

Review

Pharmacological prevention of diabetic cataract

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Abstract

Cataract — opacification of the lens — is closely related to diabetes as one of its major late complications. This review deals with three molecular mechanisms that may be involved in the development of diabetic cataract: nonenzymatic glycation of eye lens proteins, oxidative stress, and activated polyol pathway in glucose disposition. Implications resulting from these mechanisms for possible pharmacological interventions to prevent diabetic cataract are discussed. The article reviews research on potential anticataract agents, including glycation inhibitors, antioxidants, and aldose reductase inhibitors. Information on possible benefits of putative anticataract agents comes from a variety of approaches, ranging from laboratory experiments, both *in vitro* and *in vivo*, to epidemiological studies in patients.

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Keywords: Diabetic cataract; Oxidative stress; Nonenzymatic glycation; Polyol pathway

1. Introduction

Cataract, the opacification of the lens of the eye, is the leading cause of blindness worldwide — it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28000 new cases are reported daily worldwide. Approximately 25% of the population over 65 and about 50% over 80 have serious loss of vision because of cataract. In the UK, half of the patients put on waiting lists for operation will die before getting surgery (Minassian et al., 2000). In the United States, over 1.3 million cataract operations are performed annually at a cost of US\$3.5 billion. In developing countries, there is simply no sufficient number of surgeons to perform cataract operations. Besides possible complications, an artificial lens just does not have the overall optical qualities of a normal lens (Spector, 2000). This is the reason for highly required biochemical solutions or pharmacological intervention that will maintain the transparency of the lens; it is estimated that a delay in cataract formation of about 10 years would reduce the prevalence of visually disabling cataract by

about 45% (Kupfer, 1984). Such a delay would enhance the quality of life for much of the world's older and diabetic population and substantially diminish both the economic burden due to disability and surgery related to cataract.

Cataractogenesis is one of the earliest secondary complications of diabetes mellitus, a severe metabolic disorder characterized by hyperglycemia. Since extracellular glucose diffuses into the lens uncontrolled by the hormone insulin, the lens is one of the body parts most affected in diabetes mellitus. The proteins of the lens are extremely long-lived, and there is virtually no protein turnover that provides great opportunities for posttranslational modification to occur.

Multiple mechanisms have been implicated in the development of cataract in diabetes. To date, the exact sequence of events that leads to opacification has not been clearly defined. Thus, the relationship of the opacity to the initiating event may be obscure. What are the molecular changes that are responsible for increasing level of lens turbidity? How may we arrest these changes? A further problem is that the appearance of opacity in model systems rarely duplicates the cataracts observed in humans.

This review deals with three molecular mechanisms that may be involved in the development of diabetic cataract: nonenzymatic glycation of eye lens proteins, oxidative stress, and activated polyol pathway in glucose disposition.

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Implications that result from these mechanisms for possible pharmacological interventions to prevent diabetic cataract are discussed because we believe that cataract is a disease that requires a biochemical and pharmacological, rather than a surgical, solution. First, tight metabolic control remains the milestone intervention in the prevention of lens opacification. However, pharmacological blockade of biochemical events triggered by disposal of excess glucose could be required. The article reviews research on potential anticataract agents, including glycation inhibitors, antioxidants, and aldose reductase inhibitors (ARIs). A variety of approaches, from laboratory experiments, both in vitro and in vivo, to epidemiological studies in patients, have yielded information on possible benefits of putative anticataract agents.

2. Anatomy and physiology of eye lens

The ocular lens is a biconvex, relatively pliable, and normally transparent tissue held in suspension by ciliary zonules between the aqueous and the vitreous humors. Its anatomical structure and location, coupled with its physical and biochemical characteristics, are geared towards maintaining an effective transmission and convergence of the visible frequencies of the electromagnetic spectrum from the environmental objects to the retina, meant for image formation and visual perception. Lens function to converge is also dependent on its pliability and consequent adjustments in its curvature. The lens also acts as an optical filter such that the access of the ultraviolet (UV) light to the retina is greatly minimized (Varma, 1991).

The lens is an avascular tissue packed with protein that provides the high refractive index necessary for the fine focusing of light onto the retina. As shown in Fig. 1, a single unicellular layer of epithelial cells — capsula lentis — is found directly under the anterior surface of the collagenous membrane in which it is encapsulated. The epithelial cells at the germinative, equatorial region of the lens divide, migrate posteriorly, and differentiate into lens fibers that lose their nucleus and other intracellular organelles. Only two cell types are found in the

lens — fiber cells and epithelial cells. The lens increases in weight and thickness throughout life. The newly formed fiber cells displace older fiber cells, which are moved in towards the center of the tissue. Thus, the central region (cortex) contains fiber cells laid down in early life, and as one moves towards the surface of the tissue, the cells become progressively younger.

There is little protein turnover in the lens, the majority of proteins consisting of long-lived α -, β -, and γ -crystallins. These proteins appear to be specific to the lens, although they contain regions of sequence and structural homology to other proteins. Lipids, approximately 1% of wet weight of the lens, are found mainly in cell membranes. The most frequent (50–60% of all lipids of the lens) is cholesterol (Girao, Mota, & Pereira, 1999; Jacob, Cenedella, & Mason, 1999; VanMarle & Vrensen, 2000).

There is a coincident dehydration of the proteins and the lens itself. Together with modification of the protein and other constituents, these changes result in less flexibility upon aging. As the lens ages, the proteins are photooxidatively damaged, aggregate, and accumulate in lens opacities. Dysfunction of the lens due to opacification is called cataract. The term “age-related cataract” is used to distinguish lens opacification associated with old age from opacification associated with other causes, such as congenital and metabolic disorders (Jacques & Taylor, 1991; Taylor & Nowell, 1997).

