5-3. Pigment Related Pathology

Invest Ophthalmol Vis Sci. 2002 Apr;43(4):1222-7.

Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE.

Sparrow JR, Zhou J, Ben-Shabat S, Vollmer H, Itagaki Y, Nakanishi K.

Department of Ophthalmology, Columbia University, New York, New York 10032, USA. jrs88@columbia.edu

PURPOSE: The lipofuscin fluorophore A2E is known to be an initiator of blue-light-induced apoptosis in retinal pigment epithelial cells (RPE). The purpose of this study was to evaluate the role of oxidative mechanisms in mediating the cellular damage. METHODS: Human RPE (ARPE-19) cells that had accumulated A2E were exposed to blue light in the presence and absence of oxygen, and nonviable cells were quantified. Potential suppressors (histidine, azide, 1,4-diazabicyclooctane [DABCO], and 1,3-dimethyl-2-thiourea [DMTU]) and enhancers (deuterium oxide [D(2)O] and 3-aminotriazole [3-AT]) of oxidative damage, were also screened for their ability to modulate the frequency of nonviable cells. A2E in PBS, with and without an oxygen-depleter or singlet-oxygen quencher and A2E-laden RPE, were exposed to 430-nm light and examined by reversed-phase high performance liquid chromatography (HPLC) and fast atom bombardment mass spectrometry (FAB-MS). RESULTS: The death of blue-light-illuminated A2E-laden RPE was blocked in oxygen-depleted media. When A2E-laden RPE were transferred to D(2)O-based media and then irradiated (480 nm), the number of nonviable cells was increased, whereas the latter was decreased in the presence of histidine, DABCO, and azide. Conversely, no affect was observed with 3-AT and DMTU. When A2E, in either acellular or cellular environments, was irradiated at 430 nm, FAB-MS revealed the generation of a series of higher molecular mass derivatives of A2E. The sizes of these species increased by increments of mass 16. The generation of these photo-products was accompanied by the consumption of A2E, the latter being diminished, however, when illumination was performed after oxygen depletion and in the presence of a singlet-oxygen quencher. CONCLUSIONS: The augmentation of cell death in the presence of D(2)O and the protection afforded by quenchers and scavengers of singlet oxygen, indicates that the generation of singlet oxygen may be involved in the mechanisms leading to the death of A2E-containing RPE cells after blue light illumination. The finding that irradiation also produces oxygen-dependent photochemical changes in A2E, indicates that the effects of singlet oxygen may be mediated either directly or through the generation of reactive photo-products of A2E.

PMID: 11923269 [PubMed - indexed for MEDLINE]

Free Radic Biol Med. 2001 Jul 15;31(2):217-25.

Lipofuscin-formation in retinal pigment epithelial cells is reduced by antioxidants.

Sundelin SP, Nilsson SE.

Division of Ophthalmology, Department of Neuroscience and Locomotion, Linkoping University, Linkoping, Sweden. staffan.sudelin@lio.se

The accumulation of lipofuscin by retinal pigment epithelium may be an important feature in the pathogenesis of age-related macular degeneration, suggesting the possibility that this common cause of blindness might be prevented or delayed by antioxidants. In support of this idea, we now report significantly reduced formation of lipofuscin when the antioxidant substances lutein, zeaxanthin, lycopene (carotenoids), or alpha-tocopherol were added to rabbit and bovine (calf) retinal pigment epithelial (RPE) cells exposed to normobaric hyperoxia (40%) and photoreceptor outer segments. Rabbit and calf RPE cells were grown for 2 weeks with addition of one of the test substances every 48 h. The cellular uptake of carotenoids and alpha-tocopherol was assayed by HPLC after 2 weeks. The lipofuscin-content was measured by static fluorometry (rabbit cells) or by image analysis (calf cells). Both rabbit and calf RPE showed similar results with significantly lower amounts of lipofuscin in antioxidant-treated cells. The effect of carotenoids is especially interesting, since the result is not dependent on their protective effect against photo-oxidative reactions. The chain-breaking abilities of these antioxidants in peroxidative reactions of lipid membranes and quenching of free radicals seem to be of importance for inhibition of lipofuscin formation.

PMID: 11440833 [PubMed - indexed for MEDLINE]

Exp Eye Res. 2004 Sep;79(3):313-9.

A mechanistic study of the photooxidation of A2E, a component of human retinal lipofuscin.

