This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Free Radicals and Cataract

By
Hualei Li

B-180 Medical Laboratories
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2003
For May 8, 2003

Abbreviations:

CAT	catalase
CuZnSOD	copper znic superoxide dismutase
EDTA	ethylenediaminetetraacetic acid
GP	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GSSG	glutathione disulfide
HPLC	high-performance liquid chromatography
MnSOD	manganese superoxide dismutase
ppp	pentose phosphate pathway
PSH	protein thiol
PSSG	mixed disulfide
PSSP	protein-protein disulfide
RSH	compounds containing thiol groups.
RSSR	disulfide compounds
UV	Ultraviolet

Table of Contents

Abstract

A cataract is the clouding of the lens of the eye. It is a leading cause of blindness in the world. Cataracts are the result of damage to the proteins and lipids in the lens of the eyes. At first, vision is distorted due to light sensitivity (photophobia), particularly during night driving or in very bright light. As the cataract progresses, severe visual impairment develops. Cataracts are more likely to occur in those who smoke, have diabetes, or are exposed to excessive sunlight. Its formation partially is due to a lifetime of cumulative damage from free radicals in our modern diet, and ultraviolet light also plays an important role. Many antioxidants such as vitamin C and vitamin E and antioxidant enzymes may help to protect you against cataract by reducing free radical damage.

Introduction

The eye is a complex organ composed of many small parts, each vital to normal vision. The ability to see clearly depends on how well these parts work together. A cataract is the clouding or opacity that develops in the eye's lens. The crystalline lens is comprised primarily of protein and water. Normally, the protein is bonded in a way that allows light to pass through it. A cataract forms when bonding changes and protein molecules clump together. Eventually, these clumps cloud the lens and block light. If left untreated, cataracts may eventually cause blindness. Fifty million persons are blind due to cataract in the world and there are 1.2 million cataract surgeries performed at an annual cost of cover $3.2 billion in the United States [1]. In the United States, about 50% of those between the ages 65 and 74, and 70% of those over age 75 have a cataract. Women are affected more frequently than men. One in every 10,000 babies is born with congenital cataracts. Cataract extraction is the most frequently performed surgery among the elderly [1]. The most common cause is aging. Others include diabetes, eye injury, metabolic diseases, drugs and hereditary conditions. Upon aging, lens constituents are damaged and precipitate in opacities

called senile cataracts. Laboratory and epidemiologic data indicate that the damage is due in part to light and active forms of oxygen and antioxidant nutrients – ascorbate, carotenoids, and tocopherol – can offer protection against cataract [1]. In this paper, I am going to focus on the role of free radicals in cataract and antioxidants in protection against cataractogenesis.

Anatomy and physiology of human lens

How the Eye Sees

Light is reflected off a particular object to the person's eye and enters the eye through the cornea. Next, light rays pass through an opening in the iris (colored part of the eye), called the pupil. Light then reaches the crystalline lens. The lens focuses light rays onto the light-sensitive retina by bending (refracting) them. The cornea does most of the refraction and the crystalline lens fine-tunes the focus. In a healthy eye, the lens can change its shape (accommodate) to provide clear vision at various distances. If an object is close, the ciliary muscles of the eye contract and the lens become rounder. To see a distant object, the same muscles relax and the lens flattens. All of the tissues in front of retina including lens must be transparent to visible light if good vision is to be maintained [2, 3].

Structure of human lens

The eye lens is a transparent, avascular tissue that transmits and focuses light on the retina [2]. It is suspended in the eye between the aqueous humour and the vitreous body [3] (Figure 1).

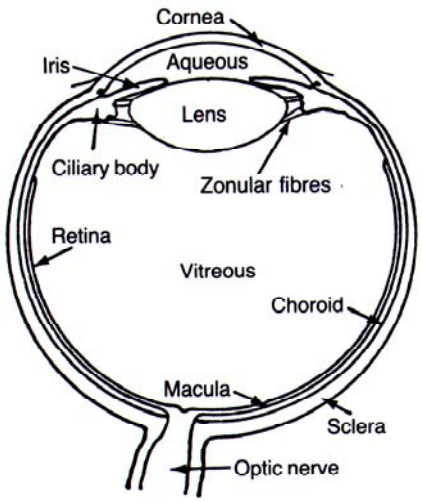

Figure 1: Diagrammatic section through the eye. The lens is a biconvex structure behind the pupil. It is held by zonular ligaments and surrounded by humour, which supplies nutrients to the lens. Adapted from [3].

The crystalline lens is comprised primarily of protein and water. The lens contents are held within a thin transparent basement membrane, the lens capsule, which controls its shape and allow the passage of small molecules [3] (Figure 2). Beneath the anterior surface of the capsule is a single layer of epithelial cells. These epithelial cells generate new lens cells and are the major site of metabolic activity. They actively divide and are located from anterior capsule to equator [2] (Figure 2). They differentiate into long mature fiber cells. The long mature fiber cells extend from the front of the lens curving round to the back of the lens. They meet in a region called the lens sutures [3] (Figure 2).

