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Journal of Photochemistry and Photobiology B: Biology 64 (2001) 136–143

Journal of Photochemistry and Photobiology B:Biology

www.elsevier.com/locate/jphotobiol

Ocular phototoxicity

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Abstract

The human eye is constantly exposed to sunlight and artificial lighting. Therefore the eye is exposed to UV-B (295–320 nm), UV-A (320–400 nm), and visible light (400–700 nm). Light is transmitted through the eye and then signals the brain directing both sight and circadian rhythm. Therefore light absorbed by the eye must be benign. Damage to the young and adult eye by intense ambient light is avoided because the eye is protected by a very efficient antioxidant system. In addition, there are protective pigments such as the kynurenines, located in the human lens, and melanin, in the uvea and retina, which absorb ambient radiation and dissipate its energy without causing damage. After middle age there is a decrease in the production of antioxidants and antioxidant enzymes. At the same time, the protective pigments are chemically modified (lenticular 3-hydroxy kynurenine pigment is enzymatically converted into the phototoxic chromophore xanthurenic acid; melanin is altered from an antioxidant to pro-oxidant) and fluorescent chromophores (lipofuscin) accumulate to concentrations high enough to produce reactive oxygen species. We have known for some time that exposure to intense artificial light and sunlight either causes or exacerbates age-related ocular diseases. We now know many of the reasons for these effects, and with this knowledge methods are being developed to interfere with these damaging processes.

Keywords: Pterygia; Cataracts; Macular degeneration; Uveal melanoma; Oxidative stress

1. Introduction

Aside from the skin, the organ most susceptible to sunlight induced damage is the eye. While light transmission through the eye is fundamental to its unique biological functions of directing vision and circadian rhythm, at the same time exposure to the intense light of the sun can pose a particular hazard: it can lead to impaired vision and, eventually, blindness. Such exposure can come with outdoor employment as well as living at low latitudes or high altitudes and/or from the reflection of light off of water, sand, or snow [1–4].

1.1. Structure of the eye

The human eye is composed of several layers. The outermost layer contains the sclera, whose function is to protect the eyeball, and the cornea, which focuses incoming light onto the lens. Beneath this layer is the choroid containing the iris which is known as the uvea. This region contains melanocytes which contain the pigment melanin, whose function is to prevent light scattering. The opening in the iris, the pupil, expands and contracts to control the amount of incoming light. The iris and the lens are bathed in the aqueous humor, a fluid that maintains intraocular pressure; this fluid also contains various antioxidants. Transport to the lens is through the aqueous. The lens is positioned behind the iris. The function of the lens is to focus light onto the retina.

Behind the lens is the vitreous humor, a fluid that supports the lens and the retina and also contains antioxidants. The retina is composed of the photoreceptor cells (rods and cones) that receive light and the neural portion (ganglion, amacrine, horizontal and bipolar cells) that transduces light signals through the retina to the optic nerve. Behind the photoreceptor cells are the retinal pigment epithelial cells, Bruchs' membrane, and the posterior choroid. The photoreceptor cells are avascular and their nutrient support (ions, fluid and metabolites) is provided by the retinal pigment epithelial cells. There is transport to the retinal pigment epithelial cells across the Bruch's membrane by the choriocapillaris.

2. Factors that determine light damage

The effect of ambient light on the eye must be largely benign, as it serves fundamental biological functions. However, there are several conditions under which am-

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1011-1344/01/$ – see front matter
PII: S1011-1344(01)00196-8

bient light exposure becomes harmful. To determine whether light is damaging, one must consider the following factors: intensity, wavelength, site of damage, oxygen tension, chromophores, defense systems, and repair mechanisms.

2.1. Intensity

The greater the intensity of light the more likely it is to damage the eye. Light that may not be ordinarily harmful can do acute damage if it is sufficiently intense. For example, it is well known that the eye can be damaged (temporarily or permanently) by exposure to reflective sunlight from snow (snow blindness), or from staring at the sun during an eclipse. Similarly, the eye can sustain damage from medical light sources, such as lasers during surgery or from accidental exposure to laser pointers [5]. Cumulative light damage results from less intense exposure over a longer period of time and is often a result of an underlying age related loss of protection [1–3].

2.2. Wavelength

Ambient radiation from the sun or from artificial light sources contains varying amounts of UV-C (220–290 nm), UV-B (290–320 nm), UV-A (320–400 nm), and visible (400–700 nm) light. The shorter the wavelength, the greater the energy and therefore the greater the potential for biological damage. However, although the longer wavelengths are less energetic, they penetrate the eye more deeply [6].