3. Factors implicated in cataractogenesis

Half-lives of many of the lens proteins are measured in decades. The sunlight and oxygen that the lens is exposed to are associated with extensive damage to the long-lived lens proteins and other constituents. With progressive damage, the altered proteins accumulate, aggregate, and precipitate in opacities, or cataracts. The young lens has substantial reserves of antioxidants (e.g., vitamins C and E, carotenoids, and glutathione — GSH) and antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione reductase/peroxidase) that may prevent damage. Proteolytic enzymes, called proteases, may selectively remove obsolete proteins and provide a second level of defense. Compromises of function of the lens upon aging are associated with and may be causally related to depleted or diminished primary antioxidant reserves, antioxidant enzyme capabilities, and diminished secondary defenses such as proteases. Environmental stress such as smoking and excessive UV-light exposure appear to provide an additional oxidative challenge associated with the depletion of antioxidants as well as with enhanced risk for cataract (Taylor & Nowell, 1997). Other risk factors for cataract formation include diabetes, galactosemia, electromagnetic radiation, life-threatening diarrhea, renal failure, and many drugs (Cerami & Crabbe, 1986). Drugs and related compounds implicated in cataract

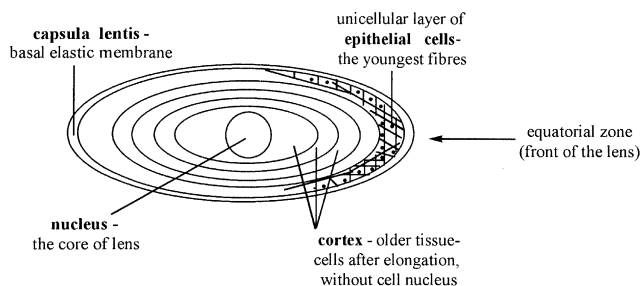


Fig. 1. Structure of the eye lens.

Table 1

Drugs and related compounds implicated in cataract formation (Cerami & Crabbe, 1986)

Cataract in experimental animals

Cyanate, methylisocyanate, *N*-methyl-*N*-nitrosourea, bisulphan, dinitrophenol, 3-aminotriazole, naphthalene, triparanol and other inhibitors of cholesterol synthesis, ecothiopate iodide (phospholine iodide) and other cholinesterase inhibitors, diquat, chloroquine, chlpromazine and phenotiazines, adrenaline and morphine, steroids, and bleomycin

Cataract in humans

Barbiturates, alcohol, dinitrophenol, triparanol and other inhibitors of cholesterol synthesis, cholinesterase inhibitors, phenotiazines and major tranquilizers, diuretics, and steroids

formation, in humans and in experimental animals, are given in Table 1.

4. Diabetes mellitus as a risk factor for cataract development

Chronic elevation of blood glucose in diabetes plays a critical role in the development and progression of major diabetic complications. Prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable macromolecules. The injurious effects of hyperglycemia are characteristically observed in tissues that are not dependent on insulin for glucose entry into the cell (e.g., eye lens, kidneys) and, hence, they are not capable of down-regulating glucose transport along with the increase of extracellular sugar concentrations.

From multiple mechanisms that have been proposed to explain how hyperglycemia might cause these abnormalities, this review is restricted to the following, with special attention to cataract development:

- nonenzymatic glycation;
- oxidative stress; and
- polyol pathway.

5. Nonenzymatic glycation

Under hyperglycemic conditions, part of the excess glucose reacts nonenzymatically with proteins or other tissue or blood constituents, thus increasing the physiological rate of nonenzymatic glycation (Fig. 2) (Brownlee, 1996). Chronic, irreversible abnormalities unaffected by normalization of blood glucose levels primarily involve long-lived molecules including extracellular matrix, eye lens crystallins, and chromosomal DNA. Due to their characteristic chemical properties, advanced products of nonenzymatic glycation play a critical role in the evolution of diabetic complications.

The formation of advanced glycation end products (AGEs) begins with the attachment of glucose carbonyl group to a free amino group of proteins or amino acids to form a labile Schiff base adduct as the first step of the complex Maillard process. Levels of the unstable Schiff base increase rapidly, and equilibrium is reached after several hours. Once formed, Schiff base adducts undergo a slow chemical rearrangement over a period of weeks to form a more stable, but still chemically reversible, Amadori product (Monnier et al., 1992). Finally, AGEs are formed as a rather heterogeneous mixture of protein-bound, nitrogen- and/or oxygen-containing heterocyclic compounds through a complex cascade of dehydration, condensation, fragmentation, oxidation, and cyclization reactions of the intermediate Amadori ketoamine. The AGEs are frequently pigmented or fluorescent, and — most importantly for diabetic complications — they participate in glucose-derived cross-link formation (Brownlee, 1996; Schinzel, Münch, Heidland, & Sebekova, 2001; Westwood & Thornalley, 1997).

Specific chemical characterization of AGE proteins has been difficult, as Amadori products can theoretically undergo a large number of potential rearrangements. Immunological and chemical evidence indicates that progressive accumulation of AGEs in diabetic eye lens contributes to accelerated cataractogenesis in hyperglycemic experimental animals and diabetic humans (Araki, Ueno, Chakrabati, Morino, & Horiuchi, 1992; Duhaiman, 1995; Lyons, Silvestri, Dunn, Dyer, & Baynes, 1991; Nagaraj, Sell, Prabhakaram, Ortwerth, & Monnier, 1991; Shamsi, Sharkey, Creighton, & Nagaraj, 2000).