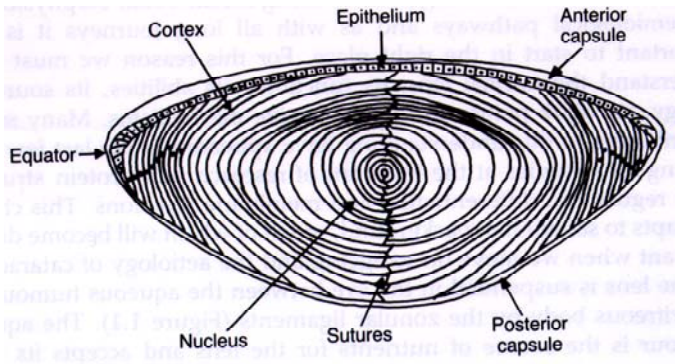

Figure 2: Diagrammatic section through the lens. Adapted from [3].

Physiological features

As epithelial cells differentiate and migrate towards equator, they elongate to form fiber cells and lose nuclei and many organelles including mitochondria and ribosomes to make the cells transparent. The loss of mitochondria makes these cells dependent on glycolysis for energy production. So their energy production is limited. The absence of nuclei and DNA and RNA makes cells unable to renew proteins. So their capacity for protein synthesis is limited and they can not replace damaged proteins. New lens cells are continuously generated, but the old fiber cells are not lost. They are compressed to form nucleus [2, 3] (Figure 2). The lens grows throughout life, so the lens weight and thickness increase according to the increase of age [3] (Figure 3).

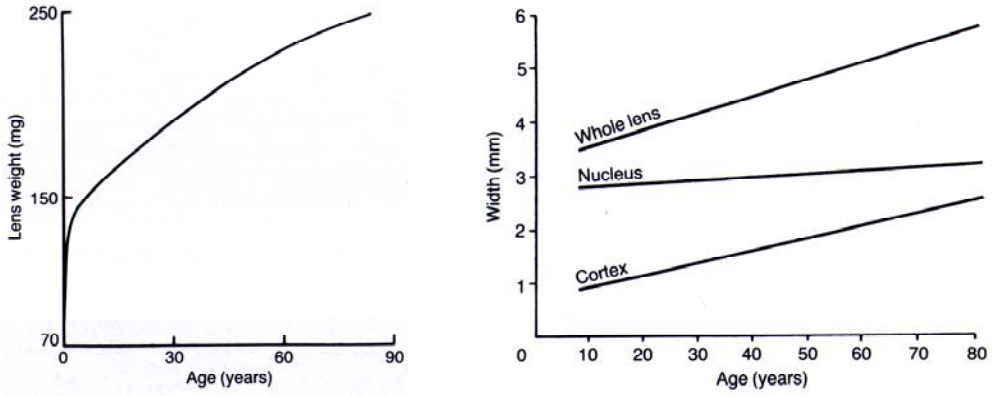

Figure 3: Human lens growth with age. Lens weight and width are increasing steadily with age. Adapted from [3].

Since the lens is transparent and has no vascular structures, the aqueous humour is the source of nutrients for the lens and accepts its waste products [3]. So the lens is vulnerable to certain damages due to lack of blood supply, limited metabolism, and lack of protein synthesis in the central region.

Pathology of cataract

A cataract is the clouding or opacity that develops in the eye's lens. Many causes can lead to cataract such as aging ("senile cataracts"), diabetes mellitus, radiation therapy or UV rays, ocular diseases (uveitis, glaucoma, intraocular tumors, retinitis pigmentosa), skin diseases (atopic dermatitis, scleroderma), drugs (corticosteroids) and trauma. The lens consists of three parts: the nucleus (center of the lens), the lens cortex (periphery), and the capsule (membrane that envelops the lens). Cataracts can form in any of these parts [2]. Nuclear cataracts develop in the nucleus and are the type most commonly found in older patients. This kind of cataract results from compression of lens fibers in the central (nuclear) portion of the lens. They can take years to develop and often give the nucleus a yellow tint. Cortical cataracts form in the lens cortex (peripheral area). They eventually extend like spokes on a wheel into the nucleus of the lens. Subcapsular cataracts develop in the envelope of the lens, and often in the center. The onset of this type is rapid and symptoms can develop over months, rather than years. A mixed cataract might involve opacities in two or three regions of the lens [2]. Three main types of the lens opacity are usually found in senile cataract [4]. Pure forms of cataract are found more frequently in early less advanced stages of the diseases, but as the cataract becomes severe, mixed cataract occurs [4].

Oxidative stress in cataractogenesis

Cataract is a major problem in the world, and the etiology of the cataract is not well understood. Surgical removal of the opaque lens is the only treatment. Various studies were carried out to understand the molecular mechanism of cataractogenesis. It was revealed that oxidative damage to lens played a significant role in the pathogenesis of many forms of cataract. Free radical induced lens peroxidation products were detected in the course of cataract development. Many oxygen species including free radicals are the major candidates involved in cataract formation.

Epidemiological studies

Age is a well-known and powerful factor for cataract. Light was also studied as a risk factor for cataract and lens protein damage [1]. Various epidemiological studies show that persons living or working in environments with higher intensities of incident and/or reflected ultraviolet (UV) light, living closer to equator or higher elevations have elevated risk of various forms of cataract [1]. Nuclear cataract was observed in patients treated with hyperbaric oxygen treatment [1]. Two large and controlled cancer prevention trials have been conducted in China using vitamin-mineral supplements [4]. One of the studies has 2142 patients with esophageal dysplasia. The patients were divided into two groups randomly. One group of patients took placebo, the other group took a mineral-multivitamin supplement reinforced with β-carotene that is an antioxidant. The other study has 3249 participants that were randomized to one of 4 possible treatments (retinal/zinc, riboflavin/niacin, ascorbic acid/molybdenum, selenium/α-tocopherol/β-carotene) or to placebo. It was turned out that the treatment group of the first study and the group treated with riboflavin/niacin in the second study had a significant reduction of the prevalence of nuclear cataract compared to controls [4]. Many epidemiological studies have demonstrated a correlation between oxidative stresses induced by sunlight exposures and oxygen with cataract formation.

