetylcarnosine (NAC) has recently been developed for clinical use.[49] Babizhayev et al. found that after 6 months of NAC treatment, 96% of the treated eyes showed improvement in lens clarity as based on slit-lamp images and retroillumination photographs. Significantly, NAC was found to be able to reverse lens opacity in canine eyes, and a “melting snow” phenomenon was described.[46] Other studies also showed improvements in best-corrected visual acuity or lens opacity. Nevertheless, there remain some barriers to widespread adoption of this experimental therapy, such as the small number of participants, high dropout rate, too little progression to show convincing worsening of untreated cataracts, and insufficient baseline measurements to compare the efficacy of NAC. A larger trial is needed to justify the benefit of long-term NAC therapy.[50]

6.2. Irradiation cataract and bendazac

Bendazac has been demonstrated to have a protective effect against lens protein denaturation both in vitro and in vivo.[51] Pandolfo et al. found that bendazac was especially effective in protecting against X-ray-induced cataracts in the rabbit lens with preservation of the antioxidant enzyme system.[52] In addition, bendazac’s main metabolite, 5-hydroxybendazac, was found to be an effective hydroxyl radical scavenger.[53] Unfortunately, the reliability of these results on bendazac is limited because the present conclusions are based on studies of subjective criteria, high dropout rate, and small participant numbers. The results were concluded to be ambiguous.

6.3. Ascorbic acid

In the lens, ascorbic acid acts as a reductant and a free radical scavenger. Ascorbic acid was reported to be capable of preventing photooxidation-induced protein cross-linking.[54] Likewise, aspirin works as an acetylating agent, which prevents protein denaturation within the lens.[55] Even if ascorbic acid is proven to be beneficial in cataract prevention in case-controlled studies, other retrospective studies have not arrived at a favorable conclusion.[56–59]

6.4. Diabetic cataract and aldose reductase inhibitors

Aldose reductase is the key enzyme in the polyol pathway. Some drugs have been designed as aldose reductase inhibitors to prevent cataracts. Sorbinil, an experimental aldose reductase inhibitor, reduces polyol production and prevents NADPH from being oxidized. Sorbinil is also capable of maintaining GSH in the reduced state in experimental cataracts. However, sorbinil showed little beneficial effect on cataract formation and many studies were stopped due to adverse effects.[60,61]

7. Conclusion

The crystalline lens is constantly subjected to oxidative stress from free radicals, which can potentially lead to lens protein damage. The lens has several protective mechanisms such as the GSH system and Trx, which work as repair systems to protect the lens protein from oxidation and further cataractogenous

changes. The relationship between UV light and cataract was first discovered by epidemiological studies, while further surveys showed that 3OHKyn is a crucial mediator of UV light absorption. UV light-induced cross-linking of lens proteins also contributes to UV-related cataracts. Research is revealing that the levels of CAT, NO and proteins in the aqueous humor may be correlated with cataractogenesis in the human lens. Further investigation of cataractogenesis is of clinical value and may lead to the development of potential medical therapy as an alternative to surgery.