In order for a photochemical reaction to occur in the eye, the light must be absorbed in a particular ocular tissue. The primate/human eye has unique filtering characteristics that determine in which area of the eye each wavelength of light will be absorbed. All light below 295 nm is cut off by the human cornea. This means that the shortest, most energetic wavelengths of light (all UV-C and some UV-B) are filtered out before they reach the human lens. Most UV light is absorbed by the lens, but the exact wavelength range depends upon age. In adults, the lens absorbs the remaining UV-B and all UV-A (295–400 nm) and therefore only visible light reaches the retina. However, the very young human lens transmits a small window of UV-B light (320 nm) to the retina, while the elderly lens filters out much of the short blue visible light (400–500 nm). Transmission also differs with species; the lenses of mammals other than primates transmit ultraviolet light longer than 295 nm to the retina [7–9].

2.3. Site of damage

In order for a photochemical reaction to occur in the eye, it is necessary that the light be transmitted to a particular ocular tissue and then be absorbed by a chromophore located at that site.

2.3.1. Cornea

Corneal epithelial and endothelial cells may be easily damaged leading to keratitis [10,11]. However, these cells have a very efficient repair mechanism and the damage is rarely permanent.

2.3.2. Uvea

Iris pigment epithelial cells and uveal melanocytes [12] are exposed to ultraviolet radiation and visible light. However these cells are highly pigmented (melanin) and are protected against damage unless that exposure is long term or the cells are aged.

2.3.3. Lens

The lens is composed of two parts that are susceptible to damage: the (outer) epithelial cells and the (inner) fiber membrane. The epithelial cells control transport to the lens. They have direct contact with the aqueous and are most vulnerable to phototoxic damage. Damage to these cells would readily compromise the viability of the lens [13]. The fiber membrane can be photochemically damaged through damage to the lipids and/or to the main intrinsic membrane protein [14,15]. Phototoxic reactions can lead to a modification of DNA and certain amino acids (histidine, tryptophan, cysteine) and/or a covalent attachment of the sensitizer to cytosol lens proteins [16–18]. Covalently bound chromophores may then act as endogenous sensitizers and produce prolonged sensitivity to light. In addition, there is non-photochemically induced modification of lens proteins associated with diabetes. A high glucose concentration in the lens has been found to lead to the glycosylation of epsilon-amino groups of lysine residues. All of these types of damage will result in a change in the refractive index of the lens material, leading to aggregation and ultimately opacification (cataractogenesis) [19]. Since there is little turnover of lens proteins this damage is cumulative.

2.3.4. Retina

Phototoxic damage can occur in retinal pigment epithelial tissues, the choroid, and the rod outer segments, which contain the photoreceptors. If the damage is not extensive, there are repair mechanisms to allow for recovery of retinal tissues. However, extensive phototoxic damage to the retina can lead to permanent blindness [20,21].

2.4. Oxygen tension

In general the greater the oxygen content of tissues the more susceptible they are to oxidative and photooxidative damage. The cornea is highly oxygenated. The retina is supplied with oxygen by the blood, so it has varying but generally high oxygen content in different portions of the retinal tissues. Although the oxygen content in the aqueous and the lens is generally low, it is sufficient for photooxidation to occur [22–24].

2.5. Chromophores

A chromophore is a substance that absorbs light. An ocular chromophore can be either an endogenous compound naturally present in the eye or an exogenous agent that has passed through blood–ocular barriers and penetrated to a particular site. In order for light to damage the eye it must first be absorbed by a chromophore present in the ocular tissue.

2.5.1. Endogenous chromophores

2.5.1.1. Cornea

The main absorbing species in the cornea are DNA and proteins [11].

2.5.1.2. Uvea

Proteins and melanin are the chromophores of the uveal tract [25].

2.5.1.3. Lens

There is actually little damage to the human eye from light before middle age because the adult human lens contains yellow chromophores (3-hydroxykyurenines) that absorb light but dissipate its energy [26]. 3-Hydroxykyurenines thus serve to protect the retina by filtering UV light and preventing it from reaching and damaging the retina [27]. After middle age an enzyme (kynurenine amino transferase) produced in increasing amounts converts the protective 3-OH kynurenine pigment into a destructive chromophore, xanthurenic acid [28]. When xanthurenic acid absorbs light it produces the reactive oxygen species singlet oxygen and superoxide [29], which damage lens proteins. Another chromophore, *N*-formyl kynurenine, formed from the photooxidation of endogenous tryptophan [30], also produces singlet oxygen or superoxide and damages lens proteins [31,32]. Thus xanthurenic acid and *N*-formyl kynurenine are likely candidates for the chromophores responsible for age-related cataract formation.