Hydrogen peroxide

Some works show that oxidation of lens protein is an early event in the development of cataract [5]. Hydrogen peroxide levels were determined *in vivo* in the aqueous humour from cataract patients and normal human cases. It was found that mean aqueous hydrogen peroxide concentration in cataractous patients was 82 ± 155 μM while for normal patients it was 24 ± 7 μM [5]. The exogenous aqueous (exogenous) H_2O_2 concentration influences the endogenous lens H_2O_2 level. A correlation was found between lens (endogenous) and aqueous (exogenous) H_2O_2 [5]. This correlation would suggest that exogenous aqueous H_2O_2 can sufficiently penetrate the lens and affect oxidation to both membrane and cytoplasm proteins in the lens [5]. Further studies were

carried out about peroxide-induced formation of mixed disulfides in rat and monkey lenses incubated with H_2O_2 [4]. It is probably because protein thiol (PSH) reacts with glutathione disulfide (GSSG), produced by oxidized glutathione (GSH) by H_2O_2 (Figure 4) (Reaction 1) [3].

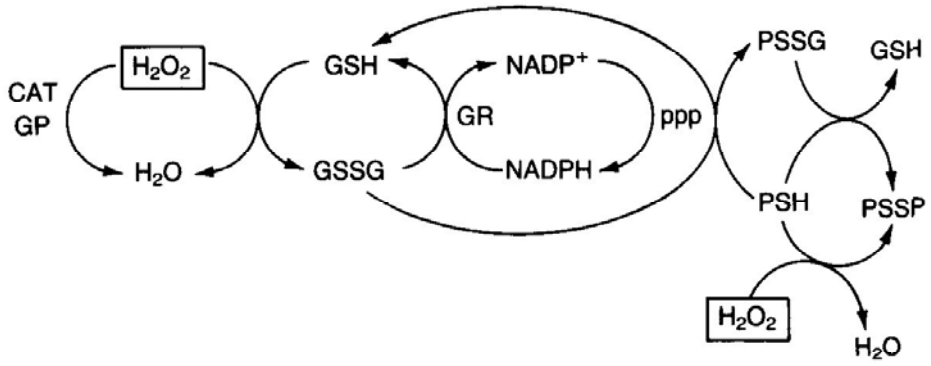

Figure 4: Effects of hydrogen peroxide on glutathione and protein thiol in the lens. On the left part, there are various mechanisms to dispose of H_2O_2 without damage. However, the right part comes to play if there is higher concentration of H_2O_2. So if lenses are incubated with high concentration of H_2O_2, lowered protein thiol contents and mixed disulfides and disulfide-crosslinked aggregates are detected in protein extracts from lenses. GR = glutathione reductase; ppp = pentose phosphate pathway; PSH = protein thiol; PSSP = protein-protein disulfide; PSSG = mixed disulfide; CAT = catalase; GP = glutathione peroxidase. Adapted from [3].

$$PSH + GSSG \leftrightarrow PSSG + GSH \quad (1)$$

Some evidences show that various cataractogenic agents such as selenium, glucose and adrimycin are able to increase H_2O_2 in ocular humors *in vivo* prior to cataract formation compared to their respective controls [6]. UV exposure, a source of oxidative stress, is known to contribute to cataract formation. Hydrogen peroxide, a downstream product of the photochemical reaction following UV exposure, is used in the lab to mimic oxidative stress.

Hydroxyl radical

Hydroxyl radical partly derives from hydrogen peroxide through the transition-metal ion catalyzed Fenton reactions [7] (reaction 2). It is possible that protein modifications linked with cataract could be the result of a reaction of lens crystallins with other oxidizing agents such as the hydroxyl

radical. It is demonstrated that nuclear cataract is associated with the extensive hydroxylation of protein-bound amino acid residues, which increases with the development of cataract by up to 15-fold in the case of DOPA [7]. Oxidized and hydroxylated amino acids such as DOPA, 3-hydroxyvaline (Val.OH1) and 5-hydroxyleucine (Leu.OH2) was measured after exposing lens protein to Fenton-derived hydroxyl radicals (Figure 5). The products include representatives of the hydroperoxide and DOPA pathways of protein oxidation [7]. Hydroxyl radical can also induce cross linking among proteins.