6. Oxidative stress and diabetes mellitus

Diabetes mellitus was found to be inextricably connected with increased oxidative stress both in diabetic humans and hyperglycemic animals (Baynes, 1991; Cameron, Cotter, &

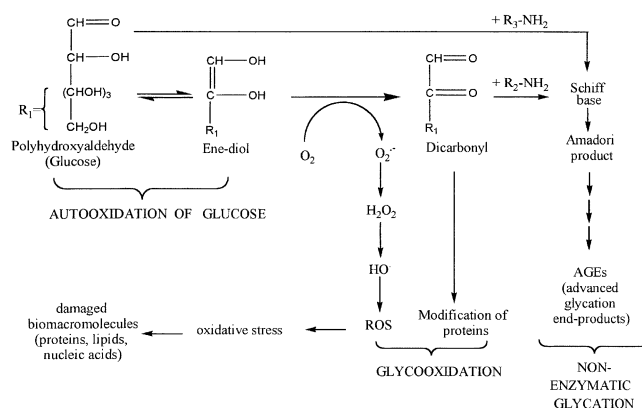


Fig. 2. Nonenzymatic reactions of excessive glucose in diabetic state: O_2 — oxygen; O_2^- — superoxide anion radical; H_2O_2 — hydrogen peroxide; HO — hydroxyl radical; ROS — reactive oxygen species; R_2-NH_2 , R_3-NH_2 — polypeptidic chains.

Archibald, 1995; Dai & McNeill, 1995; Kowluru & Kennedy, 2001). Among the number of mechanisms proposed as a pathogenic link between hyperglycemia and diabetic complications, oxidative stress is an equally tenable hypothesis as the Maillard advanced glycation hypothesis or the AR-mediated osmotic hypothesis. Irreversible AGEs were shown to be formed via a sequence of glycation and oxidation reactions (Kowluru, Kern, & Engerman, 1996; Wohaieb & Godin, 1987). Under physiological conditions, glucose, like other alpha-hydroxyaldehydes, can enolize and thereby reduce molecular oxygen, catalyzed by transition metals yielding reactive alpha-ketoaldehydes and oxidizing free radical intermediates (Fu et al., 1994). The ketoamine Amadori product undergoes autooxidation as well, contributing to the oxidative damage of proteins exposed to hyperglycemia (Baynes, 1991; Baynes & Thorpe, 1999).

Hyperglycemia would not only generate more reactive oxygen species (ROS) but also attenuate endogenous antioxidative mechanisms through glycation of scavenging enzymes and depletion of low molecular antioxidants, for example, glutathione. Shifts in redox balances due to derangement in energy metabolism of carbohydrates and lipids also contribute to the overt oxidative stress in diabetic individuals.

Reactive dicarbonyls, products of carbohydrate autooxidation, contribute to covalent attachment of monosaccharide to protein with high cross-linking potential. Indeed, glycation and oxidation are closely connected and the complex process is often referred to as glycooxidation (Baynes, 1991).

A hypothesis — parallel to the aforementioned glucose autooxidation theory — has been recently proposed, suggesting that the initial event leading to the oxidative stress in hyperglycemia would be the enhanced generation of ROS occurring at the mitochondrial level as a consequence of the increased intracellular glucose metabolism (Mario & Pugliese, 2001; Nishikawa, Edelstein, & Brownlee, 2000). As shown in Fig. 3, under these conditions, the increased proton gradient produced by the accelerated electron flow through the respiratory chain associated with excess glucose disposal is capable of generating ROS. The blockade of ROS production by manganese superoxide dismutase and by an inhibitor of pyruvate transport (Nishikawa, Edelstein, & Du, 2000) indicated the role of superoxide anion radical ($O_2^{\cdot-}$) and pyruvate, the substrate of the tricarboxylic or Krebs' cycle (Nishikawa, Edelstein, & Brownlee, 2000). The mechanism by which the polyol pathway activity is increased at elevated $O_2^{\cdot-}$ concentrations under hyperglycemia may be linked to its ability to quench nitric oxide which inhibits aldose reductase (AR) by *S*-thiolation of a cysteine-298 residue located at the active site (Chandra et al., 2002; Dixit et al., 2001). However, at this posttranslational level, AR could equally well be activated via nitrosation of the sensitive cysteine-298 residue, depending on the nature of the NO donor (Dixit et al., 2001). In addition, nitric

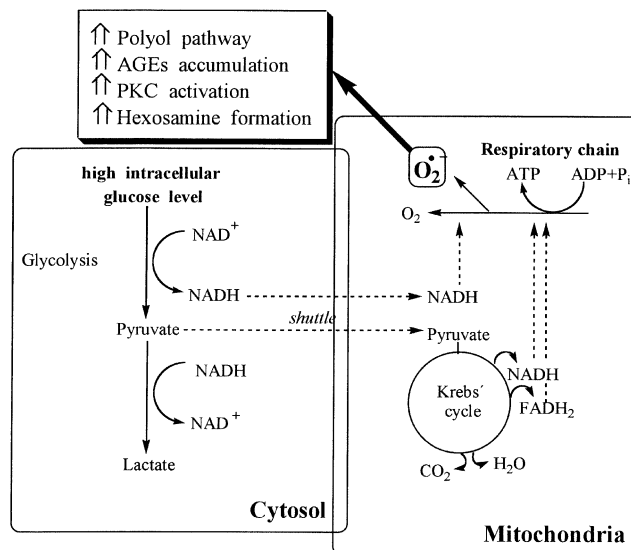


Fig. 3. Increased intracellular glucose metabolism enhance mitochondrial superoxide generation (Mario & Pugliese, 2001; Nishikawa, Edelstein, & Brownlee, 2000; Nishikawa, Edelstein, & Du, 2000), which would in turn be responsible for increased glucose flux through the polyol pathway (Giugliano et al., 1996), AGE accumulation (Baynes & Thorpe, 1999), PKC activation (Koya & King, 1998), and hexosamine formation (Schleicher & Weigert, 2000) (details are in the text): $O_2^{\cdot-}$ — superoxide anion radical; AGEs — advanced glycation end products; PKC — protein kinase C; ATP — adenosine triphosphate; ADP — adenosine diphosphate; P_i — inorganic phosphate; O_2 — oxygen, NAD^+ — oxidized nicotinamide adenine dinucleotide; NADH — reduced nicotinamide adenine dinucleotide; $FADH_2$ — reduced flavinadenine dinucleotide phosphate; \uparrow — enhancement of the respective process.

