Documenta Ophthalmologica **88:** 339–344, 1995.

UV-B as a pro-aging and pro-cataract factor

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Key words: Light damage, UV-B, Aging, Cortical cataract, Cataract location, Ozone

Abstract. One of the functions of the human lens is filter light between 300–400 nm from reaching the retina. The lens is therefore continually under photooxidative stress. In the young lens the primary absorbing species is the O-beta glucoside of 3-hydroxykynurenine (3-HKG) which has a maximum at 365 nm. Photophysical studies have demonstrated that absorptions by this compound in the short term are relatively benign to the lens, but in the long term can lead to the photochemical loss of 3-HKG with the concomitant yellowing of lens proteins. It will be proposed that part of this yellowing is due to the photochemically induced attachment of 3-HKG to lens proteins. The yellowing of lens proteins leads to a drastic increase in the number of photons absorbed by the lens. This, along with the age-related losses of antioxidants such as GSH will increase the photooxidative stress on the lens. Considering the foregoing and various epidemiological, model and biochemical studies, it can be concluded that light is most likely one of the causative factors in cataractogenesis.

Besides the skin, the eye is the only part of the body that is continuously subjected to ambient radiation. In the eye the main function of the anterior ocular tissue, the cornea and the lens, is to transmit and focus light on the retina undistorted. They perform a further function, though, which is to filter out short ultraviolet wavelengths of light from the retina. In the case of the human eye, this means that wavelengths below 400 nm do not reach the retina. The wavelengths of light filtered out, however, are absorbed by the cornea and lens, and can produce deleterious effects in those tissues. These effects may occur at ambient radiation over an extended period or at high doses over a short period of time. This paper examines the possible implications of ambient radiation impinging on the human lens and its possible role in aging and senile disease processes in that tissue.

Each absorption by the lens can lead to a photochemical change which can eventually result in biological malfunction. Unlike other tissues like the skin, cornea and retina, the lens contains little repair or turnover. It has therefore developed various strategies, some of which are unique to the lens, to minimize photic damage.

Scavengers and anti-oxidant enzymes. The lens contains glutathione, ascorbic acid, and vitamin E; all of which quench various photochemical or oxidative reactions. These decrease phototoxic effects of light by either interacting directly with the excited sensitizer; thus bringing it back down to the ground state or by neutralizing reactive intermediates that have been formed. In addition various enzymes such as catalase and superoxide dismutase scavenge hydrogen peroxide and superoxide respectively.

Oxygen levels in the lens are low. This parameter is important to any photochemical process since many reactions are slowed down or actually negated at low O_2 tensions. In general, oxygen enters the eye in part through the cornea. From there to the lens a gradient exists with decreasing concentrations of oxygen with a possible range of from 24–72 mm Hg. This is approximately 20% the concentration found in blood.

The photophysics of the chromophores in the lens. The two main chromophores that absorb radiant energy transmitted by the cornea in the young human lens are protein bound tryptophan and the O-Beta Glucoside of 3-hydroxkynurenine (3-HKG), which absorb 5 and 95% of the photons respectively (using the spectral output of the sun).

3-HKG is a very inefficient sensitizer of lens [1]. Since 3-HKG has an emission at 430 nm, time resolved fluorescence studies were performed in an attempt to ascertain a photophysical explanation for its relative inactivity. Experiments were performed on the isolated 3-HKG, an extract from a human lens homogenate and on an intact lens. It has a lifetime of 31 ± 4 ps (almost within the excitation pulse profile) while the other two samples had lifetimes of 44 ± 5 and 61 ± 5 ps respectively [2]. The increase in lifetimes presumably mirrors an increasingly rigid environment. These results are consistent with studies on simpler analogous compounds (e.g. ortho hydroxybenzophenones) where an excited state intramolecular proton abstraction has been proposed to provide an efficient deactivation pathway.

Thus, the primary function of this compound in the human lens appears to be to protect the young human retina from radiation between 295–400 nm while minimizing damage to the lens. This is an important conclusion for the viability of both the lens and the retina.