$$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-} \quad (2)$$

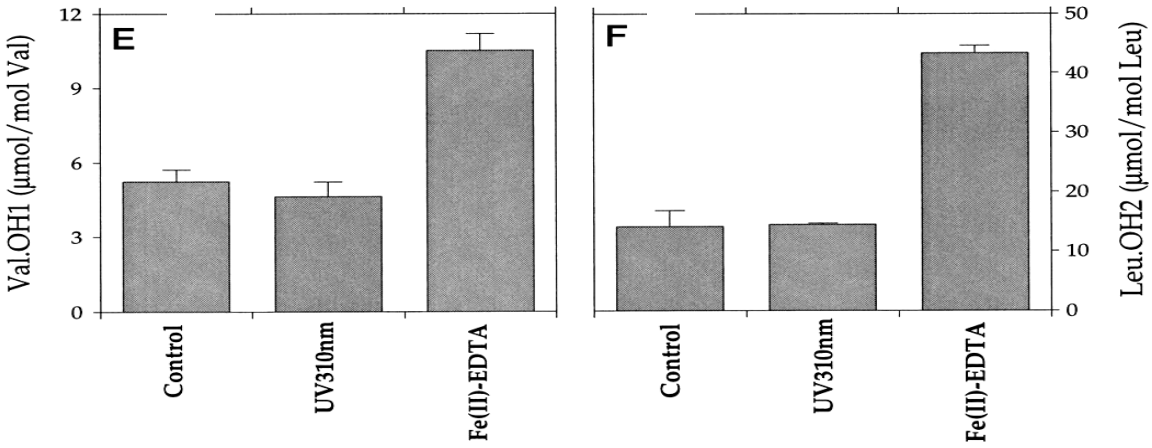

Figure 5. Oxidation of calf lens protein by UV irradiation and a Fenton system. The lens protein samples were irradiated with UV light. To expose lens protein to Fenton-derived hydroxyl radicals, the protein sample was incubated with Fe(II)-EDTA. The results make it very unlikely that the formation of hydroxyvaline and hydroxyleucine in the cataractous lens is due to photoxidation, but rather suggest an important role for hydroxyl radicals, or a closely related species. (Adapted from [7])

The ability of early stage (type II) and advanced (type IV) nuclear cataractous lens homogenates to catalyze $HO^{\cdot}$ production in the presence of H_2O_2 was investigated using electron paramagnetic resonance (EPR) spectroscopy with the free radical trap, 5,5-dimethyl-1-pyrroline- N -oxide (DMPO) [8]. $HO^{\cdot}$ signal was significantly more intense in the nuclear region of the type IV compared to the type II lenses after incubating cataractous lens homogenates with 1 mM H_2O_2 [8].

Ethanol inhibited the DMPO-HO· signal competitively and the metal ion chelator, diethylenetriaminepentaacetic acid, also inhibited HO· formation. It is suggested the DMPO-HO. signal was due to HO· formation *via* Fenton reaction. It was also shown that more advanced type IV lenses tended to have higher Fe levels compared to the type II lenses. These data support the hypothesis that transition metal-mediated HO. production may play a role in the etiology of age-related nuclear cataract [8].

The polyol pathway is known as theprimary cause of cataractogenesis in diabetics. The formation of hydroxyl radical was detected in sugar cataracts induced by galactose in rats using ESR spin-trapping method with a spin trapping agent DMPO [9]. Polyol accumulation of lenses in the same group simultaneously peaked. It is suggested that hydroxyl radical was produced in proportion to polyol accumulation in the early cataract stage [9]. Then, hydroxyl radical may induce oxidative damage to epithelial cells and cortical fibers of the lens (Figure 6).

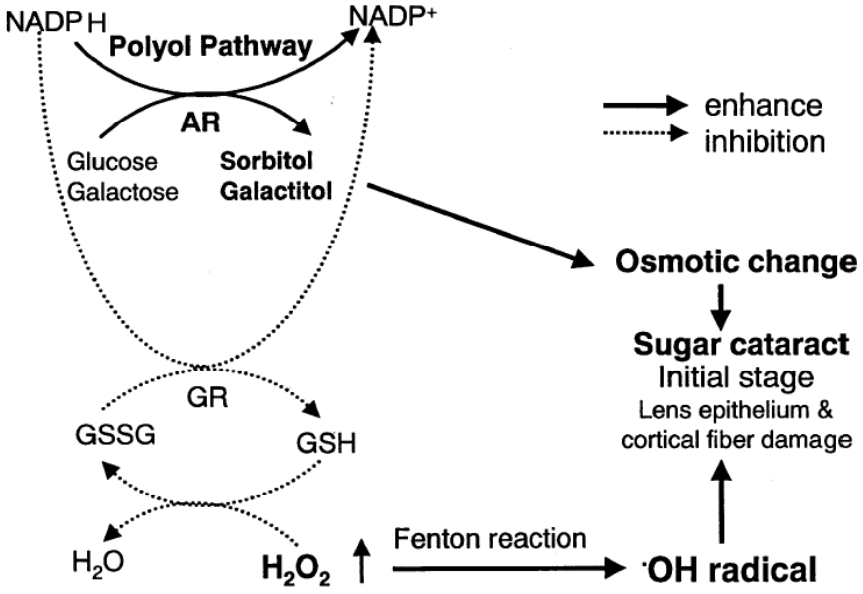

Figure 5. Schematic representation of radical production in rats fed with galactose. The active polyol pathway seems to increase oxidative stress such as H_2O_2 and consequently may produce [OH via the Fenton reaction. The •OH and the polyol accumulation may co-initiate damage to epithelial cells and cortical fibers of the lens. NADP+, nicotinamide-adenine dinucleotide phosphate; AR, aldose reductase. (Adapted from [9])

Superoxide

The mechanism of oxidative damage to the lens through intraocular photogeneration of superoxide and its derivatives has been studied [10]. Superoxide can lead to lens damage as well as its derivatives including hydroxyl radical, H_2O_2 and singlet oxygen. Peroxidative effect of superoxide on lens membrane lipids and proteins leads to their eventual oxidation [10]. The addition of SOD and catalase leads to a substantial inhibition of photoperoxidation. However, photoperoxidative damage was still observed *in vitro* after addition of SOD and catalase in the culture media. The possibility of membrane damage due to superoxide in aqueous humor is much greater. Superoxide generation plays a pivotal role in lens lipid peroxidation and oxidative degradative processes since almost all oxidants are derived secondarily from it [10].