oxide and, more generally, oxidative stress, can also affect the transcription of the AR gene resulting in the up-regulation of the rate-limiting AR enzyme (Seo, Nishinaka, & Yabe-Nishimura, 2000; Spycher, Tabataba-Vakii, O'Donnell, Palomba, & Azzi, 1997), which is coupled with depletion of reduced glutathione leading to further enhancement of the oxidative stress (Giugliano, Ceriello, & Pao-lisso, 1996). Superoxide anion radical is responsible for inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Buchanan & Armstrong, 1978; Marin, Maus, Bockaert, Glowinski, & Premont, 1995; Rivera-Nieves, Thompson, Levine, & Moss, 1999; Salvemini & Cuzzocrea, 2002). Inhibition of GAPDH would be responsible for an increased formation of the AGE-forming compound methylglyoxal (Baynes & Thorpe, 1999; Nishikawa, Edelstein, & Brownlee, 2000; Thornalley, 1996), for an elevated production of the activator of endogenous protein kinase C (PKC) diacylglycerol (Koya & King, 1998), and the activation of the hexosamine pathway (conversion of fructose-6-phosphate into glucosamine-6-phosphate by the glucosamine-fructose-amidotransferase; Schleicher & Weigert, 2000). As recently shown by Chang et al. (2002), methylglyoxal is also responsible for substrate-induced up-regulation of AR, which may further facilitate development of diabetic complications.

It is now widely accepted that oxidative free-radical damage is an initiating or very early event in the overall sequence that leads to cataract (Sarma, Brunner, Evans, & Wormald, 1994). Oxidative stress may cause direct modification of the inner lens proteins, such as cross-linking, aggregation, and precipitation (Reddy, Giblin, Lin, & Chakrapani, 1998; Young, 1991). Toxic aldehydes generated by peroxidation of lens epithelium and by oxidative damage of the vulnerable retina may contribute to the final damage of lens proteins yielding opacity (Altomare et al., 1997).

7. Polyol pathway

Under physiological conditions, the bulk of glucose is metabolized through the glycolytic pathway and the pentose shunt. When hyperglycemia occurs, glucose disposal through these pathways tends to increase (Pugliese, Tilton, & Williamson, 1991). In addition, an increased amount of glucose is converted into sorbitol by the enzyme AR via the polyol pathway, normally operating for converting aldehydes into alcohols at physiological glucose concentrations (Williamson et al., 1993). The glucose conversion into sorbitol by utilizing NADPH results in the reduction of the NADPH/NADP⁺ ratio. This reaction uses NADPH as a hydrogen donor. Moreover, sorbitol oxidation to fructose by sorbitol dehydrogenase (SD) using NAD⁺ as a cofactor is associated with an increased NADH/NAD⁺ ratio (Fig. 4). Sorbitol does not easily cross cell mem-

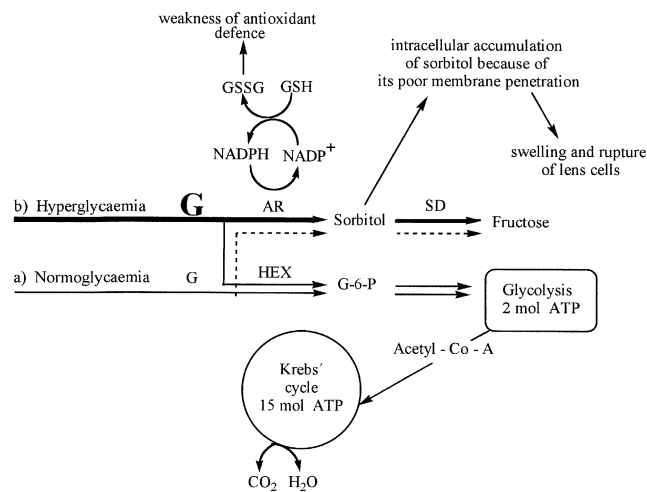


Fig. 4. Polyol pathway in hyperglycemic and normoglycemic state: G — glucose; G-6-P — glucose-6-phosphate; AR — aldose reductase; SD — sorbitol dehydrogenase; HEX — hexokinase; ATP — adenosine triphosphate; Acetyl-Co-A — acetyl coenzyme A; GSSG, — oxidized glutathione; GSH — reduced glutathione; NAD⁺ — oxidized nicotinamide adenine dinucleotide; NADH — reduced nicotinamide adenine dinucleotide; NADP⁺ — oxidized nicotinamide adenine dinucleotide phosphate; NADPH — reduced nicotinamide adenine dinucleotide phosphate; bold line arrow — accelerated processes; dotted line arrow — physiological rate of processes.