Gaillard ER, Avalle LB, Keller LM, Wang Z, Reszka KJ, Dillon JP.

Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb 60115, USA. gaillard@niu.edu

A major constituent of human retinal lipofuscin is A2E (2-[2,6-dimethyl-8-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1E,3E,5E,7E-octatetraenyl]-1-(2-hydroxyethyl)-4-[4-methyl-6(2,6,6-trimethyl-1-cyclohexen-1-yl)-1E,3E,5E,7E-hexatrienyl]-pyridinium). Light transmitted by the lens is absorbed by A2E and the processes initiated by this absorption has been implicated in several maculopothies. The purpose of this study was to evaluate the dominant photochemical mechanisms involved in these reactions, whether through free radical or singlet oxygen intermediacy. The photodestruction of A2E occurs faster in water vs. chloroform and hydrogenated vs. perdeuterated methanol. Both results suggest a free radical mechanism. Product distributions indicate sequential oxygen addition rather than the addition of two oxygen atoms which would be expected if singlet oxygen was an intermediate. Finally, EPR trapping studies lead to the detection of superoxide as the primary intermediate in the photochemical reactions. It is concluded that if singlet oxygen is involved in these photochemical processes it is of minor importance.

PMID: 15336493 [PubMed - indexed for MEDLINE]

Arch Biochem Biophys. 2001 Jul 15;391(2):160-4.

Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes.

Junghans A, Sies H, Stahl W.

Institut fur Physiologische Chemie I and Biologisch-Medizinisches Forschungszentrum, Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, D-40001, Germany.

Lutein and zeaxanthin are the predominant carotenoids in the human macula lutea. Epidemiological data suggest that an increased intake of a lutein-rich diet correlates with a diminished risk for age-related macular degeneration, a major cause of impaired vision in the elderly. Filtering of blue light has been proposed as a possible mechanism of protection. Here, the blue light filter efficacy of carotenoids was investigated in unilamellar liposomes loaded in the hydrophilic core space with a fluorescent dye, Lucifer yellow, excitable by blue light. Carotenoids were incorporated into the lipophilic membrane. Fluorescence emission in carotenoid-containing liposomes was lower than in carotenoid-free controls when exposed to blue light, indicating a filter effect. Filter efficacy was in the order lutein > zeaxanthin > beta-carotene > lycopene. Some of the difference in blue light filter efficacy of carotenoids is attributable to differences in extinction coefficients, and a major further contribution is suggested to be related to the orientation of the incorporated molecules in the liposomal membrane. Copyright 2001 Academic Press.

PMID: 11437346 [PubMed - indexed for MEDLINE]

Curr Eye Res. 1998 Aug;17(8):851-7.

Lipofuscin accumulation in cultured retinal pigment epithelial cells reduces their phagocytic capacity.

Sundelin S, Wihlmark U, Nilsson SE, Brunk UT.

Department of Ophthalmology, Linkoping University, Sweden.

PURPOSE: Retinal pigment epithelial (RPE) cells slowly accumulate lipofuscin pigment within their acidic vacuolar apparatus as a result of extra- and/or intralysosomal oxidative alterations of phagocytosed photoreceptor outer segments (POS) with consequent imperfect degradation of these structures. In old age, lipofuscin accumulation may become quite substantial. It has been suggested that pronounced accumulation of lipofuscin is related to decreased RPE function and, possibly, to age-related macular degeneration. The aim of the present investigation was to study whether heavy loading with lipofuscin of RPE acidic lysosomes would affect the further phagocytic ability of the cells. METHODS: In the first section of the investigation, cultures of rabbit RPE cells were exposed daily to bovine UV-irradiated POS (artificial lipofuscin) for 4 weeks, resulting in a pronounced lipofuscin accumulation of the cells. Fluorescent latex beads (labelled with a red fluorophore) were added to unloaded control cultures at O and 4 weeks after their establishment, and to lipofuscin loaded cells after 4 weeks of feeding with artificial lipofuscin. Cellular amounts of lipofuscin, and their phagocytotic activity, were quantified by static fluorometry measuring lipofuscin-specific and red bead-specific fluorescence, respectively. The intracellular location of the beads was verified by confocal laser scanning microscopy. RESULTS: Unloaded, and thus almost lipofuscin-free, control cells exposed to latex beads showed numerous cytoplasmic particles emitting reddish fluorescence, while few particles were taken up by cells initially loaded with artificial, POS-derived, lipofuscin. Measurement of the latex bead-specific fluorescence showed significantly ($p < 0.001$) higher levels in unloaded control cells than in lipofuscin-loaded ones. In the second part of the investigation, unloaded control cultures and lipofuscin-loaded cultures were exposed to native bovine Texas Red-X-labelled POS 4 weeks after the establishment of the cultures. Unloaded control cells showed a large number of cytoplasmic POS emitting reddish fluorescence, while fewer POS were phagocytosed by cells loaded with artificial lipofuscin. Measurement of the Texas Red-X-specific fluorescence, thus quantifying the phagocytic ability of the cells, showed significantly ($p < 0.001$) higher levels in control cells than in lipofuscin-loaded ones. CONCLUSIONS: Severe lipofuscin accumulation of RPE cells appears to result in a greatly decreased phagocytic capacity. The resulting reduction in ability to cope with the needs of the overlying photoreceptor cells, in order to eliminate the obsolete tips of their POS, may well be of significance in the development of age-related macular degeneration.