References

1. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, et al. Global data on visual impairment in the year 2002. *Bull World Health Organ* 2004;**82**:844–51.
2. Vision Research. *A National Plan 1990–2002. Report of the National Advisory Council*. Washington, DC: US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Eye Institute; 1998. pp. 59.
3. Erie JC, Raecker MA, Baratz KH, Schleck CD, Burke JP, Robertson DM. Risk of retinal detachment after cataract extraction, 1980–2004: a population-based study. *Ophthalmology* 2006;**113**:2026–32.
4. Cheng JW, Wei RL, Cai JP, Xi GL, Zhu H, Li Y, et al. Efficacy of different intraocular lens materials and optic edge designs in preventing posterior capsular opacification: a meta-analysis. *Am J Ophthalmol* 2007;**143**:428–36.
5. Javitt JC, Tielsch JM, Canner JK, Kolb MM, Sommer A, Steinberg EP. National outcomes of cataract extraction. Increased risk of retinal complications associated with Nd:YAG laser capsulotomy. The Cataract Patient Outcomes Research Team. *Ophthalmology* 1992;**99**:1487–97.
6. Tsai SY, Hsu WM, Cheng CY, Liu JH, Chou P. Epidemiologic study of age-related cataracts among an elderly Chinese population in Shih-Pai, Taiwan. *Ophthalmology* 2003;**110**:1089–95.
7. West SK, Valmadrid CT. Epidemiology of risk factor for age related cataract. *Surv Ophthalmol* 1995;**39**:323–34.
8. Abraham AG, Condon NC, Gower EW. The new epidemiology of cataract. *Ophthalmol Clin North Am* 2006;**19**:415–25.
9. Truscott RJ. Age-related nuclear cataract—oxidation is the key. *Exp Eye Res* 2005;**80**:709–25.
10. Shui YB, Fu JJ, Garcia C, Dattilo LK, Rajagopal R, McMillan S, et al. Oxygen distribution in the rabbit eye and oxygen consumption by the lens. *Invest Ophthalmol Vis Sci* 2006;**47**:1571–80.
11. Wu SY, Leske MC. Antioxidants and cataract formation: a summary review. *Int Ophthalmol Clin* 2002;**40**:71–81.
12. Brennan LA, Kantorow M. Mitochondrial function and redox control in the aging eye: role of MsrA and other repair systems in cataract and macular degenerations. *Exp Eye Res* 2009;**88**:195–203.
13. Matsui H, Lin LR, Ho YS, Reddy VN. The effect of up- and downregulation of MnSOD enzyme on oxidative stress in human lens epithelial cells. *Invest Ophthalmol Vis Sci* 2003;**44**:3467–75.
14. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B, Orrenius S. Cytochrome c release from mitochondria proceeds by a two-step process. *Proc Natl Acad Sci U. S. A.* 2002;**99**:1259–63.
15. Lin D, Barnett M, Grauer L, Robben J, Jewell A, Takemoto L, et al. Expression of superoxide dismutase in whole lens prevents cataract formation. *Mol Vis* 2005;**11**:853–8.
16. Spector A, Garner WH. Hydrogen peroxide and human cataract. *Exp Eye Res* 1981;**33**:673–81.
17. Giblin FJ, McCready JP, Kodama T, Reddy VN. A direct correlation between the levels of ascorbic acid and H_2O_2 in aqueous humor. *Exp Eye Res* 1984;**38**: 87–93.
18. Beal MF. Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol* 2005;**58**:495–505.
19. Kinoshita JH. Selected topics in ophthalmic biochemistry. *Arch Ophthalmol* 1964;**72**:554–72.
20. Halliwell B, Gutteridge JM. The antioxidants of human extracellular fluids. *Arch Biochem Biophys* 1990;**280**:1–8.
21. David LL, Shearer TR. State of sulfhydryl in selenite cataract. *Toxicol Appl Pharmacol* 1984;**74**:109–15.
22. Calvin HI, Medvedovsky C, Worgul BV. Near-total glutathione depletion and age-specific cataracts induced by buthionine sulfoximine in mice. *Science* 1986;**233**:553–5.
23. Giblin FJ, Nies DE, Reddy VN. Stimulation of the hexose monophosphate shunt in rabbit lens in response to the oxidation of glutathione. *Exp Eye Res* 1981;**33**:289–98.
24. Sweeney MH, Truscott RJ. An impediment to glutathione diffusion in older normal human lenses: a possible precondition for nuclear cataract. *Exp Eye Res* 1998;**67**:587–95.
25. Moffat BA, Landman KA, Truscott RJ, Sweeney MH, Pope JM. Age-related changes in the kinetics of water transport in normal human lenses. *Exp Eye Res* 1999;**69**:663–9.
26. Stutchbury GM, Truscott RJ. The modification of proteins by 3-hydroxykynurenine. *Exp Eye Res* 1993;**57**:149–55.
27. Truscott RJ, Augusteyn RC. Oxidative changes in human lens proteins during senile nuclear cataract formation. *Biochim Biophys Acta* 1977;**492**: 43–52.
28. Borkman RF, McLaughlin J. The molecular chaperone function of alpha-crystallin is impaired by UV photolysis. *Photochem Photobiol* 1995;**62**: 1046–51.
29. Reddan JR, Giblin FJ, Dziedzic DC, McCready JP, Schrimscher L, Reddy VN. Influence of the activity of glutathione reductase on the response of cultured lens epithelial cells from young and old rabbits to hydrogen peroxide. *Exp Eye Res* 1988;**46**:209–21.
30. Hightower KR, McCready JP, Borchman D. Membrane damage in UV-irradiated lenses. *Photochem Photobiol* 1994;**59**:485–90.
31. Holmquist WR, Schroeder WA. A new N-terminal blocking group involving a Schiff base in hemoglobin AIc. *Biochemistry* 1966;**5**:2489–503.
32. Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. N epsilon-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. *Biochemistry* 1995;**34**:10872–8.
33. Monnier VM, Cerami A. Nonenzymatic browning in vivo: possible process for aging of long-lived proteins. *Science* 1981;**211**:491–3.
34. Kador PF, Kinoshita JH. Diabetic and galactosaemic cataracts. *Ciba Found Symp* 1984;**106**:110–31.
35. Stevens VJ, Rouzer CA, Monnier VM, Cerami A. Diabetic cataract formation: potential role of glycosylation of lens crystallins. *Proc Natl Acad Sci U. S. A.* 1978;**75**:2918–22.
36. Lee JH, Shin DH, Lupovitch A, Shi DX. Glycosylation of lens proteins in senile cataract and diabetes mellitus. *Biochem Biophys Res Commun* 1984;**123**:888–93.
37. Liang JN, Hershorin LL, Chylack Jr LT. Non-enzymatic glycosylation in human diabetic lens crystallins. *Diabetologia* 1986;**29**:225–8.
38. Liang JN, Rossi MT. In vitro non-enzymatic glycation and formation of browning products in the bovine lens alpha-crystallin. *Exp Eye Res* 1990;**50**:367–71.
39. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;**39**:44–84.
40. Sawada H, Fukuchi T, Abe H. Oxidative stress markers in aqueous humor of patients with senile cataracts. *Curr Eye Res* 2009;**34**:36–41.
41. Reckelhoff JF, Kellum JA, Blanchard EJ, Bacon EE, Wesley AJ, Kruckeberg WC. Changes in nitric oxide precursor, L-arginine, and metabolites, nitrate and nitrite, with aging. *Life Sci* 1994;**55**:1895–902.
42. Sato I, Kaji K, Murota S. Age related decline in cytokine induced nitric oxide synthase activation and apoptosis in cultured endothelial cells: minimal involvement of nitric oxide in the apoptosis. *Mech Ageing Dev* 1995;**81**:27–36.
43. McCann SM, Licinio J, Wong ML, Yu WH, Karanth S, Rettorri V. The nitric oxide hypothesis of aging. *Exp Gerontol* 1998;**33**:813–26.
44. Paik DC, Dillon J. The nitrite/alpha crystallin reaction: a possible mechanism in lens matrix damage. *Exp Eye Res* 2000;**70**:73–80.
45. Kao CL, Chou CK, Tsai DC, Hsu WM, Liu JH, Wang CS, et al. Nitric oxide levels in the aqueous humor in cataract patients. *J Cataract Refract Surg* 2002;**28**: 507–12.
46. Babizhayev MA, Deyev AI, Yermakova VN, Brikman IV, Bours J. Lipid peroxidation and cataracts: N-acetylcarnosine as a therapeutic tool to manage age-related cataracts in human and in canine eyes. *Drugs R D* 2004;**5**:125–39.
47. Cotlier E, Obara Y, Toftness B. Cholesterol and phospholipids in protein fractions of human lens and senile cataract. *Biochim Biophys Acta* 1978;**530**: 267–78.
48. Babizhayev MA, Seguin MC, Gueyne J, Evstigneeva RP, Ageyeva EA, Zheltukhina GA. L-carnosine (beta-alanyl-L-histidine) and carcinine (beta-alanylhistamine) act as natural antioxidants with hydroxyl-radical-scavenging and lipid-peroxidase activities. *Biochem J* 1994;**304**:509–16.
49. Babizhayev MA, Deyev AI, Yermakova VN, Semiletov YA, Davydova NG, Doroshenko VS, et al. Efficacy of N-acetylcarnosine in the treatment of cataracts. *Drugs R D* 2002;**3**:87–103.
50. Toh T, Morton J, Coxon J, Elder MJ. Medical treatment of cataract. *Clin Exp Ophthalmol* 2007;**35**:664–71.
51. Silvestrini B, Catanese B, Barillari G, Iorio E, Valeri P. Basic data supporting the use of the l-lysine salt of bendazac in cataract. *Int J Tissue React* 1983;**5**: 217–25.
52. Pandolfo L, Livrea MA, Bono A. Effects of bendazac L-lysine salt on X-ray-induced cataract in the rabbit lens. *Exp Eye Res* 1986;**42**:167–75.
53. Guglielmotti A, Capezzone De Joannon A, Cazzolla N, Marchetti M, Soldo L, Cavallo G, et al. Radical scavenger activity of bendazac, an anticataract non-steroidal anti-inflammatory agent. *Pharmacol Res* 1995;**32**:369–73.
54. Varma SD, Morris SM, Bauer SA, Koppenol WH. In vitro damage to rat lens by xanthine-xanthine oxidase: protection by ascorbate. *Exp Eye Res* 1986;**43**:1067–76.
55. Blakytny R, Harding JJ. Prevention of cataract in diabetic rats by aspirin, paracetamol (acetaminophen) and ibuprofen. *Exp Eye Res* 1992;**54**:509–18.
56. van Heyningen R, Harding JJ. A case-control study of cataract in Oxfordshire: some risk factors. *Br J Ophthalmol* 1988;**72**:804–8.
57. Mohan M, Sperduto RD, Angra SK, Milton RC, Mathur RL, Underwood BA, et al. India-US case-control study of age-related cataracts. India-US Case-Control Study Group. *Arch Ophthalmol* 1989;**107**:670–6.

58. West SK, Muñoz BE, Newland HS, Emmett EA, Taylor HR. Lack of evidence for aspirin use and prevention of cataracts. *Arch Ophthalmol* 1987;**105**:1229–31.
59. Paganini-Hill A, Chao A, Ross RK, Henderson BE. Aspirin use and chronic diseases: a cohort study of the elderly. *BMJ* 1989;**299**:1247–50.
60. Suzen S, Buyukbingol E. Recent studies of aldose reductase enzyme inhibition for diabetic complications. *Curr Med Chem* 2003;**10**:1329–52.
61. Hotta N, Kakuta H, Ando F, Sakamoto N. Current progress in clinical trials of aldose reductase inhibitors in Japan. *Exp Eye Res* 1990;**50**:625–8.