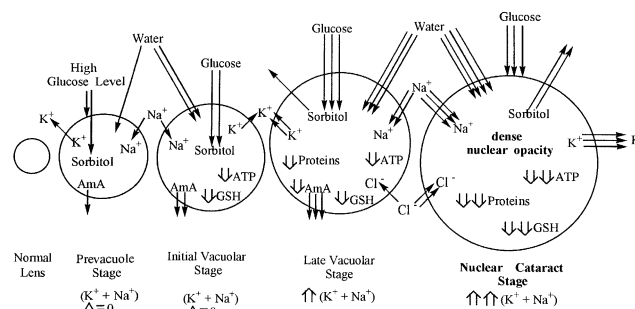


Fig. 5. Biomorphological changes during cataract formation. Theory of osmotic imbalance — modified according to Kinoshita et al. (1981): K⁺ — potassium; Na⁺ — sodium; Δ=0 — no change in total K⁺Na⁺; ATP — adenosine triphosphate; Cl⁻ — chlorine; AmA — amino acids; GSH—reduced glutathione; ↑ — moderate increase; ↑↑ — severe increase; ↓ — moderate decrease; ↓↓ — severe decrease.

branes, and it can accumulate in cells and cause damage by disturbing osmotic homeostasis. Intralenticular accumulation of polyols produced in hyperglycemic conditions has long been suggested to be a major factor in acute models of sugar cataract.

The fact that AR is responsible for initiating the cataractous process provides an explanation for the difference in the rate of cataract progression observed between diabetic and galactosemic rats. First, galactose is a better substrate than glucose for AR, so that more polyol is formed per unit time from galactose than from glucose. Second, galactitol formed in the AR reaction is not further metabolized by SD, as is sorbitol in the diabetic state. Since fructose can be further metabolized and can leak from the lens, the sorbitol pathway intermediates in the diabetic state never accumulate to the level of polyol found in the galactosemic lens. Therefore, there is a greater osmotic change in the lens of galactosemic rats, and, consequently, the rate of cataract development is more rapid (Kinoshita, 1990; Kinoshita, Kador, & Catiles, 1981; Kinoshita & Nishimura, 1988; Ohta, Yamasaki, Goto, Majima, & Ishiguro, 1999; Ohta, Yamasaki, Niwa, & Majima, 2000; Sato, Mori, Wyman, & Kador, 1998).

As the lens begins to swell in response to the hyperosmotic effects of polyol accumulation, membrane permeability changes result in an increase of lenticular sodium and in a decrease of lenticular potassium, reduced glutathione, myoinositol, ATP, and free amino acids. Eventually, as the lenticular levels of sodium exceed those of potassium, a shutdown of protein synthesis with loss of dry weight occurs. These biochemical changes are accompanied by morphological changes (Fig. 5), which include an initial swelling of the lens epithelial cells and those in the central lens region increase in height and display aberrant vacuoles and dilution of cell contents followed by swelling of the superficial cortical fibers, which eventually rupture to form visible vacuoles (Kador, Lee, Fujisawa, Blessing, & Lou, 2000; Robison, Houlder, & Kinoshita, 1990). As lens fiber

degeneration progresses, the entire cortex becomes opaque, and, eventually, nuclear opacity formation occurs along with liquefaction of the cortical regions.

Increased flux of glucose via polyol pathway has also consequences for the overall antioxidant status of the lens leading to depletion of GSH as a result of competition between AR and glutathione reductase for NADPH (Cheng & Chylack, 1985; Varma & Kinoshita, 1990). The NADPH depletion, combined with leakage of GSH and compounds essential for its synthesis, such as amino acids and ATP, results in a significant fall in lenticular GSH levels, an important intralenticular antioxidant (Gonzalez, Sohor, & McLean, 1983; Lee & Chung, 1999; Obrosova, Gao, Greene, & Stevens, 1998).

8. Possibilities of pharmacological prevention of cataract

At present, no definitive pharmacological therapy is available, and, thus, the only solution for the patient with advanced cataract is surgery, with all its disadvantages. Nevertheless, there are certain measures and treatment modalities, resulting from the aforementioned discussion on possible molecular mechanisms of cataractogenesis, which can improve the visual outcome of this disabling eye disease (Fig. 6; Table 2).

As the first possibility of delaying cataract, protection of critical amino groups of long-lived proteins is offered. An efficient inhibitor of nonenzymatic glycation should inhibit glucose-derived AGE generation and cross-link formation.

Aminoguanidine is a hydrazine compound that was introduced to deal with the complications of diabetes, including cataract (Brownlee, Vlassara, & Kooney, 1986; Harding, 2001). It can react with compounds at different stages of glycation to prevent the formation of both early and late glycation products (Brownlee et al., 1986; Khatami, Suldan, & David, 1988; Lewis & Harding, 1990). It slowed the progression of lens opacification in moderately diabetic rats (Swamy, Green, & Abraham, 1996). Aminoguanidine

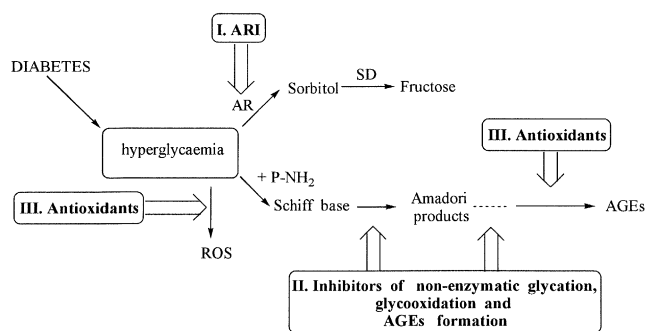


Fig. 6. Pharmacological possibilities of prevention of cataract formation: AR — aldose reductase; SD — sorbitol dehydrogenase; ARIs — aldose reductase inhibitors; ROS — reactive oxygen species; AGEs — advanced glycation end products; P-NH₂ — protein; ⇒ — possible sites of drug action.