PMID: 9724002 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2001 Feb;42(2):439-46.

Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population.

Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME.

University Department of Ophthalmology, Manchester Royal Eye Hospital, Manchester, UK. stephen@stiofanbetagh.demon.co.uk

PURPOSE: Age and advanced disease in the fellow eye are the two most important risk factors for age-related macular degeneration (AMD). In this study, the authors investigated the relationship between these variables and the optical density of macular pigment (MP) in a group of subjects from a northern European population. METHODS: The optical density of MP was measured psychophysically in 46 subjects ranging in age from 21 to 81 years with healthy maculae and in 9 healthy eyes known to be at high-risk of AMD because of advanced disease in the fellow eye. Each eye in the latter group was matched with a control eye on the basis of variables believed to be associated with the optical density of MP (iris color, gender, smoking habits, age, and lens density). RESULTS: There was an age-related decline in the optical density of macular pigment among volunteers with no ocular disease (right eye: $r(2) = 0.29$, $P = 0.0006$; left eye: $r(2) = 0.29$, $P < 0.0001$). Healthy eyes predisposed to AMD had significantly less MP than healthy eyes at no such risk (Wilcoxon's signed rank test: $P = 0.015$). CONCLUSIONS: The two most important risk factors for AMD are associated with a relative absence of MP. These findings are consistent with the hypothesis that supplemental lutein and zeaxanthin may delay, avert, or modify the course of this disease.

PMID: 11157880 [PubMed - indexed for MEDLINE]

Free Radic Biol Med. 1997;22(7):1229-34.

Lipofuscin accumulation in cultured retinal pigment epithelial cells causes enhanced sensitivity to blue light irradiation.

Wihlmark U, Wrigstad A, Roberg K, Nilsson SE, Brunk UT.

Department of Ophthalmology, University Hospital, Linkoping, Sweden.

Lipofuscin accumulates with age within secondary lysosomes of retinal pigment epithelial (RPE) cells of humans and many animals. The autofluorescent lipofuscin pigment has an excitation maximum within the range of visible blue light, while it is emitting in the yellow-orange area. This physico-chemical property of the pigment indicates that it may have a photo-oxidative capacity and, consequently, then should destabilize lysosomal membranes of blue-light exposed RPE. To test this hypothesis, being of relevance to the understanding of age-related macular degeneration, cultures of heavily lipofuscin-loaded RPE cells were blue-light-irradiated and compared with respect to lysosomal stability and cell viability to relevant controls. To rapidly convert primary cultures of RPE, obtained from neonatal rabbits, into aged, lipofuscin-loaded cells, they were allowed to phagocytize artificial lipofuscin that was prepared from outer segments of bovine rods and cones. Following blue-light irradiation, lysosomal membrane stability was measured by vital staining with the lysosomotropic weak base, and metachromatic fluorochrome, acridine orange (AO). Quantifying red (high AO concentration within intact lysosomes with preserved proton gradient over their membranes) and green fluorescence (low AO concentration in nuclei, damaged lysosomes with decreased or lost proton gradients, and in the cytosol) allowed an estimation of the lysosomal membrane stability after blue-light irradiation. Cellular viability was estimated with the delayed trypan blue dye exclusion test. Lipofuscin-loaded blue-light-exposed RPE cells showed a considerably enhanced loss of both lysosomal stability and viability when compared to control cells. It is concluded that the accumulation of lipofuscin within secondary lysosomes of RPE sensitizes these cells to blue light by inducing photo-oxidative alterations of their lysosomal membranes resulting in a presumed leakage of lysosomal contents to the cytosol with ensuing cellular degeneration of apoptotic type. The suggested mechanism may have bearings on the development of age-related macular degeneration.