Other ROS and/or ROS also account for the oxidative stress induced protein and lipid damage including singlet oxygen and nitric oxide. The accumulation of damaged proteins and lipids in lens opacities indicates that protective systems are not keeping pace with the insults that damage the proteins and lipids. The capabilities of protection *via* lens antioxidants and antioxidant enzymes exist in normal young lenses, but not in cataracts.

Antioxidants and cataract prevention

Superoxide dismutase (SOD)

Superoxide dismutase activity was assayed in normal and cataractous lenses [11]. In normal whole human lenses, SOD showed no significant difference in activity during aging. However, SOD activity in both nucleus and equator decreased with increasing age [11]. The mean values of SOD activity was significantly lower in human lenses with mature cataract than normal clear lens [11]. SOD is one of the important enzymes that protect tissue from serious damage by superoxide radicals. This enzyme detoxified superoxide to the less toxic H_2O_2, which is then completely

detoxified by catalase and/or GPx. It was suggested that antioxidant control system is abnormal in cataract. CuZn-SOD-null mouse lenses showed a doubled basal superoxide concentration, and were more prone to develop photochemical cataract with more opacification, and more hydration than lenses from wild-type mice [12]. Therefore CuZn-SOD is an important superoxide scavenger in the lens, and it may have a protective role against cataract formation [12].

Glutathione (GSH)

The GSH pool is a major source of antioxidants. Reduced GSH levels in cataract patients show that the size of GSH pool was diminished. GSH concentration in blood was lower and serum TBARS level, an indicator of lipid peroxidation concentrations, was higher in the cataract group than those of the control group [13]. Increased free radical production and oxidative stress in the diabetic cataractous group was confirmed by a significant increase in TBARS and a decrease in GSH concentrations, and a strong correlation was found between lens GSH and lens TBARS concentrations in the diabetic cataractous group [13]. Cataract can be induced by lipid peroxidation. The clouding of the lens is accompanied by a regular drop in the concentration of reduced GSH with a concomitant accumulation of fluorescing lipid peroxide products (Figure 6) [14].

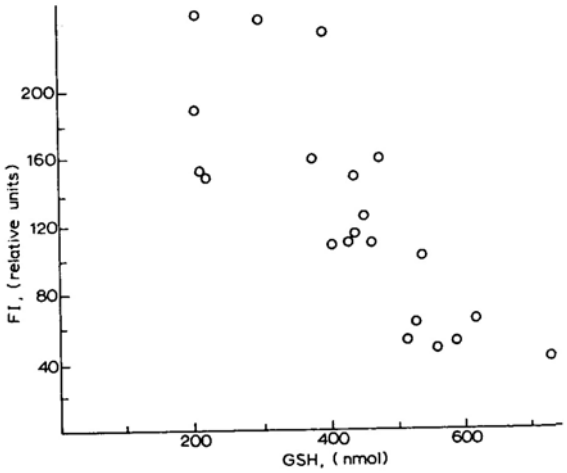

Figure 6: Level of GSH in lens as a function of the accumulation of lipid peroxidation products, FI is level of fluorescing lipid peroxidation products in lens. Adapted from [14].

Vitamin C and E

There have been a number of studies on the prevention of cataract or changes related to cataract by vitamins C and E: ascorbate and α-tocopherol [3]. Both of them act as free radical scavengers. Vitamin C is present at high levels in the human lens. The levels in the lens, which concentrates vitamin C to over 3 mmol/L, is 20-60 fold higher than plasma. Ascorbate is lowered in experimental cataracts [2]. It has also demonstrated that the concentration of vitamin C in the lens can be increased with vitamin C supplements. Some evidences show that vitamin C might play a role in prevention of cataract formation and protection of lens constituents from earlier stages of damage [2].

Vitamin E is a natural lipid-soluble antioxidant that can inhibit lipid peroxidation and help to preserve membrane integrity and critical membrane function [2]. The amount of α-tocopherol in normal human lens is approximately 1.6 μg/g wet weight of lens tissue [2]. Some experimental evidences show that vitamin E can delay galactose-induced and aminothiazole-induced cataracts in rabbits and protect against photo-peroxidation of lens lipids [2]. Some experiments are performed *in vivo* on the Emory mouse that was suggested to be a good model of senile cataract. In an experimental group, which was treated with vitamin E, the percentage of animals having

comparable opacity was substantially lower at all period of examination, indicating that vitamin E has definite protective effect (Figure 7) [10].

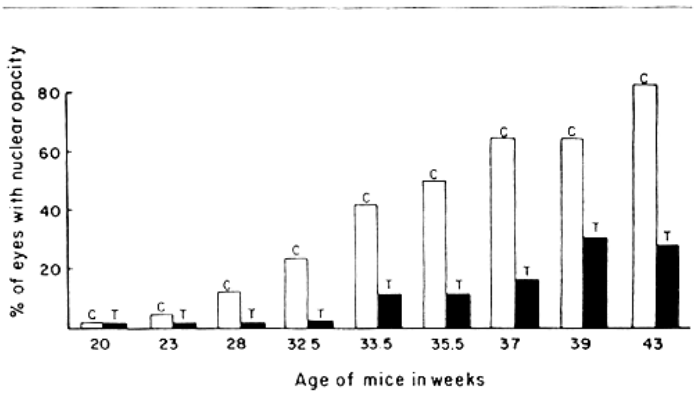

Figure 7: Prevention of the progress of cataracts by vitamin E in Emory mice. C = placebo treated mice, T = vitamin E treated mice. Adapted from [10].

