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Antioxidant vitamins in cataract prevention

H. Gerster

Department of Human Nutrition and Health, F. Hoffmann-La Roche & Co., Ltd., Basel, Switzerland

Summary: The ocular lens, which is continually exposed to light and ambient oxygen, is at high risk of photooxidative damage resulting in cataract. Oxygen free radicals appear to impair not only lens crystallins which will aggregate and precipitate forming opacities but also proteolytic enzymes whose function it would be to eliminate the damaged proteins. Apart from an enzymatic defense system consisting of superoxide dismutase, catalase and glutathione peroxidase against excited oxygen species the lens contains the antioxidant vitamins C, E and presumably β-carotene as another line of defense. In vitro and in vivo studies in different animal species have demonstrated a significant protective effect of vitamins C and E against light-induced cataract. Sugar and steroid cataracts were prevented as well. Epidemiological evidence in humans suggests that persons with comparatively higher intakes or blood concentrations of antioxidant vitamins are at a reduced risk of cataract development. These positive findings established by several research groups justify extensive intervention trials with antioxidant vitamins in humans using presenile cataract development as a model.

Zusammenfassung: Die Augenlinse kann durch einfallendes Licht und Sauerstoff photooxidativ so geschädigt werden, daß eine Trübung bzw. ein Katarakt entsteht. Sauerstoffradikale schädigen nicht nur die Kristalline, spezialisierte Linsenproteine, die Aggregate bilden und präzipitieren, sondern sie greifen auch proteolytische Enzyme an, deren Funktion es wäre, die geschädigten Proteine zu eliminieren. Neben einem enzymatischen Abwehrsystem gegen Sauerstoffradikale, bestehend aus Superoxiddismutase, Katalase und Glutathionperoxidase, enthält die Linse die antioxidativen Vitamine C und E und evtl. Betakarotin. Tierversuche an verschiedenen Spezies haben sowohl in vitro als auch in vivo eine Schutzwirkung gegen lichtinduzierte Kataraktbildung aufgezeigt. Eine ähnliche Wirkung war gegen Zucker- und Steroidkatarakte nachweisbar. Epidemiologische Studien am Menschen haben gezeigt, daß Personen mit vergleichsweise höherer Einnahme bzw. höheren Blutkonzentrationen antioxidativer Vitamine ein vermindertes Risiko der Kataraktbildung haben. Diese positiven Befunde rechtfertigen die Durchführung breit angelegter Interventionsstudien mit antioxidativen Vitaminen am Menschen.

Key words: free radicals, cataracts, antioxidant vitamins, vitamin C, vitamin E, β-carotene

Schlüsselwörter: Katarakte, antioxidative Vitamine, Vitamin C und E, Betakarotin

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Introduction

The lens of the eye is a biconvex, highly elastic crystalline body situated in the front of the eyeball and embedded in aqueous humor. The optical function of the lens consists in focussing incoming light on the retina by altering its curvature in order to accommodate the sight for near or far vision. To fulfil its function optimally the lens is avascular and, apart from water, contains a large proportion of specialized protein, the crystallins. These characteristics render it highly transparent and limit the scatter of light. Human cataract is defined as opacity in the lens with progressively impaired vision, the final stage of which is blindness. In the majority of cases opacification is a gradual process lasting one to two decades or longer, but some types of cataract may follow a different course, and in certain metabolic disorders opacification may occur rapidly with total loss of visual acuity within a year.

Prevalence of cataracts

Cataracts are far more widespread than is commonly realized. According to the 1986 statistics of the World Health Organization (WHO), 14 million of the 28 million blind worldwide are blind due to cataract (1). In developing countries the rate is higher by a factor of 10 and its appearance is earlier by 10 years than in industrialized countries. In India alone, 5.5 to 6 million persons are blind from cataracts (2). The prevalence worldwide of lens opacities of various degrees has been estimated to be roughly 50 million (3).

In the United States the renowned Framingham Heart Study was extended to investigate possible visual disability in 2477 former participants in the Heart Study. The subjects were between 52 and 85 years old, and 384 persons (15.5 %) were found to have cataract in one or both eyes in combination with a visual acuity of 20/30 or less. Within the group there was a sharp increase in prevalence with age, the rate being consistently higher in females than in males (Fig. 1) (4).

In another large survey in the USA in about 3000 subjects aged 45 to 74 years the prevalence of senile cataract was found to be 14.7 % (5). If these samples are truly representative of the American population a prevalence of more than 7 million cataract cases can be extrapolated for the elderly. In accordance with the projected increase in the population of the over-55-year-olds from 47 million in 1980, to 86 million in the year 2030, the prevalence of cataract in the USA can be expected to grow equally by about 80 % in this period. The same trend is foreseeable for other industrialized countries as well as for developing nations (2).