PMID: 9098097 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2000 Jul;41(8):2303-8.

Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin.

Schutt F, Davies S, Kopitz J, Holz FG, Boulton ME.

Department of Ophthalmology, University of Heidelberg, Germany.

PURPOSE: A fluorescent component of lipofuscin, A2-E (N-retinylidene-N-retinylethanol-amine) has been shown to impair lysosomal function and to increase the intralysosomal pH of human retinal pigment epithelial (RPE) cells. In addition to its lysosomotropic properties A2-E is known to be photoreactive. The purpose of this study was to determine the phototoxic potential of A2-E on RPE cells. METHODS: A2-E (synthesized by coupling all-trans-retinaldehyde to ethanolamine) was complexed to low-density lipoprotein (LDL) to allow for specific loading of the lysosomal compartment. Human RPE cell cultures were loaded with the A2-E-LDL complex four times within 2 weeks. A2-E accumulation was confirmed by fluorescence microscopy and flow cytometry analysis. Acridine orange staining allowed assessment of lysosomal integrity and intralysosomal pH. The phototoxic properties of A2-E were determined by exposing A2-E-free and A2-E-fed RPE cell cultures to short wavelength visible light (400-500 nm) and assessing cell viability and lysosomal integrity. RESULTS: Fluorescence microscopy and flow cytometry analysis demonstrated that the intralysosomal accumulation of A2-E in cultured RPE cells increased with the number of feedings. Acridine orange staining confirmed that the A2-E was located in the lysosomal compartment and induced an elevation of intralysosomal pH. Exposure of A2-E-fed cells to light resulted in a significant loss of cell viability by 72 hours, which was not observed in either RPE cells maintained in the dark or A2-E-free cultures exposed to light. Toxicity was associated with a loss of lysosomal integrity. CONCLUSIONS: A2-E is detrimental to RPE cell function by a variety of mechanisms: inhibition of lysosomal degradative capacity, loss of membrane integrity, and phototoxicity. Such mechanisms could contribute to retinal aging as well as retinal diseases associated with excessive lipofuscin accumulation-for example, age-related macular degeneration and Stargardt's disease.

PMID: 10892877 [PubMed - indexed for MEDLINE]

FASEB J. 2004 Mar;18(3):562-4. Epub 2004 Jan 8.

Inhibition of the ATP-driven proton pump in RPE lysosomes by the major lipofuscin fluorophore A2-E may contribute to the pathogenesis of age-related macular degeneration.

Bergmann M, Schutt F, Holz FG, Kopitz J.

Department of Molecular Pathology, University of Heidelberg, Heidelberg, Germany.

Lipofuscin accumulation in the retinal pigment epithelium (RPE) is associated with various blinding retinal diseases, including age-related macular degeneration (AMD). The major lipofuscin fluorophor A2-E is thought to play an important pathogenetic role. In previous studies A2-E was shown to severely impair lysosomal function of RPE cells. However, the underlying molecular mechanism remained obscure. Using purified lysosomes from RPE cells we now demonstrate that A2-E is a potent inhibitor of the ATP-driven proton pump located in the lysosomal membrane. Such inhibition of proton transport to the lysosomal lumen results in an increase of the lysosomal pH with subsequent inhibition of lysosomal hydrolases. An essential task of the lysosomal apparatus of postmitotic RPE for normal photoreceptor function is phagocytosis and degradation of membranous discs shed from photoreceptor outer segments (POS) and of biomolecules from autophagy. When the lysosomes of cultured RPE cells were experimentally loaded with A2-E, we observed intracellular accumulation of exogenously added POS with subsequent congestion of the phagocytic process. Moreover, the autophagic sequestration of cytoplasmic material was also markedly reduced after A2-E loading. These data support the hypothesis that A2-E-induced lysosomal dysfunction contributes to the pathogenesis of AMD and other retinal diseases associated with excessive lipofuscin accumulation.

PMID: 14715704 [PubMed - indexed for MEDLINE]

Proc Natl Acad Sci U S A. 2002 Mar 19;99(6):3842-7.

The lipofuscin component A2E selectively inhibits phagolysosomal degradation of photoreceptor phospholipid by the retinal pigment epithelium.