2.5.1.4. Retina

As visible light is used for sight (phototransduction), there are many chemical species in the retina (rhodopsin, opsin, melanin, A2E) that absorb light and either do no harm or behave as quenchers of reactive oxygen species [25,33,34]. However, with age, the retina gradually accumulates fluorescent materials, generally known as lipofuscin [35,36]. This mixture of chromophores absorbs photons in the visible range and produces reactive oxygen species [37–44]. As lipofuscin increases in the retina, the once protective melanin decreases, is modified, and may become a pro-oxidant [25,35,36,44].

2.5.2. Exogenous chromophores

Most of the damage to the eye caused by direct irradiation from the sun or artificial sources is from ultraviolet radiation. However, in the presence of a light activated (photosensitized) drug, herbal medication [6,45,46] or diagnostic dye, patients are in danger of enhanced ocular injury from both ultraviolet and visible light [6]. The extent to which a particular dye or drug is capable of producing phototoxic side-effects in the eye depends on several parameters including: (i) the chemical structure; (ii) the absorption spectra of the drug; (iii) binding of the drug to ocular tissue; and (iv) the ability to cross blood–ocular barriers.

Any compound that has a tricyclic, heterocyclic or porphyrin ring structure is a potential ocular chromophore. If a drug absorbs ultraviolet light, it may damage the lens, whereas if it absorbs visible light it may also affect the retina. When exogenous sensitizers bind to ocular tissues (lens proteins, melanin, DNA) their lifetime in the eye is extended and the hazard is enhanced. Substances that are amphiphilic or lipophilic are able to cross most blood–ocular barriers [6].

2.6. Defense systems

Because the eye is constantly subjected to ambient radiation, each portion of the eye contains very efficient defense systems. There are antioxidant enzymes (SOD and catalase) and antioxidants (e.g. vitamins E and C, lutein, zeaxanthin, lycopene, glutathione, and melanin) that serve to protect against oxidative and photoinduced damage [47–55]. Unfortunately, most of these antioxidants and protective enzymes decrease beginning at 40 years of age [25,51,56].

2.7. Repair

Even if the eye is damaged the damage does not have to be permanent. The cornea and retina have very efficient repair systems. However, damage to the lens is cumulative and not repairable [6].

2.8. Mechanisms

Ocular damage from light can occur through either an inflammatory response or a photooxidation reaction. In an inflammatory response, an initial insult to the tissue provokes a cascade of events that eventually results in wider damage to the tissue [57,58]. In photooxidation reactions, a sensitizing compound in the eye absorbs light, is excited to a singlet, then triplet state and from the triplet produces free radicals and reactive oxygen species which in turn damage the ocular tissues [59].

3. Specific ocular disorders associated with light damage

3.1. Cornea

There are three ocular diseases associated with the chronic deliverance of UV-B to the cornea: photokeratitis, pingueculae and pterygia.

3.1.1. Photokeratitis

Photokeratitis is an inflammatory response to the outer layer (endometrium) of the cornea caused by UV-B light. It is very painful but is reversible through repair, if the damage is not extensive or deep. It is caused by exposure to intense sunlight, usually as a result of reflective light from sand or snow [11].

3.1.2. Pingueculae and pterygia

Pingueculae, which comes from the Latin pingueculus meaning fatty, is a raised growth located on the nasal bulbar conjunctiva. It is most probably the earliest stage of a pterygia, which is a growth originating from the bulbar conjunctiva that has extended across the cornea. An association with UV-B and/or UV-A exposure is strongly suggested both by the location of these lesions, in the area of the eye most likely to be a focal point of incoming light, and by their high occurrence in both young outdoor workers and aged populations in areas of intense sunlight. Indeed, epidemiological studies have shown a statistically significant association between ultraviolet light exposure and these ocular lesions [60–63].

3.2. Uvea

3.2.1. Uveal melanoma

The most common malignant tumor of the eye is uveal melanoma, and there is epidemiological evidence that exposure to ultraviolet light is a factor in its etiology. Tucker [64] compared 444 uveal melanoma patients with controls and found that the melanoma patients were more likely to have spent time outdoors gardening, to have sunbathed, and to have used sunlamps. They were less likely to have used some form of eye protection while outside. The fact that iris melanoma tends to occur in the inferior sector of the iris [65–67], where exposure to sunlight is the greatest, also suggests that the occurrence of melanoma in the iris is related to UV radiation exposure. All of these findings suggest that UV radiation and sunlight exposure may be an important risk factor for uveal melanoma [68].