Table 2

Potentially effective anticataract agents that were used in animal, epidemiological, and clinical studies

I. Inhibitors of glycation

Aminoguanidine (Brownlee et al., 1986; Harding, 2001; Khatami et al., 1988; Lewis & Harding, 1990; Swamy et al., 1996), aspirin (Blakytyn & Harding, 1992; Harding, 2001; Harding et al., 1989; Huby & Harding, 1988; Qin et al., 1993; Rao & Cotlier, 1988; Swamy & Abraham, 1989), ibuprofen (Harding, 2001; Harding et al., 1989; Blakytyn & Harding, 1992), paracetamol (Blakytyn & Harding, 1992; Harding, 2001; Harding et al., 1989), pyruvate (Varma et al., 1995; Zhao et al., 1998, 2000), and LPA (Obrosova et al., 1998; Packer, 1998; Suzuki et al., 1992)

II. Antioxidants

Vitamin C^a (Harding, 2001; Jacques et al., 1997; Knekt et al., 1992; Leske et al., 1998; Mares-Perlman et al., 1994; Robertson et al., 1989; Van der Pols, 1999; Varma, 1991; Vitale et al., 1993), vitamin E (Bates et al., 1996; Knekt et al., 1992; Leske et al., 1998; Mares-Perlman et al., 1994; Ohta et al., 1996, 2000; Rouhiainen et al., 1996; Varma, 1991; Vitale et al., 1993), LPA (Borenshtein et al., 2001; Kilic et al., 1995; Scott et al., 1994; Varma, 1991), pyruvate (Devamanoharan et al., 1999; Harding, 2001; Varma et al., 1995; Zhao et al., 1998, 2000), carotenoids (Bates et al., 1996; Chasan-Taber et al., 1999; Sanderson et al., 1996; Teikari et al., 1998), trolox (Ansari et al., 1994), butylated hydroxytoluene (Linklater et al., 1986; Srivastava & Ansari, 1988), venoruton (Kilic et al., 1996), and quercetin (Sanderson et al., 1999)

III. ARIs

M-79175 (Sato et al., 1998), imirestat (AL-1576) (Kador et al., 1998, 2000), epalrestat (Constantino et al., 1999; Hamada et al., 2000; Yabe-Nishimura, 1998), zenarestat (FR-74366) (Ao et al., 1991), BAL-ARI8 (Gervasi et al., 1991), zopolrestat (Beyer-Mears et al., 1996), tolrestat (Crabbe & Goode, 1988; Yabe-Nishimura, 1998), and ponalrestat (Crabbe & Goode, 1988; Yabe-Nishimura, 1998)

^a Also a possible prooxidant effect (Lee et al., 1998; Saxena et al., 1996; Spector et al., 1998).

has progressed to clinical trials against other diabetic complications and results on cataract should follow. Derivatives of aminoguanidine and agents cleaving glycation cross-links are in the testing process (Constantino, Rastelli, Vianello, Cignarella, & Barlocco, 1999).

Forty years ago, Cotlier (1961) reported that aspirin (acetylsalicylic acid) protected patients with rheumatoid arthritis or diabetes mellitus against cataract. Experimental studies then showed that aspirin protected lens proteins against a variety of chemicals relevant to cataract formation (Ajiboye & Harding, 1989; Bucala, Manabe, Urban, & Cerami, 1985; Huby & Harding, 1988; Rao & Cotlier, 1988; Swamy & Abraham, 1989). This protective action appears to be brought about by acetylation of vulnerable groups of lens proteins (Crompton, Rixon, & Harding, 1985; Qin, Smith, & Smith, 1993), and more recently, acetylation of a single lysine in human crystalline was identified (Lin, Barry, Smith, & Smith, 1998). Aspirin, paracetamol (acetaminophen), and ibuprofen have all been shown to delay experimental cataracts in laboratory animals (Blakytyn & Harding, 1992; Gupta et al., 1984; Swamy & Abraham, 1989). In these experiments, aspirin decreased glycation of lens proteins without lowering blood glucose

levels and helped to maintain glutathione levels in the lens. Perhaps the strongest support for the view that aspirin and other nonsteroid antiinflammatory drugs protect against cataract came from case-control studies of cataract in human populations. The first of these studies elicited the unexpected protective association between the consumption of aspirin, paracetamol, and ibuprofen and protection against cataract (Harding & van Heyningen, 1988; Van Heyningen & Harding, 1986). A further study in the same area confirmed the result and provided more information on the relevant dosage, showing that even low lifetime doses were associated with protection (Harding, Egerton, & Harding, 1989). However, a trial of photocoagulation and aspirin in patients with diabetic retinopathy reported a significant number of cataracts with more than 7 years of treatment providing no protection (Chew et al., 1992).