Classification of cataracts

Cataract prevalence figures should be interpreted with caution because of the great differences in the diagnostic procedures used and because there is a lack of a simple and generally accepted classification system. A certain loss of lens transparency occurs almost universally with aging but does not necessarily progress to cataract formation. Therefore, the diag-

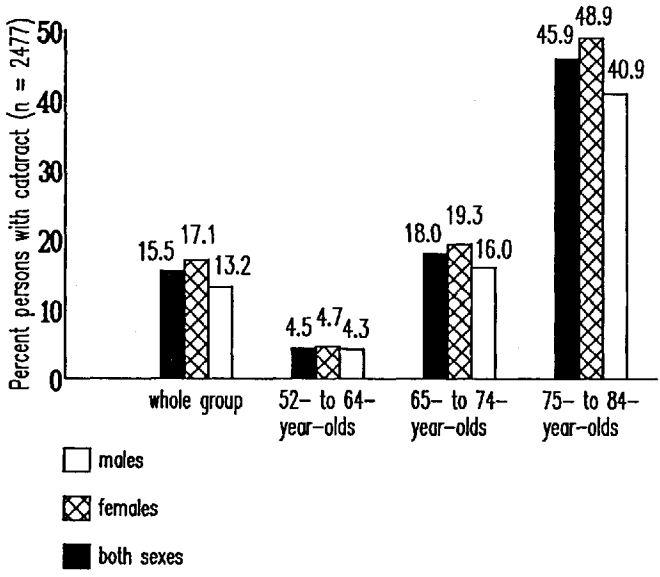

Fig. 1. Prevalence of cataract in the Framingham Eye Study (4).

nosis of cataract is often made only if opacity is accompanied by reduced vision (4, 5).

One classification system characterizes cataracts by their severity and distinguishes between immature (partial opacity), mature (total opacity with no swelling), and hypermature (total opacity with swelling) cataracts. Another characterization describes the site of the opacities in the lens, the three major ones being cortical, nuclear, and posterior subcapsular areas (6). It is also possible to distinguish cataracts by their presumed etiology as, for example, radiation cataract, degenerative or senile cataract, sugar cataract, and steroid cataract.

The methods of eye examination also vary greatly. Simple methods such as flash-light or ophthalmoscope examination may suffice for the epidemiological assessment of cataract prevalence and for the diagnosis of mature cataracts (7). More detailed, objective, and reproducible procedures are required for the diagnosis of early cataract, for studies trying to link single risk factors with specific morphological features of cataract, or for therapeutic trials in which the progression or regression of opacities needs to be closely observed. Various photographic methods and illumination techniques are available today to visualize extracted lenses or lenses in situ at various angles after dilatation of the pupils, often with the use of a slit lamp or slit lamp microscope. Computer programs have been developed to evaluate the photographs or their negatives, or standard pictures can be used for direct comparison (8).

In the USA the Cooperative Cataract Research Group (CCRG) has developed a field microscope with a simple classification system for the evaluation of extracted cataractous lenses by photographic means and has

Table 1. Risk factors for cataract formation.

Climatic conditions
Solar radiation (UV-light, 310–340 nm)
High altitude
Low latitude
Diseases
Diabetes
Diarrhea
Cardiovascular diseases
Respiratory diseases
Hypocalcemia
Galactosemia
Uremia
Phenylketonuria
Drugs
Corticosteroids
Major tranquillizers (barbiturates, MAO-inhibitors, phenothiazines)
Antihypertensives
Cytostatic agents
Occupations
Glass blowing
Steel work
Dental laboratory work
Outdoor work
Work with microwaves
Genetic predisposition
Race black > white
Indians > Bantus > whites
Oriental Israelis > European Israelis > Oxford population
Familial tendency

Adapted from (2, 13–15)

provided standard pictures (6). The system has now been adapted to cataract diagnosis in vivo and is being continually improved (9). It is useful, especially for large scale investigations.

The Scheimpflug photographic method with correction for depth produces UV-visible slit-lamp densitograms which can be evaluated by an automatic laser-scanning device. This sophisticated approach permits the detection of precataractous changes not visible by other techniques (10, 11). However, the equipment is expensive and requires highly trained personnel and at least 30 min for the examination of one lens (12).

Risk factors for cataract development

The sharp increase in the prevalence of cataract in the over 65-year-olds (Fig. 1), clearly points to age as a major risk factor. However, it is not known with certainty whether the aging process per se predisposes to cataracts or whether they are the result of the cumulative insults of a life time, or of reduced resistance or repair capacity of the lens. In the rela-

tively short period of human cataract research a large number of predisposing factors have been identified some of which are of course associated with increasing age (Table 1).