An important role in protecting eye tissues from UV radiation is played by the ocular pigment cells (pigment epithelial cells and uveal melanocytes), which contain melanin. The protective influence of pigment may be particularly important in the iris, since the iris is positioned in front of the lens, an ultraviolet filter. The color of the iris is determined by the melanin content of uveal melanocytes. There are two different types of melanin, the eumelanin (present in the skin and hair of dark-colored persons) and pheomelanin (present in the skin and hair of light colored persons). It has been reported that eumelanin is photo-protective and that pheomelanin is phototoxic in nature. In a recent report, both kinds of melanin were detected in the eye, with the ocular pigment epithelium containing mainly eumelanin and the uveal melanocytes containing both eumelanin and pheomelanin [69].

In Tucker's study [64], it was also determined that subjects with brown eyes were protected compared to subjects with blue eyes. The influence of eye color on the incidence of uveal melanoma is supported by other reports. Jeson compared 111 uveal melanoma patients to control patients and found that melanoma patients are nearly three times more likely to have light-colored irides [70]. In another report, persons with blue or gray eyes were found to have three times the risk of uveal melanoma of people with brown eyes [65]. Further bolstering the protective role of eye color is the fact that uveal melanoma is relatively common in Caucasians and rare among non-Caucasian races. In the United States, Caucasian have more than eight times the risk of developing uveal melanoma than African-Americans [68]. Different melanin content in the uveal melanocytes may be one of the factors that determine the difference of incidence of uveal melanoma among various races.

Only a decade ago, little was known about melanogenesis originating in the uveal melanocytes. In the past decade, as methods for isolation and culture of human uveal melanocytes have been developed by Hu and his colleagues [71], many pure cell lines of human uveal melanocytes have been established and could be used to study the regulation of melanogenesis of uveal melanocytes.

The modulation of melanogenesis in uveal melanocytes should be important in determining the UV radiation protecting capacity of the eye. Hu and his colleagues have reported that the adrenergic agonists endothelin and prostaglandin E stimulate melanogenesis by uveal melanocytes in vitro, while the cholinergic agonists TGF-β and interleukin-6 inhibit it [12,72–76]. The neuropeptides α-MSH and ACTH, which are regulators of melanogenesis of the skin, stimulated growth and melanogenesis of cultured epidermal melanocytes but not uveal melanocytes [12]. This finding may explain why UV radiation causes color changes in the skin but not the iris.

Finally, there may be a circadian component [77] to the induction or control of uveal melanoma. Melatonin is synthesized from tryptophan in the eye and found in the aqueous humor. Its synthesis is downregulated by light. Melatonin inhibits the growth of uveal melanoma cells at the range of endogenous melatonin concentrations (2 nM) found in the human aqueous humor. Under the same conditions, uveal melanocyte cell growth is not inhibited

[78,79]. Mel_{1b} (a receptor that is also expressed in the retina of the eye and in that location controls light-mediated processes) was detected in both uveal melanoma cells and uveal melanocytes [80]. Uveal melanoma cell growth is blocked by the agonists for the melatonin membrane receptor.

The inhibition of melanoma cell growth is probably not related to melatonin's antioxidant properties. The redox properties of melatonin are similar to those of other tryptophan metabolites [81] except that melatonin produces a small but detectable amount of singlet oxygen and quenches this reactive oxygen species with only moderate efficiency [82]. Clinical trials have found that melatonin is an efficacious treatment for metastatic dermal melanoma and may yet be found to be clinically useful against uveal melanoma. The expression of mRNA encoding Mel_{1b} receptors in normal uveal melanocytes suggests that melatonin and/or the light/dark cycle may play a role in the normal functioning of these cells [80].

3.3. Lens

3.3.1. Cataracts

The orderly arrangement of protein fibers in the lens normally causes the lens to be highly transparent [19]. Chronic exposure to sunlight damages the lens. When damage to the lens and its proteins becomes extensive, the lens becomes sufficiently cloudy to obstruct vision, and the individual is said to have a cataract. Exposure to sunlight while taking photosensitizing medication dramatically accelerates this process [6].

