

NEW DISCOVERIES AND THERAPIES IN RETINAL PHOTOTOXICITY



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INTRODUCTION

Age-related Macular Degeneration, AMD, is one of the major causes of visual impairment in industrialised countries, along with diabetic retinopathy and glaucoma. In the United States, AMD is considered to be the cause of 54.4% of visual impairments and 22.9% of cases of blindness^[1]. It is estimated that in 2010, 9.1 million Americans aged over 50 presented early-stage AMD^[2] and that this number is set to double by 2050, to reach 17.8 million. At least 12% of the American and European populations aged over 80 is affected by advanced AMD^[3-5]. Amongst risk factors for AMD identified in literature, sunlight is indicated as being a factor that can cause cumulative damage to the retina. The highest energy portion of the visible spectrum, at between 400nm and 500nm, also known as blue light, is incriminated here. Ophthalmic appliances already claim to offer protection against blue light. Spectacle lenses or intraocular implants mostly contain high-pass filters that absorb a wide band of blue light. However, such unselective filtering can lead to maladjustment of the eye's visual and non-visual functions. Colour perception is disturbed, scotopic vision is limited and the body clock of wake/sleep cycles, which is controlled by certain wavelengths of blue light, is potentially thrown out of kilter. The limited specificity of the filtering mechanisms in existence is due to a lack of information concerning the relative toxicity to the retina of each wave length within the visible spectrum. This is the reason why Essilor International and the Vision Institute went into partnership in 2008 in order to define the harmfulness of blue light to the retina more clearly and develop more selective, protective filtering lenses.

LIGHT: A RISK FACTOR FOR AMD

Since the causes of AMD are currently poorly identified, there are no efficient preventive and curative solutions. Numerous epidemiological studies demonstrate a large variety of potential risk factors. Although the first proven factors are age^[5], tobacco consumption^[5-8] and lack of carotenoids^[9], light is also being blamed as probably playing a part in the prevalence of AMD^[10-14]. One of the characteristics of AMD is the appearance of sub-retinal deposits known as drusen^[15,16]. These deposits are made up of lipofuscin, a product of the deterioration of the external segment of the photoreceptors and of the visual pigment. Lipofuscin, because of its photosensitising characteristics, is thought to be involved in the retinal damage caused by exposure to light.

BLUE LIGHT: HOW DANGEROUS IS IT FOR THE RETINA?

In the retina, light is mainly absorbed by the visual pigments contained in the external segments of the photoreceptors. The visual pigments of vertebrates are made up of a transmembrane protein, opsin, combined with a vitamin A derivative 11-cis-retinal. In the rod photoreceptors, this visual pigment is rhodopsin.

Most ultraviolet radiation is naturally filtered by ocular tissues located in front of the retina, particularly the cornea and the crystalline lens^[17,18]. The most energetic light that reaches the retina is therefore mainly blue light, at between 400nm and 500nm. Because of its high energy level, it induces and accelerates photochemical reactions and cellular damage via the production of radical species that are highly reactive in the presence of oxygen. In particular, the toxic potential of blue light on the external retina acts at two cellular levels: photoreceptors and the cells in the retinal pigment epithelium.

In the rod photoreceptors, absorption of a photon by rhodopsin causes isomerisation and the release of the 11-cis-retinal as all-*trans*-retinal. Free all-*trans*-retinal is not only toxic as a reactive aldehyde, it also presents strong sensitivity to blue light^[19,20]. Under moderate light exposure conditions, the all-*trans*-retinal is recycled continuously into 11-cis-retinal by the cells of the retinal pigment epithelium and does not cause any danger to the cell. When exposure to light happens over a longer or more intense period, the all-*trans*-retinal accumulates and its activation by blue light may be the cause of oxidative stress which damages the cellular components of the photoreceptors. This oxidative stress is normally compensated for by the presence of the numerous antioxidants in the retina. However, with age and certain genetic and environmental factors, such as tobacco consumption or a diet that is low in antioxidants, anti-oxidative defences are reduced^[21,22] and can no longer compensate for the stress caused by prolonged or intensive exposure to blue light.

The function of the cells in the retinal pigment epithelium is to ensure renewal of the external segment of photoreceptors. They eliminate the distal part of them by ingestion, or "phagocytosis", whilst the growth of these external segments occurs continuously^[23]. When the external segments are too damaged by oxidative stress, their membrane components are difficult for the retinal pigment epithelium to break down. Intracellular digestion is then incomplete and generates an accumulation of residual granular bodies, in the form of lipofuscin. The granules of lipofuscin contain a large amount of polyunsaturated fat, a target for oxidation. The lipophilic extract of lipofuscin contains a potential photosensitiser, which forms a triplet excited state with a maximum of absorption in blue at 440nm^[24,25]. One of the components of lipofuscin, A2E, has been identified as being involved in the photosensitising nature of the lipid residue. The energy of the triplet state is sufficient to be transferred and react with oxygen in the blood.