Of the many factors suggested to predispose to cataract development the damaging effect of excessive ultraviolet light is one of the most widely accepted. The etiological theory is based on findings that cataract is more prevalent in the south than in the north with the incidence being highest when long sunshine hours are combined with high altitude and low latitude, a situation that is found in the Tibet, which has indeed one of the highest cataract rates known (16). These epidemiological data are supported by experimental findings in animals showing that UV-radiation from sunlight or other sources induces cataractous changes in the exposed lens (17). The theory has however been challenged on the basis of findings in India which demonstrated no relation of cataract to sunlight but a very strong one to recurrent diarrhea and malnutrition (18).

Of the diseases associated with an increased prevalence of cataract, diabetes is the most important. Patients with adult-onset diabetes, in particular those with poor blood glucose control, appear to be at the greatest risk (19).

It has variously been stressed that the factors listed in Table 1 should not be regarded as single causes of cataract but rather as a complex of interacting factors increasing the risk of cataractogenesis. Several studies have been set up to learn more about the importance of the various cataract inducers (20).

Mechanisms of lens opacification

Great uncertainty still exists about the biochemical mechanisms leading to reduced lens transparency, especially regarding the question whether the different types of cataract develop via a common pathway or whether only one step or perhaps none is common to all forms.

One of the best understood, though still partly speculative, mechanisms is that of sugar cataract development which can be induced in laboratory animals by feeding them excessive amounts of glucose or galactose or by giving them streptozotocin, a diabetogenic agent. It is thought that the elevated blood sugar is metabolized by the enzyme aldose reductase to sugar alcohol which then accumulates leading to a high osmotic gradient with swelling of the lens and finally to lens protein oxidation, protein aggregation, and protein cross-linking. The gross changes in protein form lens opacities (19).

The mechanism leading to senile cataract could be the continual photo-oxidative insult of lens proteins by UV-radiation from sunlight. In the young healthy lens the damaged crystallins are eliminated by proteolytic enzymes. These proteases act as catalysts breaking the peptide bonds that join the constituent amino acids in proteins reducing them to their building blocks. The process of aging reduces not only the amount of proteolytic enzymes in the lens but also their capacity of removing damaged lens protein. Hence, aging decreases the repair capacity of the lens and thus promotes the aggregation and precipitation of damaged protein (3).

Table 2. Harmful effects of free radicals on cellular components.

Lipids	Peroxidation of polyunsaturated fatty acids in organelles and plasma membranes
Proteins	Oxidation of sulfhydryl-containing enzymes with inactivation of enzymes
Carbohydrates	Polysaccharide depolymerization
Nucleic acids	Base hydroxylation, cross-linkage, scission of DNA strands (causing mutation, inhibition of protein, nucleotide and fatty acid synthesis)

Adapted from (22)

In the development of senile or light-induced cataract as well as of sugar cataract, oxidation appears to occur as one of the steps. The possibility has been considered that oxidation may be involved in most or even all types of cataract, either as a primary or secondary event (21).

Oxidation reactions

Biochemical reactions involving oxygen may generate free radicals, defined as chemical species having one or more unpaired electrons in their outer orbitals. This state of imbalance renders them reactive and inclined to seizing the missing electrons from, or donating them to, other molecules that are stable. The high affinity of free radicals for other molecules induces a chain or cascade reaction of free radical generation.

Apart from the classical free radicals there exists a chemical species, singlet oxygen, which is unstable and highly reactive without being a radical. Singlet oxygen is a powerful inducer of superoxide radical formation. The cellular components that serve as targets for free radical attack include lipid membranes, proteins, carbohydrates, and nucleic acids (Table 2).

In the presence of ambient oxygen UV-light generates free radicals as well as radical precursors or intermediates as for instance hydrogen peroxide (H_2O_2) which, by acquiring an electron, forms the highly reactive hydroxyl radical. Singlet oxygen too can be produced by UV-light. Other factors generating free radicals include environmental pollutants such as ozone or nitrogen oxide, cigarette smoke, car exhaust fumes, alcohol, and certain drugs (22).

Potential protection against oxidation reactions

Oxidative stress with the formation of reactive oxygen species is a normal part of aerobic life. Intra- and extracellular metabolism of oxygen continuously produces free radicals in small amounts. In some instances activated oxygen species such as singlet oxygen can have a beneficial effect in physiological concentrations, for instance in killing aerobic bacteria, an activity which may depend on their presence. However, even low concentrations of free radicals would mostly be toxic to cells were it not

Table 3. Antioxidant action of vitamins.