α -Lipoic acid (LPA), an essential cofactor in oxidative metabolism, may serve as a possible therapeutic agent due to its potential hypoglycemic and antioxidant actions. Studies show that LPA facilitates nonoxidative and oxidative glucose metabolism and increases glucose uptake leading to improved glucose utilization in vitro and in vivo (Borenshtein et al., 2001; Jacob, Streeter, & Fogt, 1996; Klip, Volchuk, Ramlal, Ackerley, & Mitsumoto, 1994; Natraj, Gandhi, & Menon, 1984; Wagh, Natraj, & Menon, 1987). Recent reports also indicate that LPA may prevent protein glycation (Suzuki, Tschiya, & Packer, 1992) and inhibit AR activity in cultured lens under hyperglycemic conditions (Altomare et al., 1997; Ou, Nourooz-Zadeh, Tritschler, & Wolff, 1996). Furthermore, LPA may prevent oxidative damage by direct radical scavenging and metal chelation, interactions with other antioxidants (Cao & Phillis, 1995), and increasing intracellular reduced GSH (Obrosova et al., 1998; Packer, 1998; Scott, Aruoma, & Evans, 1994). These powerful hypoglycemic and antioxidant effects led to the use of LPA supplementation in treatment of diabetic cataract. The ability of LPA to prevent cataractogenesis has been demonstrated recently in vitro in rat lens cell cultures exposed to high concentrations of glucose (Kilic, Handelman, Serbinova, Paker, & Trevithick, 1995), and also in vivo in an oxidative stress model of cataract (Bantseev, Bhardwaj, Rathbun, Nagasawa, & Trevithick, 1997; Maitra, Serbinova, Trischler, & Packer, 1995) as well as in nutritionally induced Type 2 diabetes in *Psammomys obesus* rats (Borenshtein et al., 2001). Unfortunately, no clinical or even epidemiological study with LPA as an anticataract agent has been published as yet.

The second way how to prevent cataractogenesis is to reduce the oxidative stress by antioxidants. Antioxidants may generally act at different levels, for example, by preventing the formation of ROS, by eliminating already created ROS by scavenging, trapping, and quenching them, or by binding metal ions into inactive chelates. The lens may defend itself against oxidative stress by means of endogenous antioxidants like vitamin C, vitamin E, carotenoids, and antioxidant enzymes such as

superoxide dismutase (SOD), catalase (CAT), and Se-dependent GSH peroxidase (Se-GPx) (Sarma et al., 1994; Van der Pols, 1999).

Epidemiological studies and in vitro experiments suggest that antioxidants might protect the lens against cataract formation. Although most of these studies report inverse associations between the risk of cataract and at least one antioxidant nutrient — vitamin E (Jacques et al., 2001; Knekt, Heliövaara, Rissanen, Aromaa, & Aaran, 1992; Leske, Chylack, & Wu, 1991; Leske et al., 1998; Lyle, Mares-Perlman, Klein, Klein, & Greger, 1999; Mares-Perlman, Klein, Klein, & Ritter, 1994; Robertson, Donner, & Trevithick, 1989; Rouhiainen, Rouhiainen, & Salonen, 1996; Tavani, Negri, & La Vecchia, 1996; Vitale et al., 1993), vitamin C (Hankinson et al., 1992; Jacques & Chylack, 1991; Jacques et al., 1997; Leske et al., 1991; Mares-Perlman et al., 1994; Robertson et al., 1989), or carotenoids (Brown et al., 1999; Chasan-Taber et al., 1999; Hankinson et al., 1992; Jacques & Chylack, 1991; Knekt et al., 1992; Lyle et al., 1999) — they do not demonstrate consistent associations between intake or blood levels of any one nutrient and the risk of cataract. This lack of specificity may be a consequence of differences in the populations studied and the methods used.

Lens concentrations of vitamin C are many times higher than in plasma (Taylor et al., 1991; Taylor & Nowell, 1997). However, vitamin C concentrations are compromised on aging and/or cataractogenesis (Berger, Shepard, Morrow, & Taylor, 1989). Interest in the utility of vitamin C has been fueled by observations that (1) eye tissue levels of this vitamin are related to dietary intake in humans (Taylor et al., 1991) and animals (Berger et al., 1989), and (2) the concentration of vitamin C in the lens was increased with dietary supplements beyond levels achieved in persons who already consumed more than two times the recommended dietary allowance (60 mg/day) for vitamin C (Taylor et al., 1991). Vitamin C was considered in several studies (Hankinson et al., 1992; Leske et al., 1991; Mares-Perlman et al., 1994, 1995; Mohan et al., 1989; Robertson et al., 1989; The Italian–American Cataract Study Group, 1991; Vitale et al., 1993) and in one preliminary report (Jacques, Lahav, Willett, & Taylor, 1992) and was observed to be inversely associated with cataract risk.

Besides a protective role, vitamin C has also been implied to exacerbate cataractogenesis. Ascorbate can generate H_2O_2 by reducing molecular oxygen, a reaction that is catalyzed by metal ions (Garland, 1990; Halliwell & Gutteridge, 1989; Van der Pols, 1999). Radical species can be generated from the H_2O_2 by further reaction of the metal ions in a Fenton reaction, ascorbate restoring the metal ion into its original state so that it can participate in another cycle of the reactions (Garland, 1990). Recent work by Spector, Ma, and Wang (1998) showed that H_2O_2 generation in the aqueous humor is temperature and O_2 tension dependent, and that ascorbic acid and metal ions may make a major contribution to H_2O_2 production. Ascorbate has also

been shown to play a role in protein cross-linking and formation of AGEs (Ortwerth, Feather, & Olesen, 1988; Saxena, Saxena, & Monnier, 1996). It has recently been suggested that, although tempered by the low O₂ pressure in lens tissue, ascorbate can make a much larger contribution to cross-linking than lens glucose (Lee, Mossine, & Ortwerth, 1998). Consequently, in situations where oxidation of the lens tissue occurs, such as those observed in cataract formation, ascorbate could become a significant glycation agent (Lee et al., 1998) and promote cataract formation. This hypothesis will have to await confirmation by further experimental evidence.