Lipoic acid:

α-lipoic acid and its reduced from, dihydropoic acid (DHLA), are powerful antioxidants [14]. LA scavenges hydroxyl radicals, hypochlorous acid, peroxynitrite, and singlet oxygen. Dihydrolipoic acid also scavenges superoxide and peroxyl radicals and can regenerate thioredoxin, vitamin C, and glutathione, which in turn can recycle vitamin E (Figure 8). LA markedly reduced the symptoms of diabetic pathologies, including cataract formation [15].

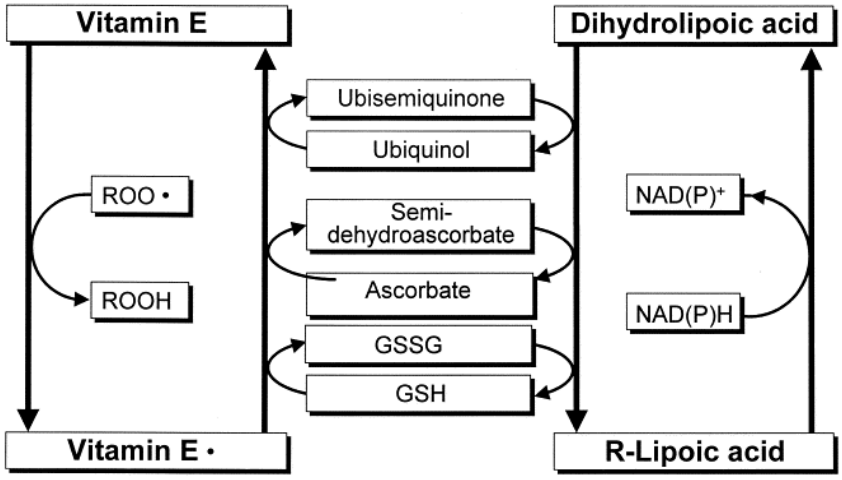

Figure 8: The antioxidant network showing the interaction between vitamin E, ubiquinol, vitamin C, glutathione, and R-lipoic acid redox cycles. When vitamin E scavenges a peroxyl radical, a vitamin E radical is formed. The vitamin E radical may be reduced at the interface between lipid

and water by several antioxidants such as ascorbate, ubiquinol, and reduced glutathione (GSH). DHLA can reduce all these antioxidants and be regenerated by several enzymes, including lipoamide reductase, GSH reductase, and thioredoxin reductase. Therefore, lipoic acid and DHLA take central positions in the antioxidant network. In addition, lipoic acid has water-soluble and lipid-membrane–soluble characteristics, thus enabling it to reduce oxidized antioxidants at the interphase between lipid and water. Adapted from [15].

Many other antioxidants such as carotenoids and some cofactors for antioxidant enzymes such as riboflavin can delay cataract progression [2]. Some potential anti-cataract agents such as paracetamol, aspirin and bendazac possess antioxidant activity and some metal-chelating activity [16]. The interaction network among free radicals, anitioxidants and lens biomolecules was proposed to affect cataract formation (Figure 9) [1].

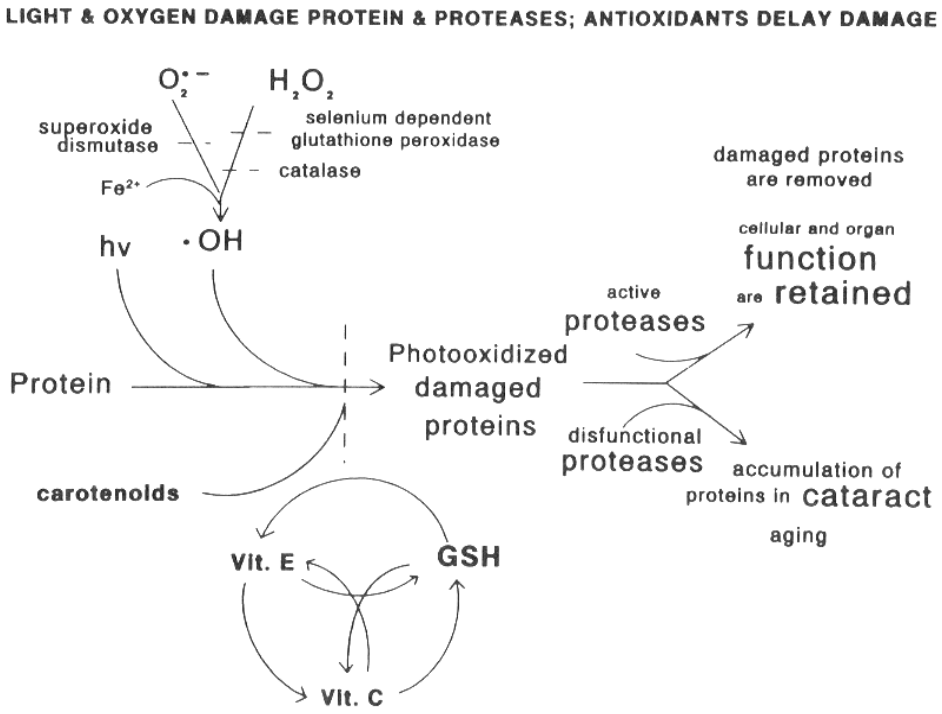

Figure 9: proposed interaction between lens proteins, oxidants, antioxidants, light, and antioxidant enzymes. Lens proteins are extremely long lived. They are subject to alteration by light and various forms of oxygen. Antioxidant enzymes such as SOD, catalase, and glutathione reductase/peroxidase protect them. These enzymes convert active oxygen to less damaging species. Direct protection is offered by antioxidants such as vitamin E, vitamin C, GSH and carotenoids. The levels of reduced form and oxidized forms of these molecules are determined by the redox status of the environment. If the antioxidative capability is not sufficient to protect the lipids and proteins in lenses, Cataractous opacities will appear. Adapted from [1].