Photoactivation of the lipofuscin granules by blue light then generates reactive oxygen species (superoxide, hydrogen peroxide, lipid hydroperoxides and malondialdehyde)^[26, 27]. When the number of these species exceeds the cellular defence capacity, the retinal pigment epithelium cells die by apoptosis. Deprived of these support cells that provide their energy supply, the photoreceptors deteriorate in turn, contributing to the loss of vision diagnosed in patients suffering from AMD.

In conclusion, the suggested mechanism by which light is involved in the appearance and progression of AMD may happen at two levels: on the one hand in photoreceptors *via* absorption of blue light by rhodopsin and then in the near ultraviolet blue by the all-trans-retinal, and, on the other, in the retinal pigment epithelium *via* absorption of blue by lipofuscin.

___ THE LIMITATIONS OF EXISTING STUDIES

The toxic effects of visible light and blue light in particular on the retina have already been demonstrated experimentally on cellular^[28-30] and animal^[31] models of degenerative retinal pathologies. However, the studies performed to date have not enabled characterisation of the respective toxicity of each wavelength. Also, they suffer from certain limitations. In fact comparisons of results are difficult from one study to another because units fluctuate between energetic and visual units. Also, the illumination systems used are not calibrated on the illumination of the light sources existing in our environment, whether natural (the sun) or artificial (neon, LED, halogen, etc.) and therefore do not reflect true conditions of exposure to light. Finally, none of the illumination systems used to date enables step by step definition of the toxic spectrum of light on the cells of the retina. The only recurrent information is that the highest toxicity levels are contained within the spectral interval [400nm; 500nm].

___ THE CONTRIBUTION MADE BY THE VISION INSTITUTE AND ESSILOR INTERNATIONAL

The objective of this contribution was, in partnership with Essilor International, to establish a photobiology laboratory at the Vision Institute, to enable us to define precisely the specific toxicity on the retina of each wavelength in the blue section of the visible spectrum. The first action taken involved the development of a cellular illumination system. This enabled the production of visible wavelengths of very narrow bandwidths and at given illumination in order to model the desired luminous spectrum. The light source to which we are the most exposed and which is the most intense is the sun and the work was therefore carried out using, for each wavelength, radiation values relative to the sun's spectrum.

The second direction for work involved development of a model of cultured cells, reproducing *in vitro* the degeneration of retinal cells, as observed in AMD, with the presence of a lipofuscin component: A2E.

___ EQUIPMENT AND METHOD

The system of illumination that has been developed is a multi-wavelength generator used to illuminate the cells being cultured inside an incubator. The light source comprises a set of light-emitting diodes (LEDs), each connected to the incubator and the cells by means of optical fibres. The range of wavelengths covered extends from 390nm to 520nm in bandwidths of 10nm (*fig. 1*). The whole unit can thus, with each optical fibre, restrict illumination to 10nm of the spectrum arriving in the retina.

In order to model the accumulation of lipofuscin in the retina, cells cultured in pig's pigmentary epithelium were treated with various concentration of A2E, one of the components of lipofuscin (*fig. 2*). These cells were then exposed to a light bandwidth of 10nm