Vitamin C (water-soluble)	Quenches singlet oxygen Stabilizes superoxide and other radicals Regenerates reduced vitamin E
Vitamin E (lipid.-soluble)	Quenches singlet oxygen Stabilizes superoxide and other radicals Stabilizes lipid membranes thus preventing production of lipid peroxidation Has sparing action on β-carotene
β-carotene (lipid-soluble)	Acts as the most efficient singlet oxygen quencher Has antioxidant properties at low oxygen pressure

Adapted from (27)

for the fact that an elaborate network of protective antioxidative factors had evolved in living organisms. Under physiological conditions the reactive oxygen species, oxygen free radicals and singlet oxygen, can be inactivated by enzymatic and non-enzymatic mechanisms. Most cells contain enzyme systems transforming free radicals to more harmless molecules; these enzymes include superoxide dismutase, catalase or glutathione peroxidase which is present in the lens in high concentrations. Another defense system involves free radical scavengers and quenchers, the most important being vitamin C, vitamin E, β-carotene, cysteine and uric acid. Depending on their solubility they are effective either in a lipid or aqueous environment. They can interrupt the chain reaction of free radicals if free radical generation is not so great as to overwhelm the defense network (23–26).

While the enzymes inactivating free radicals cannot be mobilized beyond a certain point, the level of antioxidant nutrients – vitamin C, vitamin E and β-carotene – can be increased according to the presumed needs of the organism for antioxidant protection (Table 3) (27).

The findings that oxidation reactions are important factors in cataractogenesis implies that the antioxidant vitamins may play a role in the prevention of cataract. Vitamin C and vitamin E were being studied as single entities in cataract prevention for several years before it was recognized that the antioxidant vitamins may be acting in concert in the free radical defense system. Thus only few experimental or clinical data are available considering the combination of these antioxidants in man.

Vitamin C in cataract prevention

Vitamin C concentrations in the eye

In diurnal species such as man, monkey, rabbit and guinea pig the concentration of vitamin C in aqueous humor is 10 to 50 times higher than in plasma and is also considerably higher in cornea and lens. On the other hand, nocturnal animals such as the rat have vitamin C levels in aqueous humor and lens that are hardly detectable. Human, monkey and rabbit embryos also have very low ocular vitamin C concentrations. Postnatally, however, vitamin C levels in the eye increase by a factor of 20 to 40 (29, 30).

Table 4. In vitro effect of vitamin C on cataract development.

Species	Cataractogenic agent	Remarks	Reference
Rat	photoirradiation	no damage to cation pump	29
Rat	photoirradiation	reduced lipid peroxidation	40
Rabbit	photoirradiation	protection of lens proteases	41,42

These outstandingly high vitamin C levels in eyes exposed to light and thus to oxidant stress have been considered suggestive evidence that a function of the vitamin is the antioxidative protection of structures, in particular the lens, that are sensitive to the effects of free radicals. Studies in guinea pigs indicate that it is possible to increase the concentration of vitamin C in aqueous humor and lens by dietary supplementation of the vitamin (31).

The vitamin C concentrations in the aqueous humor and lens tend to decrease with age as well as with progressing cataract formation. It has been speculated in the literature that this decrease was due to rapid oxidation of vitamin C which would form the brunescent opacities that may sometimes be seen in cataracts (32, 33). However, it was pointed out by others that for technical reasons this interpretation was not permissible because the spectra on which this hypothesis was based were unspecific (34). Moreover, a study in persons with senile cataract demonstrated that the uptake of vitamin C into aqueous humor after a loading dose of 500 mg of the vitamin was greatly delayed (35). A sluggish transport of vitamin C to aqueous humor was also suggested to occur in a study of patients with cortical cataract (36). The reduction of vitamin C in aging and cataractous eyes may thus be the expression of a degenerative process rather than of increased oxidation of the vitamin.

The damaging photochemical effect on the lens and the interaction with vitamin C can be demonstrated in different animal models (Tables 4 and 5).

Animal studies in vitro

Cataractous lens and aqueous humor of various species contain increased amounts of hydrogen peroxide (H_2O_2), a toxic metabolite of oxygen, and lenses incubated in H_2O_2 will develop cataractous changes (21). It may be formed from the superoxide anion radical by a dismutation reaction. H_2O_2, while not being a radical itself, can form the highly reactive hydroxyl radical.

Studies analysing H_2O_2 concentrations in aqueous humor of rabbit, guinea pig, and frog showed a linear relationship with vitamin C concentrations, and it was suggested that vitamin C was the primary source of H_2O_2 in this fluid (37). Vitamin C would thus have a deleterious rather than protective effect. However, this view has been contested, and it was suggested that the directly proportional concentration of H_2O_2 and vitamin C represented a dynamic equilibrium preventing the generation of more damaging oxidant species, H_2O_2 being a "relatively benign by-product of the non-enzymatic removal of more deleterious oxidant species such as free radicals or hydroperoxides" (38). No correlation between

vitamin C and H_2O_2 concentrations in aqueous humor of patients with cataract was noted by other authors (39).