Age is an important risk factor in the induction of cataracts. At middle age the eye's natural enzymatic and antioxidant protection against ultraviolet-radiation-induced damage is lost and at the same time there is an increase in production of the photochemically active chromophores, *N*-formyl kynurenine and xanthurenic acid. As the lens absorbs ambient light these chromophores are activated, and the lens proteins (α, β γ crystallins) become denatured with a resultant loss of transparency [28,29,31].

Maintenance of structural integrity is particularly important for lens α-crystallin because of its role as a molecular chaperone. α-Crystallin is an aggregate of two polypeptides, αA and αB, which are small heat shock proteins that prevent ultraviolet (A and B) induced protein aggregation [83,84]. The specific sites of damage to α-crystallin with both endogenous and exogenous chromophores have been detected using mass spectrometry [30,45,85,86].

Additional information on the mechanism of cataract induction has been possible now that human lens epithelial cells have been immortalized [13]. It has been found that both UV-A and UV-B induce precataractous changes [87]. Other specific targets for damage by UV-A radiation are lens cell membrane lipids, the antioxidant enzyme catalase, and the cytoskeletal elements of human and rabbit lens epithelial cells in culture.

Since age decreases the normal production of antioxidants in the lens, supplementation with vitamins and antioxidants present in fruits and vegetables has been suggested to replace the missing protection. Vitamin E and lutein have been shown to be particularly effective in retarding age related cataracts [88,89]. Green tea, which contains polyphenols (epigallocatechin gallate), has also been shown to retard light induced damage to the lens [90].

Ultraviolet light avoidance with appropriate sunglasses and lutein, zeaxanthin and vitamin E supplementation may help retard or eliminate this blinding disorder in the elderly.

3.4. Macular degeneration

The leading cause of irreversible blindness in the increasingly aged population (14% over 55; 37% over 75) is macular degeneration. This is caused by deterioration of the macula (central) cells in the retina. Vision first becomes blurry, lines and colors of objects are distorted and finally a circular area of total blindness occurs. Patients with macular degeneration retain a reasonable amount of peripheral vision. Although the etiology of macular degeneration is unknown, age (over 55), sex (female), blue eyes, smoking and light exposure are considered risk factors [91–93]. Visible (400–700 nm) and/or blue light (400–550 nm) are particularly toxic to the aging retina because it has lost antioxidant protection [25,47,51]. At the same time that these protective agents are becoming depleted, the aging retina also accumulates fluorescent phototoxic chromophores. Visible light activates these chromophores and produces reactive oxygen species (ROS). The production of ROS in aged RPE (retina pigment epithelial) cells leads to apoptosis and cell death. One of the functions of human RPE cells is to transport nutrients to the photoreceptor cells. With the death of the RPE cells, the photoreceptor cells are no longer nourished and they die off. The final result is a loss of vision.

The endogenous carotenoids lutein and zeaxanthin have been found to effectively prevent or retard macular degeneration in humans [94–96] when supplemented in the diet. Both are found in high concentrations in spinach, kale and collard greens. As lutein is a very efficient singlet oxygen quencher [97], these results would appear to confirm the participation of singlet oxygen and other reactive oxygen species as reactive intermediates in macular degeneration [36,37].

Early detection of macular degeneration may be helped by recent developments in ocular fluorometry [98] and the full-spectrum decay curves of chromophores associated with macular degeneration, lipofuscin and A2E, that have been determined by Cubeddu and his colleagues [35,99]. In the future, ocular fluorometry will be used as a non-invasive diagnostic tool to detect macular degeneration at a

very early stage in the disease. Early detection in conjunction with blue light avoidance with appropriate sunglasses and lutein supplementation may help retard or eliminate this blinding disorder in the elderly.

4. Conclusion

We have known for some time that exposure to intense sunlight either causes or exacerbates age-related ocular diseases. Clarification of the underlying mechanisms of induction of ocular disease has been helped by the immortalization of human corneal [10] and lens epithelial cells [13] and the establishment of pure uveal melanocyte cultures [12]. Also, it is now clear that light damage can be enhanced by certain dyes and drugs [6], and be enhanced or prevented by herbal supplements [45,78,90] and vitamins [94–96]. In the future, earlier diagnosis, in conjunction with ocular protection against light (sunglasses) and prescribed specific supplementary antioxidants (lutein, zeaxanthin, vitamin E) may retard or eliminate most blinding disorders in the elderly.

Acknowledgements

The author wishes to thank Dr Ann Motten, NIEHS North Carolina, for help in preparing this paper and the Hugoton Foundation for its financial support.

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