Vitamin E is present in the lens in very low concentrations (Bates, Chen, Macdonald, & Holden, 1996; Yeum, Taylor, Tang, & Russell, 1995). Several *in vitro* experiments have suggested a protective role against cataract, possibly through protection of membrane lipids against peroxidation (Ohta, Okada, Majima, & Ishiguro, 1996; Sanderson, McLauchlan, & Williamson, 1996; Varma, Chand, Sharma, Kuck, & Richards, 1984), but very little evidence is available from *in vivo* experiments. Human epidemiological studies have suggested a protective effect of high plasma vitamin E levels (Knekt et al., 1992; Leske et al., 1995, 1998; Rouhiainen et al., 1996), but a recent intervention study did not show a protective effect of vitamin E supplementation (Teikari et al., 1998).

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox) is a structural analog of vitamin E possessing both hydrophilic and lipophilic properties. This compound is reported to scavenge peroxy radicals better than vitamin E from artificial system (Castle & Perkins, 1986) as well as from cultured hepatocytes and rat liver exposed to oxyradicals, generated by the xanthine oxidase–hypoxanthine system (Wu et al., 1989). Ansari, Bhatnagar, Fulep, Khanna, and Srivastava (1994) demonstrated that trolox prevented sugar-induced lens opacification in cultured lenses and could be of greater use in preventing diabetic and galactosemic cataracts and possibly the other diabetic complications than vitamin E.

Varma, Ramachandran, Devamanoharan, Morris, and Ali (1995) have reported a delay in the development of galactose- and selenite-induced cataract in rats by pyruvate, considered to be an antioxidant. Ethyl pyruvate may give better penetration for topical application (Devamanoharan, Henein, & Ali, 1999). Glucose-6-phosphate dehydrogenase is inactivated by fructose and other sugars (Ganea & Harding, 1995), and pyruvate could protect against this inactivation (Zhao, Devamanoharan, & Varma, 1998). It also protected α -crystalline against reaction with fructose and against formation of AGEs (Zhao, Devamanoharan, & Varma, 2000).

Linklater, Dzialiszynski, McLeod, and Trevithick (1986) have investigated the effects of butylated hydroxytoluene (BHT) in reducing protein leakage from lenses in diabetic rats. They found that the addition of BHT to the diet results in improved general body condition, reduction in cataracts,

decrease of gamma-crystalline leakage into the vitreous humour, and weight gain. BHT was qualified to prevent or delay also galactosemic cataractogenesis in rats (Srivastava & Ansari, 1988).

The effect of a novel flavonoid, venoruton (a mixture of mono-, di-, tri-, and tetrahydroxyethylrutinosides), has been investigated in healthy rat lenses and compared with diabetic cataract modeled *in vitro*. The protective effect of venoruton was suggested to be related to antioxidant activity against ROS (Kilic, Bhardwaj, & Trevithick, 1996).

Sanderson, McLauchlan, and Williamson (1999) showed that low micromolar concentrations of naturally occurring flavonoid, quercetin, have inhibited cataractogenesis in a rat lens organ cultured model exposed to the endogenous oxidant hydrogen peroxide. Quercetin was active both when incubated in the culture medium, together with hydrogen peroxide, and was also active when the lenses were pre-treated with quercetin prior to oxidative insult.

The third approach is the development of potential new agents to interfere with cataract formation through the inhibition of accumulation of polyols in eye lens cells. A wide variety of molecules were synthesized to inhibit AR (ARIs), which themselves may be acting in ways other than lowering the sorbitol pathway. Progress has been made in this area with reports of delaying cataract formation by zenarestat (FR74366) (Ao, Kikuchi, & Ono, 1991), BAL-ARI8 (Gervasi, Bartoli, & Catalani, 1991), zopolrestat (Beyer-Mears, Mistry, & Diecke, 1996), and imirestat (AL-1576) (Kador, Inoue, & Secchi, 1998), all of which are classified as ARIs. Clinical trials of ARIs have been started, but they concentrated on diabetic complications other than cataract, and, moreover, major problems were encountered (Stribling, 1990). Clinical trials of ponalrestat in diabetic complications proved to be disappointing, whereas epalrestat and tolrestat showed some promise (Crabbe & Goode, 1988; Yabe-Nishimura, 1998). Sorbinil, the leading compound, was withdrawn because of unacceptable adverse effects (Hotta, Kakuta, Ando, & Sakamoto, 1990). Tolrestat was withdrawn for lack of efficacy, and after 16 years of clinical trials, only epalrestat remains in contention (Constantino et al., 1999; Hamada et al., 2000; Yabe-Nishimura, 1998).

9. Conclusions

Despite the fact that there is a variety of agents, including glycation inhibitors, antioxidants, and ARIs, that have the potential of preventing cataract in animals, the recommendation for future interventions in humans to slow the development of diabetic cataract is premature. Glycation inhibitors, antioxidants, and ARIs have a potential of correcting biochemical and metabolic abnormalities in the hyperglycemic milieu of the diabetic individual or to combat oxidative stress, which altogether might help to inhibit irreversible biochemical and morphological

changes of the eye lens crystallines and lipids, eventually leading to cataract.

The possible anticataract agent is expected to slow the progression of cataract, thus involving prolonged treatment. Minimal adverse effects are therefore desirable. Apart from knowledge of the pathogenic links between hyperglycemia and lens opacity at the molecular level, successful development of an anticataract agent for humans would further require a better understanding of transparency in the normal eye lens, of mechanisms of drug penetration into human lens systemically or topically to achieve reasonable biological availability, and effective noninvasive methods for monitoring cataract progression in humans.

Thus, supplementation with a potential anticataract agent is envisaged as an adjunct therapy to help preserve vision in diabetic patients and future clinical trials are needed to assess the benefits of pharmacological interventions in lowering the risk of cataract development. Significant improvement of the quality of life and reduction of the cost of caring for diabetic patients may be expected.

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