A trial exposing rat lens to light in the presence of a photosensitizer demonstrated that the photochemical production of oxygen radicals led to physical damage of the lens reducing cation pump capacity. This damaging effect could be prevented by the addition of vitamin C as well as of superoxide dismutase (29).

Another expression of photooxidative damage of the lens is lipid peroxidation of lens membrane. This effect can be assessed by measuring malonedialdehyde (MDH), a breakdown product of lipid peroxides. In an in vitro study using rat lenses, lipid peroxidation was shown to be prevented by the enzymes superoxide dismutase and catalase as well as by vitamin C again supporting a protective effect of the antioxidant systems in the eye (40).

A new model for the assessment of photooxidative damage to the lens and its prevention is the measurement of protease function. Photooxidative action appears to reduce the capacity of lens proteases – among them of leucine aminopeptidase – to eliminate damaged protein which will then aggregate. A recent study demonstrated that the reduction of aminopeptidase activity, caused by photoirradiation of rabbit lens, could be delayed by vitamin C, and consequently protein aggregation was delayed as well. Another proteolytic pathway in the lens, the degradation of high molecular weight ubiquitin-protein conjugates has been shown in the same in vitro study also to be protected by the addition of vitamin C (41, 42).

While these in vitro models have the inherent disadvantage of not representing interacting processes of living organisms they do provide suggestive evidence for a protective role of vitamin C against free radical-induced damage to the cation pump, lipid peroxidation and against peptidase inactivation and protein aggregation in isolated lenses. This means that successive steps in the process of cataract formation, i.e., protein alteration and lipid damage may be modified by vitamin C (41).

Animal studies in vivo/ex vivo

An ex vivo study in guinea pigs appears to confirm the findings obtained in vitro. The animals were fed a diet either low (2 mg/day) or high (50 mg/day) in vitamin C for 21 days, and the lenses were then extracted and exposed to UV-light. Compared with low-dose vitamin C there was a clear protective effect in the high-dose vitamin C-group on the integrity of lens crystallins and proteases (43).

A protective effect of vitamin C has been demonstrated also against the development of cataracts induced by agents other than UV-light. Steroids are recognized to increase the risk of cataract formation, presumably also by oxidative damage to the lens. In chick embryos who were administered glucocorticoid, cataracts developed within 48 h. Vitamin C concentrations in the lens decreased rapidly and lipid peroxide levels increased. These developments could be prevented by giving vitamin C 3, 10 and 20 h after glucocorticoid administration (44, 45). Interestingly, the local administration of cortisone in the form of eye drops significantly reduces the vitamin C concentration in aqueous humor of albino rabbits (46).

Another animal model is the production of cataract in scorbutic guinea pigs by giving high concentrations of galactose, a form of sugar. The same type of cataract is found in patients with galactosemia, an inborn error of metabolism which leads to galactose accumulation in the eye due to impaired galactose biotransformation. In guinea pigs galactose cataracts could be delayed by the addition of vitamin C to the diet (47).

These studies provide some insight into the cataract-preventive effect of vitamin C, but obviously more detailed studies using in vivo models would be desirable. Recently, the Emory mouse with genetic cataract has been proposed as a model resembling human senile cataract (48). The model may be suitable but it must be remembered that the mouse is a nocturnal animal and, for studies investigating the effect of vitamin C, that in contrast to man this species can synthesize vitamin C according to its needs.

Studies in humans

Data on a possible role of vitamin C in the prevention or therapy of human cataract is scarce even though some pharmaceutical preparations contain vitamin C as one of several components. The reasons for the inclusion of the vitamin appear to be restitution of low vitamin C concentrations in aqueous humor associated with cataract formation and unspecific "biostimulation" (49).

Newer preparations have been designed on the basis of a therapeutic rationale such as the protection of the lens against oxidative damage. One prepartation tested in double-blind trials in 100 patients contains amino acids, vitamin B-6, which is necessary for amino acid metabolism, and vitamin C. The evaluation of the study was carried out by densitometric analysis of Scheimpflug photographs taken at the beginning and every 3 months of the trial. The results showed that treatment, which included about 350 mg vitamin C daily for 9 months, reduced or even halted the progression of cataract development in comparison with placebo (50).

The scientific rationale suggesting a role of vitamin C in cataract prevention and the corresponding findings in animal studies indicate that extensive investigations of the vitamin in man would be desirable.

Table 5. In vivo effect of vitamin C on cataract development.