Future studies

Many previous studies show that many oxidative species many induce cataract formation and many antioxidants may protect lens from getting opacities. At present, two main research directions thus seem to be promising sources of new insights into cataract formation. The following are two hypotheses waiting to be tested.

Hypothesis 1: Some significant changes in gene expression may be induced in vitro upon exposure to oxidants such as hydrogen peroxide in human lens cataractous lenses.

Oxidative stress is believed to be responsible for age-related cataract. To test this hypothesis, several researches are worthy to be carried out.

1. Molecular analysis of the tissue samples.

The tissue specimen many be obtained during surgical phacoemulsification. Careful classification of cataract type is very important for this experiment; since different type of cataract may has different gene expression and different pathogenic mechanisms responsible for cataract formation. We need to collect various samples and try to figure out different gene expression between normal lenses and various types of cataractous lenses.

2. Molecular analysis of lens epithelial cells.

As the lens contents are held within a thin transparent basement membrane, the lens capsule, which controls its shape and while allowing the passage of small molecules including some free radicals. Immediately behind the anterior capsule is a single layer of lens epithelial cells, which divide and move towards the equator where they elongate to form fiber cells. As they elongate they lose many organelles such as nuclei, mitochondria, ribosomes. So lens epithelial cells are much more accessible to free radials than the lens core and readily to change their gene expression upon changes of redox environment because of the presence of nuclei and mitochondria and ribosomes.

Since all lens cells are derived from lens epithelial cells, the gene expression and differentiation of lens epithelial cells have great significance to understand the effects that free radicals have on lens. We should try to assess the molecular changes that occur during oxidative stress such as hydrogen peroxide and identify differentially expressed genes in lens epithelial cells of cataract patients compared to normal donors. One of the up-regulated genes in the cataract samples was the anti-oxidant enzyme metallothionein, a free radical scavenger. It is possible that metallothionein is up regulated in human lens epithelial cells in response to oxidative insult. It is believed that many other anit-oxidant enzymes are waiting to be identified in lens. Likewise, redox-regulated transcription factors and redox-regulated genes such as NK-κB and AP-2 are likely to be modulated. Since maintaining an optimal redox environment needs energy and ionic balance, if the Na^{+}-Ka^{+} ATPase is out of order, detrimental gene regulation and expression will occur. So the normal function of Ca^{2+}-ATPase and Na^{+}-Ka^{+} ATPase is worthy to be tested.

The regulatory mechanisms of expression of genes encoding lens fiber membrane channel proteins, which are essential for maintaining the transparency of the lens, are our interest too.

Since mitochondrial gene expression is readily affected by oxidative reagents, using tissue culture cells of human lens epithelia to test mitochondrial gene expression when these cells are exposed to hydrogen peroxide is a good way to try. Many electron transport proteins' expression may be altered by oxidative stress in lenses.

3. Animal studies

Many models of cataract can be evaluated in animals such as galactose-induced cataract, adrimycin-induced cataract, and UV-induced cataract. For example, rat lenses are exposed to reactive oxygen species via an oxygen generating system. These lenses become opaque after several hours. Some antioxidant and redox-regulated gene expression are worthy to be analyzed in the control and oxidatively stressed lenses.

Hypothesis 2: normal expression and overexpression of antioxidants and antioxidant enzymes can inhibit free radical induced cataract formation.

Over exposure to UV light and radiation leads to cataract formation. Various studies showed that decreased level of antioxidants was observed in aqueous humor in cataract patients and SOD knockout mice readily formed cataract. The concept is widely accepted that cataract is formed because of imbalance between oxidative and anti-oxidative capabilities in lens. Based on this concept, the restoration of antioxidants can inhibit or at least retard cataractgenesis. Enhanced biological defense against free radical processes is an important way to avoid pathological conditions in cataracts.

1. Tissue culture experiments on lens epithelial cells

Some antioxidants such as GSH are good candidates to start these experiments. Since the loss of Trp and His content during all stages of brunescent cataract development was observed after UV irradiation [17] because they are the major targets for singlet oxygen, we may expose lens epithelial cells to singlet oxygen. These lens epithelial cells with addition of free radical scavengers should have less Trp and His destruction compared to lens epithelial cells with low antioxidant level.

Some antioxidant enzyme such as CuZnSOD, MnSOD, catalase, and GPx may play a role in prevention of peroxide formation which is implicated in cataract initiation. The lens epithelial cells are prone to free radical damage. The overexpression of antioxidant enzyme may decrease peroxide level in lens epithelial cells.

2. Animal experiment on cataract prevention.

Since the antioxidant and antioxidant enzyme levels are dramatically decreased during cataract formation, restoration of protective antioxidants and antioxidant enzymes should be able to prevent or retard cataract formation.