Since the constituents of the lens do not turnover, any changes that occur tend to accumulate. For the lens cytosol proteins, these include polymerization, charge changes and the formation of lower molecular weight polypeptides (reference [3] for a review). In addition, the concentration of 3-HKG decreases with age, reaching approximately one third of that found in the young human lens by the fourth decade. During that period of time the total filtering capacity of the human lens actually increases due to the generalized yellowing of the lens proteins. This color increases the absorptive characteristics of the lens between 300 and 400 nm with end absorptions out to 550 nm, adding to the possible initiators of photochemical processes.

At the same time that the absorptive characteristics of the lens is increasing, some of the enzyme systems and scavengers that protect it from photochemical insult decrease. This includes the glutathione enzymes, GSH and superoxide dismutase.

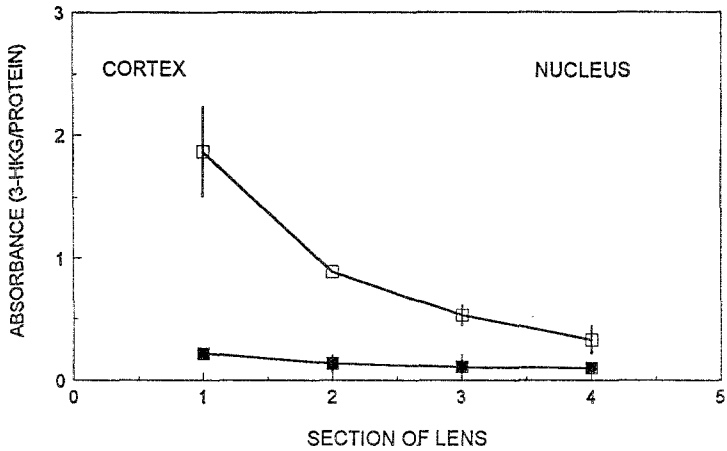

Fig. 1. The ratio of 3-HKG to yellow lens protein as measured at 365 nm for thirty (□) and sixty year olds (■) from the outer cortex to the nucleus.

Two questions will be addressed:

1) How much and at what age do the presence of these new chromophores increase the photic stress on the lens.

2) What is the mechanism of their formation.

The relative abundance of these absorbing species varies with age and as a function of position within the lens. In the young human lens absorptions between 295 and 400 nm are due primarily to 3-HK and 3-HKG with minor contributions from tryptophan. By middle age the yellow material competes for absorptions with the kynurenines. Fig. 1 presents the ratio of 3-HKG to yellow lens protein as measured at 365 nm for human lenses in the 3rd through 6th decades. In thirty year olds, in the outer cortex, the kynurenines absorb much of the ambient radiation. This is gradually reversed in favor of the yellow compounds, spatially, towards the nucleus. In sixty year olds, absorptions are due almost exclusively to the age related components [4].

In an attempt to estimate the relative importance of the various lens chromophores *in vivo*, the relative quanta of each species at each wavelength was calculated. For the purpose of this study the spectra will be presented with equal absorptions at 365 nm. By taking into account the transmission characteristics of the cornea, the absorption of each species and assuming the sun as a light source, it can be calculated that by the fifth decade, there is a doubling of the total photons absorbed by the lens.

The substitution of the yellow compounds for the kynurenines as the main absorbing species in the lens would increase the overall number of photons absorbed by that organ. Therefore at high intensities or over long periods of time, yellow lens protein can accelerate the damage to the lens over that

due to the kynurenines. This, taken with the fact that there is an age related decrease in various radical scavengers in the lens gives a plausible explanation for the epidemiological correlation between light exposure and cataracts. It must be emphasized that this long term damage is not due to the age related formation of an intrinsically efficient sensitizer attached to lens protein but to the increased absorption of light by an inefficient sensitizer.

The age related loss of 3-HKG and concomitant formation of yellow material attached to lens proteins suggests a causal relationship. We therefore investigated the possible photochemical attachment of 3-HKG to lens proteins as a possible model for aging of the human lens. 3-HK (as a model for 3-HKG) was photolyzed in the presence of 1 M glycine and absence and presence of oxygen. Glycine was used as a mimic for the lens milieu since 1) high concentrations can be attained and 2) it contains a free amino group [5].