Species	Cataractogenic agent	Vitamin C dose	Remarks	Reference
Guinea pig	UV light	2 mg or 50 mg/day	protective effect of high-dose vitamin C	43
Chick embryo	steroid	3 times 20 μmol/egg	reduced lipid peroxides	44
Guinea pig (scorbutic)	galactose	1.1 g/day	delayed cataractogenesis	47

Vitamin E in cataract prevention

Vitamin E concentrations in the eye

Much less is known about the distribution of vitamin E in the organism than about that of vitamin C, as the transport and diffusion of vitamin E is considerably more complex. It appears that vitamin E is not concentrated in aqueous humor and lens as preferentially as vitamin C (51). A study of the uptake of vitamin E in kittens has shown that the concentration of the vitamin in the eye can be increased by oral, intramuscular, and intravenous administration. However, the levels were not predictable and could vary significantly between the eyes of the same animal. No clear correlation with plasma vitamin E levels was noted (52). The analysis of extracted human eyes, lacking the lens, showed that the vitamin E concentrations were comparable to those in other non-adipose tissues (53).

The production of diabetic cataract in rats with the administration of streptozotocin led to decreasing vitamin E concentrations in the lens which was accompanied by an increase in lipid peroxide levels. These changes occurred before any significant opacity was notable (54).

Animal studies in vitro

A role of vitamin E in the maintenance of lens transparency was suggested by findings in rats. One-third of the offspring of mothers who had received a diet deficient in vitamin E and tryptophan had lens opacities in one or both eyes (55). In a study assessing a possible protective effect of vitamin E on photooxidative lenticular damage, rat lenses maintained in culture were exposed to light of visible frequency, and the extent of peroxidative degradation of lipids was assessed by measurements of malonedialdehyde (MDH). The addition of vitamin E to the medium substantially reduced the generation of MDH. This was interpreted as supporting the hypothesis that vitamin E could prevent the light-induced generation of oxygen-free radicals (56).

Cataract formation can be induced in isolated rat lenses with different forms of radiation including ionizing and microwave radiation. An in vitro experiment was conducted in which cataract development with damaged lens fiber cell membranes was achieved within 24 h of using ionizing radiation, a process that normally takes several months in animal experiments. As could be shown, membrane damage was greatly diminished by the addition of vitamin E to the medium. It was postulated that the protective effect consisted either in free radical scavenging or in increasing membrane fluidity and prevention of cell membrane leakiness, considered an early event in cataractogenesis (57).

In other in vitro studies cataract formation was induced by incubating rat and gerbil lenses with glucose. Opacities in gerbil lenses developed within 24 h, those in rat lenses within 96 h. The faster formation in gerbil lenses was attributed to the higher aldose reductase activity in gerbils leading to a faster enzymatic transformation of sugar to sugar alcohol and thus osmotic stress. In this model the participation of oxidative damage was excluded by the finding that the antioxidants vitamin E and glutathione did not prevent cataract formation (58). Others did however demonstrate a protective effect of vitamin E in vitro against cataracts induced by elevated glucose and

Table 6. In vitro effect of vitamin E on cataract development.

Species	Cataractogenic agent	Remarks	Reference
Rat	photoirradiation	reduced photoperoxides	56
Rat	ionizing radiation	reduced cell membrane damage	57
Rat/gerbil	glucose	no effect	58
Rat	glucose/galactose	effect against glucose, partial effect against galactose cataract	59
Rat	steroid	reduced lens damage	60
Rat	high temperature	prevention of cataractogenesis	61

partly against those induced by elevated galactose levels (59). These findings suggest that sugar cataract formation is a complex process, probably involving both oxidative and osmotic stress.

Still other in vitro models in which a protective effect of vitamin E was observed include steroid cataracts and cataracts induced by elevated temperatures (60, 61) (Table 6).

As in the case of vitamin C, the in vitro findings of a beneficial effect of vitamin E on the lens have been confirmed using in vivo animal models (Table 7).

Animal studies in vivo

Cataractogenesis by oxidative damage was studied in the Emory mouse with the use of the Scheimpflug camera. The addition of 30 mg vitamin E/kg of the diet and the weekly intraperitoneal administration of 10 mg vitamin E considerably delayed the occurrence of opacities compared with controls (62). When 3-aminotriazole, an inhibitor of catalase, was used in rabbits as a prooxidant cataractogenic substance it could be shown that the progression of cataract formation, assessed by slit-lamp microscopy, was arrested in about 50 % of the animals after 2 to 16 weeks of parenteral vitamin E administration in doses of 50 mg/kg body weight daily. Superoxide was 70 % lower in aqueous humor and MDA was 33 % lower in the lens of vitamin E-treated animals than of untreated animals. Vitamin C levels in aqueous humor, which were reduced by 60 % in untreated cataractous rabbits, were normalized by vitamin E treatment (63). These findings underline the importance of free radical mechanisms in cataractogenesis and the defense afforded by antioxidants such as vitamin E (64).