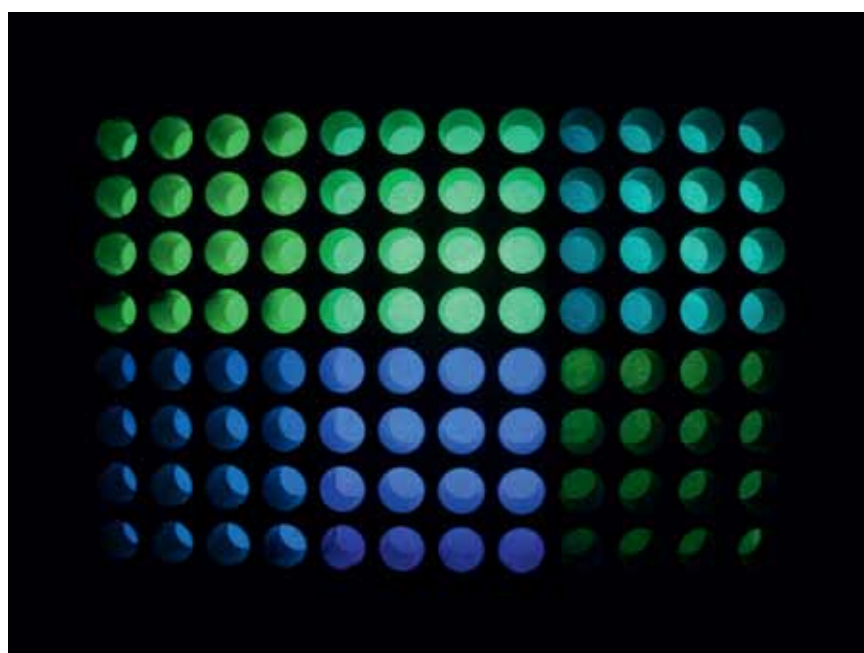


FIG. 1 | View from above of a cell culture plate lit by various wavelengths, from 390 to 520nm.

→ for 18 hours. Six hours after exposure, the effects of the light on the cells were characterised according to three parameters: the percentage of live cells, apoptotic activity of the cells and the percentage of cells undergoing necrosis.

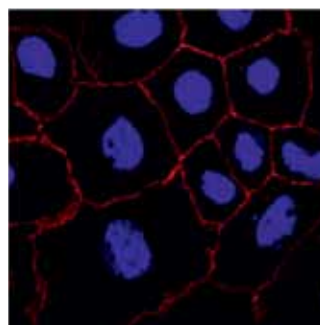
RESULTS

Quantification of live cells shows that exposure to light leads to cell death only when the cells have been treated with A2E (*fig. 3*). This phototoxicity is shown by activation of an enzyme, caspase-3, which is involved in programmed death processes (apoptosis). On the other hand, we did not observe any cell necrosis under these experimental conditions. Our results also show that the greater the concentration of A2E, the greater the toxic effect of light. These results demonstrate that an A2E dose-dependent effect exists, and therefore probably one of lipofuscin too, in induction of phototoxicity. This can be related to the influence of age in AMD, because it has been observed that drusen and lipofuscin accumulate with age and are present in greater quantities in elderly patients suffering from AMD^[15,16,32,33].

CONCLUSION AND PROSPECTS

The joint work carried out by the Vision Institute and Essilor International has resulted in the establishment of an experimental process using a cellular model of AMD to define the precise spectrum of sunlight toxicity on the retina. These results provide information of capital importance in terms of the need to be protected from highly specific blue light wavelengths. It is important to note that these wavelengths are also present in variable proportions in the various sources of artificial light (neon, LED, xenon, halogen, etc.) and that the potential effects of lengthy exposure should not be neglected. This project supplies elements of understanding of the physiopathological processes taking place in AMD, with the possibility of therapeutic or preventative solutions for this major pathology. This type of therapeutic solution could be extended to other retinal pathologies involving oxidative stress processes leading to degeneration of the photoreceptors, such as pigmentary retinitis and Stargardt's disease. The association of the respective skills of the Vision Institute in terms of the cellular biology of the retina, and of Essilor International in optics was essential in setting up this innovative ophthalmological project. •

Non-treated cells



Cells + A2E

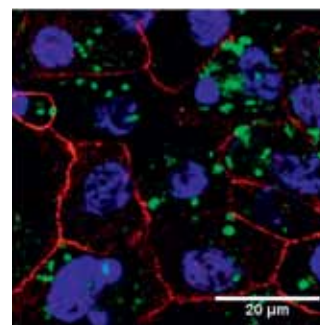
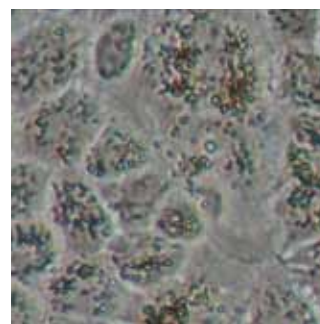


FIG. 2 | Pig's retinal pigment epithelium cells with or without treatment with A2E, one of the components of lipofuscin.

The cells' nuclei are visible in blue and inter-cellular junctions are coloured red. On the left the A2E internalised by the cells is visible by autofluorescence in green when it is illuminated with blue light.

Dark



Light

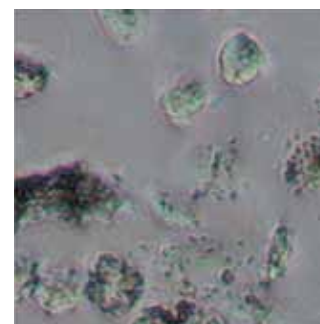


FIG. 3 | Pig's retinal pigment epithelium cells treated with A2E kept in the dark or exposed to light. On the left the cells kept in the dark are healthy because they are hexagonal in shape and will join to each other (at confluence).

On the contrary, exposure to light (on the right) causes cell death, visible from their rounded shape and reduced density.

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