Finnemann SC, Leung LW, Rodriguez-Boulan E.

Margaret M. Dyson Vision Research Institute, Department of Ophthalmology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021, USA.

Daily phagocytosis of spent photoreceptor outer segments is a critical maintenance function performed by the retinal pigment epithelium (RPE) to preserve vision. Aging RPE accumulates lipofuscin, which includes N-retinylidene-N-retinylethanolamine (A2E) as the major autofluorescent component. We studied the effect of physiological levels of A2E in RPE cultures on their ability to phagocytose outer segments. A2E localized to lysosomes in cultured RPE as well as in human RPE in situ. A2E-loaded RPE cells in culture bound and internalized identical numbers of outer segments as control RPE indicating that A2E does not alter early steps of phagocytosis. A2E-loaded RPE degraded outer segment proteins efficiently but, strikingly, failed to completely digest phospholipids within 24 h. Because of the circadian rhythm of RPE phagocytosis in the eye, a delay in lipid degradation would likely result in a build up of undigested material in RPE that could contribute to the development of age-related macular degeneration.

PMID: 11904436 [PubMed - indexed for MEDLINE]

J Biol Chem. 2000 Dec 15;275(50):39625-30.

Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells.

Suter M, Reme C, Grimm C, Wenzel A, Jaattela M, Esser P, Kociok N, Leist M, Richter C.

Institute of Biochemistry, Swiss Federal Institute of Technology, Universitatstr. 16, CH-8092 Zurich, Switzerland.

10-20% of individuals over the age of 65 suffer from age-related macular degeneration (AMD), the leading cause of severe visual impairment in humans living in developed countries. The pathogenesis of this complex disease is poorly understood, and no efficient therapy or prevention exists to date. A precondition for AMD appears to be the accumulation of the age pigment lipofuscin in lysosomes of retinal pigment epithelial (RPE) cells. In AMD, these cells seem to die by apoptosis with subsequent death of photoreceptor cells, and light may accelerate the disease process. Intracellular factors leading to cell death are not known. Here we show that the lipophilic cation N-retinyl-N-retinylidene ethanolamine (A2E), a lipofuscin component, induces apoptosis in RPE and other cells at concentrations found in human retina. Apoptosis is accompanied by the appearance of the proapoptotic proteins cytochrome c and apoptosis-inducing factor in the cytoplasm and the nucleus. Biochemical examinations show that A2E specifically targets cytochrome oxidase (COX). With both isolated mitochondria and purified COX, A2E inhibits oxygen consumption synergistically with light. Inhibition is reversed by the addition of cytochrome c or cardiolipin, a negatively charged phospholipid that facilitates the binding of cytochrome c to membranes. Succinate dehydrogenase activity is not altered by A2E. We suggest that A2E can act as a proapoptotic molecule via a mitochondria-related mechanism, possibly through site-specific targeting of this cation to COX. Loss of RPE cell viability through inhibition of mitochondrial function might constitute a pivotal step toward the progressive degeneration of the central retina.

PMID: 11006290 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2000 Jun;41(7):1981-9.

The lipofuscin fluorophore A2E mediates blue light-induced damage to retinal pigmented epithelial cells.

Sparrow JR, Nakanishi K, Parish CA.

Department of Ophthalmology, Columbia University, New York, New York 10032, USA. jrs88@columbia.edu

PURPOSE: To determine whether the lipofuscin fluorophore A2E participates in blue light-induced damage to retinal pigmented epithelial (RPE) cells. METHODS: Human RPE cells (ARPE-19) accumulated A2E from 10, 50, and 100 microM concentrations in media, the levels of internalized A2E ranging from less than 5 to 64 ng/10(5) cells, as assayed by quantitative high-performance liquid chromatography (HPLC). Restricted zones (0.5-mm diameter spots) of confluent cultures were subsequently exposed to 480 +/- 20-nm (blue) or 545 +/- 1-nm (green) light for 15 to 60 seconds. Phototoxicity was quantified at various periods after exposure by fluorescence staining of the nuclei of membrane-compromised cells, by TdT-dUTP terminal nick-end labeling (TUNEL) of apoptotic cells and by Annexin V labeling for phosphatidylserine exposure. RESULTS: Nonviable cells were located in blue light- exposed zones of A2E-containing RPE cells, whereas cells situated outside the illuminated areas remained viable. As shown by fluorescence labeling of the nuclei of membrane-damaged cells and by the presence of TUNEL-positive cells, the numbers of nonviable cells increased with exposure duration and as a function of the concentration of A2E used to load the cells before illumination. The numbers of blue light-induced TUNEL-positive cells also increased in advance of the increase in labeling of membrane-compromised cells, a finding that, together with Annexin V labeling, indicates an apoptotic form of cell death. Conversely, blue light-exposed RPE cells that did not contain A2E remained viable. In addition, illumination with green light resulted in the appearance of substantially fewer nonviable cells. CONCLUSIONS: These studies implicate A2E as an initiator of blue light-induced apoptosis of RPE cells.