In the case of sugar cataracts the findings in different in vivo studies are partly contradictory. In one study the administration of vitamin E in doses of 10 and 20 mg/100 g body weight to rats and 15 and 30 mg/kg body weight to rabbits together with galactose as cataractogenic agent resulted in a rate of cataract formation that, compared with controls, was reduced by 50 % to 70 %, as well as in a delay in the appearance of various cataract stages as judged by naked eye and ophthalmoscopic evaluation (65). No effect of vitamin E (5 g/kg diet) was noted on galactose-induced cataracts in a study in rats, but cataracts caused by glucose were successfully prevented by vitamin E. Cataract formation was assessed by scanning electron microscopy (59), a method that has been severely criticized in the context of cataract studies (66).

Table 7. In vivo effect of vitamin E on cataract development.

Species	Cataractogenic agent	Vitamin E dose	Remarks	References
Emory mouse	genetic degeneration (senile cataract)	30 mg/kg diet plus weekly 10 mg i.p.	delayed opacities	62
Rabbit	aminotriazole	50 mg/kg body weight	• arrest of development • reduced H_2O_2 in aqueous • reduced lens peroxides • normalized vitamin C in aqueous	63
Rat	galactose	10 and 20 mg/100 g body weight 15 and 30 mg/kg body weight	reduced rate and delayed occurrence	65
Rat	galactose glucose	5 g/kg diet 5 g/kg diet	no effect protective effect	59
Rat	streptozotocin	1 g/kg body weight s.c.	reduced lens damage	67

Streptozotocin-diabetic rats with highly increased blood glucose levels again responded to treatment with vitamin E (subcutaneous injections of 1 g/kg body weight) by showing only minimal lens changes compared with an untreated control group which had greatly damaged lenses. The protective effect of vitamin E was evident in spite of unchanged levels of fructose and glucose in the lenses (67).

The reasons for the contradictory findings in galactose cataracts are not yet known. The fact that a partial response to vitamin E was noted in some studies and that an effect of the antioxidant vitamin C was also demonstrated may be an indication that oxidation participates at one stage of galactose cataractogenesis though other factors may be more important.

The known biochemical functions of vitamin E as a lipid antioxidant in cell membranes and the positive findings of a protective effect of the vitamin against cataract development induced by various agents justify extensive investigations in human cataract.

β-carotene in cataract formation

The idea that β-carotene may be involved in the antioxidant defense system regarding cataract formation stems from its light-protective function. The idea is however quite new so that no data have so far become available on the concentration of β-carotene in the eye, and no in vitro and in vivo animal studies of cataract prevention have been performed.

An important biochemical function of β-carotene is the quenching of singlet oxygen (68, 69). It has moreover been shown also to act as a lipid antioxidant inhibiting free radical induced lipid peroxidation in membranes (70). β-carotene is unusual in that it is most effective as an antioxidant at low oxygen pressure (71), and it could thus complement the antioxidant action of vitamin C, vitamin E, and the antioxidant enzymes.

Photoprotective effect of β-carotene

Indirect evidence for a photoprotective effect of β-carotene was obtained in a study investigating changes in β-carotene plasma levels in human volunteers after repeated exposure to UV-radiation. The levels were found to be significantly reduced. This was thought to result from an interaction of β-carotene with singlet oxygen and free radicals generated by photooxidation (72, 73).

More directly, a photoprotective effect was demonstrated in patients with erythropoietic protoporphyria, a genetic disorder with high skin sensitivity to light (380–560 nm). Since the findings in the early 1970s that β-carotene considerably improved the tolerance of the majority of patients to sunlight (74) β-carotene has remained the treatment of choice in erythropoietic protoporphyria. It is important to adjust the dose of β-carotene to the individual requirements of the patients, which may vary considerably (75).

It would not be permissible, of course, to deduct from these positive findings a protective effect of β-carotene also against photooxidative damage to the lens. Nevertheless, the biochemical role of β-carotene as a singlet oxygen quencher and antioxidant and the clinical findings of a

photoprotective effect in the skin strongly support the performance of animal studies investigating various types of cataract formation in analogy to the studies with vitamin C and vitamin E. Studies using a combination of antioxidant vitamins will be valuable as well. If an anticataract effect of β-carotene can be confirmed, investigations in humans will be indicated.