Photolysis leads to the formation of two fluorescent photoproducts with emissions at 460 nm (blue) and 520 nm (green). These form in the presence but not in the absence of Glycine. Neither of these fluorophores form in the dark and the green fluorophore is a photoproduct of the blue compound. In addition, the formation of the green, but not the blue fluorophore is oxygen dependent and formation of both are retarded by the presence of penicillamine, a glutathione mimic.

Compounds that have these fluorescent characteristics have been detected by numerous lens investigators and have been used as markers of age related changes in the human lens. In general they have found that the blue fluorophore forms early in life and it increases throughout. The green fluorophore apparently forms later [6] and in one study (see below) its increased presence has been associated with the formation of cataracts. These results strongly suggest that many of the age related changes in the human lens are light dependent.

The major pathology of the lens is cataract formation. This is a multifactoral disease, whose etiology has been investigated on a number of levels; epidemiologically, in model systems, by an assessment of the biochemical changes during the course of cataractogenesis and the location of the cataract.

Epidemiological. An increased risk of cataract has been found for a number of variables; disease (diabetes), malnutrition, less anti-oxidant index and greater exposure to light. This last parameter has been an area of contention, but the preponderance of evidence suggests that increased light exposure is a verifiable factor in the early onset of at least cortical cataractogenesis [7]. An additional factor that has been advanced is episodic diarrhea. The extent to which this is involved appears to be determined by the number and duration of life threatening diarrhea and subsequent dehydration.

Model systems. A second approach is to assume a cataractous mechanism and develop a model system to test that hypothesis. Light models have been tested on the molecular, tissue and animal levels. Many of the studies on tissue and whole animals may not be relevant to humans since lower animals (e.g. rats, rabbits) were used in the studies. Unlike skin, the cornea and retina, the optical characteristics of the young primate lens is considerably different than that of lower species. This is due to the presence of 3-HKG. As the lens ages these differences become even greater.

The only lower species whose optical characteristics are similar to the primate is the gray diurnal squirrel. In a recent study, Zigman *et al.* [8] exposed gray squirrels to subsolar light for an extended period of time (2 yrs). The lenses were examined for morphological, histological and biochemical changes. They found many of the same changes found in the human lens during cataractogenesis.

Biochemical parameters. A third approach is to examine the chemical changes that occur during cataractogenesis and attempt to interpret them in terms of the mechanism by which they were formed. During cataract formation there is extensive oxidation of the sulfhydral residues; cysteine to cystine and methionine to methionine sulfoxide. In more severe cataracts this oxidation may go further to cysteic acid and methionine sulfone respectively. These are the same residues that react with singlet oxygen, a photochemically generated reactive intermediate.

In addition Yappert *et al.* [9] recently reported that the green fluorophores that we generated in our photochemical model system are increased in cataracts. Both of these molecular studies suggest the causal relationship of light in cataract formation.

Location. A final approach is to assess the location of cataracts and correlate their production with light exposure. Almost all light that reaches the lens is scattered. In an experiment assessing the optics of the cornea, Coroneo *et al.* [10] showed that light passing through the cornea is preferentially focused onto the lower nasal portion of the lens. In addition, epidemiological studies [11, 12] clearly demonstrated that most (65%) cortical cataracts formed initially in that area of the lens.

Conclusions

Light is a continual stress on the lens.

This stress increases with age, due to both increased absorptions and the loss of some anti-oxidants.

Based on epidemiological, model, and molecular studies, and the location of cortical cataracts, light is most likely one of the causal factors in cataractogenesis.

It must be emphasized that a clear causal epidemiological relationship between increased light and cataract formation need not be established for light to be a major factor in cataractogenesis. At constant light levels other factors are equally or more important such as the loss of anti-oxidant ability (due to nutrition or disease), increased absorptions (due to drugs) or increased oxygen. All of these factors would increase the likelihood of light damage.

The prudent course then is to assume that light is a factor in cataractogenesis and take proper precautions.

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