PMID: 10845625 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2001 May;42(6):1356-62.

Blue light-induced apoptosis of A2E-containing RPE: involvement of caspase-3 and protection by Bcl-2.

Sparrow JR, Cai B.

Department of Ophthalmology, Columbia University, 630 W. 168th Street, New York, NY 10032, USA. jrs88@columbia.edu

PURPOSE. The lipofuscin fluorophore A2E has been shown to mediate blue light-induced damage to retinal pigment epithelial (RPE) cells. The purpose of this study was to evaluate caspase-3 and Bcl-2 as executor and modulator, respectively, of the cell death program that is initiated in A2E-containing cells in response to blue light. METHODS. Human RPE cells (ARPE-19) that had accumulated A2E were exposed to blue light. Caspase-3 activity was assayed by observing cleavage of a fluorogenic peptide substrate, and the effect of a peptide inhibitor of caspase-3 (Z-DEVD-fmk) on the quantity of apoptotic nuclei was determined. ARPE-19 cells were transfected with either a neomycin-selectable expression vector containing Bcl-2 cDNA or a control neomycin-selectable expression vector without Bcl-2 cDNA. Expression of Bcl-2 transcripts by independently derived clones was established by in situ hybridization, and Bcl-2 protein expression was confirmed by Western blot analysis. Cell viability was assayed by TdT-dUTP terminal nick-end labeling (TUNEL) in conjunction with 4'6'-diamidino-2-phenylindole (DAPI) staining and by fluorescence staining of the nuclei of membrane-compromised cells. RESULTS. In RPE cells that had previously accumulated A2E, caspase-3 activity was detected within 5 hours of blue light exposure. The incidence of apoptotic nuclei was attenuated when A2E-containing RPE cells were exposed to blue light in the presence of caspase-3 inhibitor and in A2E-loaded RPE cells that had been stably transfected with Bcl-2. CONCLUSIONS. Blue light illumination of RPE in the setting of intracellular A2E initiates a cell death program that is executed by a proteolytic caspase cascade and that is regulated by Bcl-2.

PMID: 11328751 [PubMed - indexed for MEDLINE]

Photochem Photobiol. 2004 Feb;79(2):127-36.

A2E: a component of ocular lipofuscin.

Lamb LE, Simon JD.

Department of Chemistry, Duke University, Durham, NC 27708-0346, USA.

The presence of lipofuscin in postmitotic cells is considered a hallmark of the aging process. In the retinal pigment epithelium (RPE), lipofuscin is found as micrometer-sized spherical particles and characterized by its yellow autofluorescence when exposed to blue light. This exposure to light is also known to produce reactive oxygen intermediates (ROI), but the particular molecular constituent(s) responsible for this phototoxicity have yet to be completely identified. Resulting mostly from the autophagocytosis of intracellular organelles, the composition of lipofuscin is poorly defined but known to contain protein, lipids and several fluorophores. The subsequent identification of one of the fluorophores in lipofuscin, A2E, generated much interest and resulted in a variety of studies to understand its potential role in the phototoxicity of lipofuscin. Several modes of toxicity have been suggested through which A2E can affect the health of RPE cells. These modes include photoinduced production of ROI, which places additional oxidative stress on RPE cells, the disruption of membrane integrity through its natural role as an amphiphilic detergent and inhibition of key cellular functions. This article presents the current understanding of the photochemistry of A2E and its involvement as a phototoxic agent in RPE cells.

Publication Types:

- Review
- Review, Tutorial

PMID: 15068025 [PubMed - indexed for MEDLINE]

Exp Eye Res. 2001 Mar;72(3):215-23.

Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye.

Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY, Katz NB.

Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah School of Medicine, Salt Lake City, UT 84132, USA. paul.bernstein@hsc.utah.edu

There is increasing evidence that the macular pigment carotenoids, lutein and zeaxanthin, may play an important role in the prevention of age-related macular degeneration, cataract, and other blinding disorders. Although it is well known that the retina and lens are enriched in these carotenoids, relatively little is known about carotenoid levels in the uveal tract and in other ocular tissues. Also, the oxidative metabolism and physiological functions of the ocular carotenoids are not fully understood. Thus, we have set out to identify and quantify the complete spectrum of dietary carotenoids and their oxidative metabolites in a systematic manner in all tissues of the human eye in order to gain better insight into their ocular physiology.Human donor eyes were dissected, and carotenoid extracts from ocular tissues [retinal pigment epithelium/choroid (RPE/choroid), macula, peripheral retina, ciliary body, iris, lens, vitreous, cornea, and sclera] were analysed by high-performance liquid chromatography (HPLC). Carotenoids were identified and quantified by comparing their chromatographic and spectral profiles with those of authentic standards.Nearly all ocular structures examined with the exception of vitreous, cornea, and sclera had quantifiable levels of dietary (3R,3'R,6'R)-lutein, zeaxanthin, their geometrical (E / Z) isomers, as well as their metabolites, (3R,3'S,6'R)-lutein (3'-epilutein) and 3-hydroxy-beta,epsilon-caroten-3'-one. In addition, human ciliary body revealed the presence of monohydroxycarotenoids and hydrocarbon carotenoids, while only the latter group was detected in human RPE/choroid. Uveal structures (iris, ciliary body, and RPE/choroid) account for approximately 50% of the eye's total carotenoids and approximately 30% of the lutein and zeaxanthin. In the iris, these pigments are likely to play a role in filtering out phototoxic short-wavelength visible light, while they are more likely to act as antioxidants in the ciliary body. Both mechanisms, light screening and antioxidant, may be operative in the RPE/choroid in addition to a possible function of this tissue in the transport of dihydroxycarotenoids from the circulating blood to the retina. This report lends further support for the critical role of lutein, zeaxanthin, and other ocular carotenoids in protecting the eye from light-induced oxidative damage and aging.
Copyright 2001 Academic Press.

PMID: 11180970 [PubMed - indexed for MEDLINE]

Free Radic Biol Med. 2001 Jan 1;30(1):74-81.

Lipofuscin-formation in cultured retinal pigment epithelial cells is related to their melanin content.

Sundelin SP, Nilsson SE, Brunk UT.

Divisions of Division of Ophthalmology, Department of Neuroscience and Locomotion, Linkoping University, Linkoping, Sweden. staffan.sundelin@lio.se

Age-related macular degeneration (AMD), the leading cause of blindness in the developed world, is accompanied by degeneration of the retinal pigment epithelial (RPE) cells. There is an inverse correlation between the melanin content of the eye and the incidence of AMD. Lipofuscin (LF)-accumulation in RPE cells accompanies the process of aging, and may also be related to AMD. This study was designed to evaluate the effect of melanin/melanosomes on the rate of LF formation in cultured rabbit and bovine RPE cells subjected to oxidative stress (40% normobaric O(2)) and daily supplementation with photoreceptor outer segments for 4 weeks. The LF content was measured at 0, 2, and 4 weeks in RPE cells from pigmented and albino rabbits, as well as in pigment-rich and pigment-poor bovine cells. Albino rabbit and pigment-poor bovine cells accumulated significantly higher amounts of LF than pigmented rabbit cells and pigment-rich bovine RPE cells after both 2 and 4 weeks of exposure. Autometallography of melanin-containing cells, without previous exposure to ammonium sulfide, showed a positive outcome, indicating either the occurrence of pre-existing iron-sulphur clusters or an extremely high intrinsic reducing capacity. These results suggest that melanin acts as an efficient antioxidant, perhaps by interacting with transition metals.

PMID: 11134897 [PubMed - indexed for MEDLINE]

Photochem Photobiol. 2001 Sep;74(3):483-8.

Environmental effects on the photochemistry of A2-E, a component of human retinal lipofuscin.

Ragauskaite L, Heckathorn RC, Gaillard ER.

Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, IL 60115, USA.