These experiments can be performed on rats. When adult rats were injected with an adenovirus (Ad) vector encoding human CuZnSOD or catalase cDNA, a mixture of both Ad vectors or a control Ad vector containing no exogenous gene into aqueous humor, the expression of human catalase and CuZnSOD should be increased in lens epithelial cells after infection with corresponding adenovirus. UV irradiation or any other free radical generating methods could be used to accelerate the formation of cataract. The retardation of cataract formation should be greater in rats injected with catalase and/or CuZnSOD Ad vector than in control rats when all of them are exposed to free radical damage.

Increased ascorbic acid and other antioxidant level in aqueous humor is also implicated in prevention of cataract formation and peroxide generation. These protective antioxidants can be delivered orally or intravenously or directly injected into aqueous humor. Control rats were given some placebo or distilled water. When both groups of rat cataracts are exposed to free radical generating agents, the group with higher antioxidants should develop less cataract formation compared to control groups. If this is the truth, there are further evidences to demonstrate that free radical is implicated in cataract formation and antioxidant and antioxidant enzymes have protective effects on lens. Some promising treatment that could be used in clinical trials may be developed based on this general idea.

Summary

A cataract is a whitening opacity of the normally transparent lens (and/or lens capsule) of the eye. This condition is the result of damage to the protein or lipid structure of the lens. Cataracts commonly accompany aging. They can also be congenital or precipitated by infection, injury, and side reactions to drugs, diabetic complications, cigarette smoking, and overexposure to radiation. The lens of the eye has two factors that limit its nutritional status: (1) it has an unusually high requirement for vitamin C; (2) the lens does not receive the nutrients it needs from the bloodstream but must get them indirectly, from adjacent tissues. These two factors make the lens of the eye especially vulnerable nutritionally. In cataract formation, the body's normal protective mechanisms are unable to prevent damage from free radicals. For its protection against free radicals, the lens has two lines of defence: one is the antioxidant nutrients (vitamin C, vitamin E, selenium, beta carotene, glutathione). Individuals with higher intakes of dietary antioxidants have a much lower risk of developing cataracts. The other protection is the enzymatic defences produced within the body (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase). However the mechanisms of cataract formation and protection are not completely discovered. Further studies are in great need in this field.

References:

1. Taylor A. (1992) Effect of photooxidation on the eye lens and the role of nutrients in delaying cataract. In *Free Radicals and aging.* ED. Emerit I, Chance B. PP: 266-279, Birkhauser Verlag. Switzerland.

2. Jacques PF. (1997) Nutritional antioxidants and prevention of age-related eye disease. *Antioxidants and disease prevention.* ED. Garewal HS. PP: 149-173. CRC Press. NY.

3. Harding J. (1991) Cataract (biochemistry, epidermiology and pharmacology). St Edmundsbury Press. NY.

4. Ottonello S, Foroni C, Carta A, Petrucco S, Maraini G. (2000) Oxidative stress and age-related catract. *Ophthalmologica.* **214:** 78-85.

5. Spector A, Garner WH. (1981) Hydrogen peroxide and human cataract. *Exp Eye Res*. **33**:673-681.

6. Bhuyan KC, Bhyuan DK. (1984) Molecular mechanism of cataract cataractogenesis: toxic metabolites of oxygen as initiators of lipid peroxidation and cataract. *Cur Eye Res.* **3:** 67-81.

7. Fu S, Dean R, Southan M, and Truscott R. (1998) The Hydroxyl Radical in Lens Nuclear Cataractogenesis. *J. Biol. Chem*. **273:** 28603-28609.

8. Garner B, Davies MJ, Truscott RJ. (2000) Formation of hydroxyl radicals in the human lens is related to the severity of nuclear cataract. *Exp Eye Res*. **70:** 81-8.

9. Kubo E, Miyoshi N, Fukuda M and Akagi Y. (1999) Cataract Formation through the Polyol Pathway is associated with Free Radical Production. *Exp. Eye Res.* **68:** 457-464.

10. Varma SD, Chand D, Sharma YR, Kuck JF, Richard RD. (1984) Oxidative stress on lens and cataract formation: role of light and oxygen. *Cur Eye Res.* **3:** 35-57.

11. Ohrloff C, Hockwin O. (1984) Superoxide dismutase (SOD) in normal and cataractous human lenses. *Graefe's Arch Clin Exp Ophthalmol*. **222:** 79-81.

12. Behndig A, Karlsson K, Reaume AG, Sentman ML and Marklund SL. (2001) In vitro photochemical cataract in mice lacking copper-zinc superoxide dismutase. *Free Radi Biol Med.* **31:** 738-744.

13. Donma O, Yorulmaz EO, Pekel H, Suyugul N. (2002) Blood and lens lipid peroxidation and antioxidant status in normal individuals, senile and diabetic cataractous patients. *Cur Eye Res*. **25**: 9-16.

14. Babizhayev MA, Deyev AI. (1989) Lens opacity induced by lipid peroxidation products as a model of cataract associated with retinal disease. *Biochimica et Biophysica Acta*. **1004:** 124-133.

15. Packer L, Kraemer K and Rimbach G. (2001) Molecular aspects of lipoic acid in the prevention of diabetes complications**.** *Nutrition.* **17:** 888-895.

16. Woollard AC, Wolff SP, Bascal ZA. (1990) Antioxidant characteristics of some potential anticataract agents. *Free Radi Biol Medi*. **9:** 299-305.

17. OrtwerthH BJ, Casserly TA and Olesen PR. (1998) Singlet Oxygen Production Correlates with His and Trp Destruction in Brunescent Cataract Water-Insoluble Proteins. *Exp Eye Res.* **67**: 377-380 .