Combined antioxidants in human cataract prevention

As the concept of a protective role of an antioxidant defense system against cataract formation is quite new hardly any trials have been carried out in man. A notable exception is a recent study in which the antioxidant status of 112 persons – 77 with and 35 without cataract – was determined by the analysis of the enzymes superoxide dismutase, glutathione peroxidase and glucose-6-phosphate dehydrogenase in erythrocytes as well of vitamin E, vitamin C and β-carotene in plasma. The eye examinations included tests of visual acuity and slit-lamp biomicroscopy.

The enzyme activities in erythrocytes did not differ between those with and those without cataract. However, subjects with cataract had significantly lower plasma levels of at least two antioxidant vitamins indicating that persons with higher levels of antioxidant vitamins may be at a reduced risk of cataract development. The lack of an inverse correlation between enzyme activity and risk of cataract development was unexpected. A possible explanation is that while the concentrations of the antioxidant vitamins in plasma and lens have a direct relationship, the enzyme activity in red blood cells may not reflect enzyme activity in the lens (76).

Recently, a case control study examining the supplementary intake of vitamin C and vitamin E was concluded in 175 cataract patients needing surgery and in an equal number of matched disease-free controls. Most subjects who did take supplements used doses of 300–600 mg vitamin C and 400 mg vitamin E. The analysis of the comparison between supplement users and non-users showed that those taking them had a significantly lower incidence of cataract, or conversely that the group of cataract surgery patients included significantly fewer subjects who had taken supplementary vitamin C and vitamin E (77).

These studies provide preliminary evidence for a protective role of the antioxidant defense system in cataractogenesis in humans justifying double-blind intervention studies with an antioxidant combination. A suitable model may be the selection of persons with presenile cataract in whom the progression of opacities can be followed with or without prophylactic treatment.

Conclusions

The role of the lens is to collect and focus light on the retina. It is therefore permanently exposed to photooxidative stress which may cause, or at least contribute to, the development of cataracts. Lens opacities can be initiated also by agents other than light as for instance high blood sugar, steroids or ionizing radiation. It is possible that in these cases too oxidation occurs at one point in the chain of events leading to cataract.

Aging appears to be the greatest risk factor for the development of lens opacities. The reason may be cumulative effects of photooxidative insults leading to the generation of excited oxygen species, i.e., free radicals and singlet oxygen. The lens contains elaborate antioxidant defense systems to respond to oxidative attack. One system comprises enzymes which deactivate specific excited oxygen species. They include superoxide dismutase, catalase, and glutathione peroxidase. The process of aging is associated with progressive reduction in the activity of these enzymes which in turn increases the susceptibility of aging lenses to oxidative damage (78).

Another line of antioxidant defense comprises vitamin C, vitamin E, and β-carotene. They act as free radical scavengers or singlet oxygen quenchers or both. Particularly vitamin C is present in the lens and aqueous humor in high concentrations. The antioxidants not only act singly against oxidation reactions but may also support each other in their function. Vitamin E, which has an important role in the protection of lipid cell membranes by interacting with free radicals, is regenerated by vitamin C. It can maintain its scavenging activity as long as vitamin C is present (79–81). Recently, it has been shown that vitamin C and glutathione can exert their protective effect in the induction phase of microsomal lipid peroxidation only if vitamin E is present in the membrane (82).

The process of cataract formation involves photooxidative damage to lens proteins, the crystallins, which aggregate and cross-link forming opacities. Normally, damaged proteins and protein fragments are eliminated by proteolytic enzymes. As these proteases are proteins themselves they may undergo the same photooxidative damage as the crystallins. They have been shown to lose their activity in the lens with increasing age and oxidative stress. It is therefore of great interest that vitamin C was shown to protect lens proteases in vitro against photooxidative damage.

The clinical relevance of the protective antioxidant network against free radicals which can be demonstrated so elegantly in experimental models has been questioned (83). However, in the case of cataracts induced by photooxidative effects, steroids, glucose and partly galactose, the evidence of a protective effect of vitamin C and E both in vivo and in vitro is quite convincing. β-carotene should theoretically also prevent cataract formation but it still needs to be tested in the various animal models. The situation in human beings has so far not been investigated in depth. The only evidence for a protective effect of the antioxidant vitamins comes from an epidemiological study which showed that persons with a higher intake of at least two antioxidant vitamins were at a lower risk of cataract formation than those with lower intakes, and from a case-control study in which cataract patients were found to have taken significantly fewer supplements of vitamin C and vitamin E than healthy controls.

Large-scale intervention studies in humans are now indicated to determine whether dietary supplementation with the vitamins will afford antioxidative protection and delay the occurrence of lens opacities.

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Author's address:

H. Gerster, F. Hoffmann-La Roche & Co. Ltd., Grenzacher Straße 124, 4002 Basel, Schweiz