Several retinal dystrophies are associated with the accumulation of lipofuscin, a pigment mixture, in the retinal pigment epithelium (RPE). One of the major fluorophores of this mixture has been identified as the bis-retinoid pyridinium compound, A2-E. Because this compound absorbs incident radiation that is transmitted by the anterior segment of the human eye, photophysical and photochemical studies were performed to determine if A2-E could photosensitize potentially damaging reactions. Steady-state fluorescence measurements indicate that the fluorescence emission maximum and quantum yield are very sensitive to the chemical environment and a correlation between these two parameters and the solvent dielectric constant is observed. Time-resolved absorption experiments of A2-E in pure organic solvents showed no formation of transient species on the timescale of our experiments. However, when these measurements were repeated for A2-E in Triton X-100 micelles, a short-lived (tau approximately 14 microseconds), weak absorption was observed. This species is quenched by oxygen (k = 2 x 10(9) M-1 s-1) and by the addition of the antioxidants, cysteine and N,N,N',N'-tetramethylphenylenediamine. Quenching of this species by 2,3,5-trimethylhydroquinone results in the formation of the 2,3,5-trimethylsemiquinone free radical and an increase in yield of the A2-E-derived species. Sensitization of the A2-E triplet excited state indicates that the species observed in micelles upon direct excitation is not consistent with the triplet excited state. Based on these data we tentatively assign this absorption to a free radical. In the RPE these initial processes can ultimately lead to damage to the tissue through the formation of peroxides and other oxidized species.

PMID: 11594065 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2001 Nov;42(12):3041-6.

Inhibition of RPE lysosomal and antioxidant activity by the age pigment lipofuscin.

Shamsi FA, Boulton M.

Cell and Molecular Biology Unit, Department of Optometry and Vision Sciences, Cardiff University, Wales, United Kingdom.

PURPOSE: To determine whether lipofuscin is detrimental to lysosomal and antioxidant function in cultured human retinal pigment epithelial (RPE) cells. METHODS: Isolated lipofuscin granules were fed to confluent RPE cultures and the cells maintained in basal medium for 7 days. Parallel cultures were established that did not receive lipofuscin. Cultures were either exposed to visible light (390-550 nm) at an irradiance of 2.8 mW/cm(2) or maintained in the dark at 37 degrees C for up to 24 hours. Cells were subsequently assessed for cell viability, lysosomal enzyme activity, and antioxidant capacity. RESULTS: There was no loss of cell viability during the first 3 hours of light exposure, whereas a 10% loss of viability was observed in lipofuscin-fed cultures after 6 hours' exposure to light. Activities of acid phosphatase, N-acetyl-beta-glucuronidase, and cathepsin D were decreased by up to 50% in lipofuscin-fed cells exposed to light compared with either unfed cells or cells maintained in the dark. There was also a decrease in the antioxidant potential of RPE cells. Catalase and superoxide dismutase activities decreased by up to 60% and glutathione levels by 28% in light-exposed lipofuscin-fed cells compared with unfed cells or cells maintained in the dark. CONCLUSIONS: Lipofuscin has the capacity to reduce the efficacy of the lysosomal and antioxidant systems in RPE cells that may play an important role in retinal ageing and the development of age-related macular degeneration.

PMID: 11687553 [PubMed - indexed for MEDLINE]

Gerontology. 1995;41 Suppl 2:201-12.

Accumulation of lipofuscin within retinal pigment epithelial cells results in enhanced sensitivity to photo-oxidation.

Brunk UT, Wihlmark U, Wrigstad A, Roberg K, Nilsson SE.

Department of Pathology, University of Linkoping, Sweden.

Retinal pigment epithelial (RPE) cells are largely postmitotic. They continuously phagocytose the outer tips of the photoreceptor outer segments (POS). Over the life span of an individual, this activity results, although surprisingly slowly, in the intralysomal accumulation of lipofuscin, or age-pigment. Native lipofuscin shows orange-red autofluorescence when exposed to blue light. The loss of energy resulting from the conversion of excitatory blue light into emitted orange-red light may induce photo-oxidative reactions. We exposed neonatal rabbit RPE cells in culture to purified POS from cow eyes. The material were either native or peroxidized by irradiation with UV-light before being added to the RPE cultures. Lipofuscin accumulation was studied by transmission electron microscopy and measured by microfluorometric registration of its autofluorescence. Cells exposed to peroxidized POS accumulated much more lipofuscin than those exposed to native POS, indicating that peroxidized outer segments are not digestable by lysosomal enzymes. Furthermore, lipofuscin-loaded RPE cells were considerable more sensitive to visible blue light than unloaded control cells. The former ones showed lysosomal membrane destabilization with ensuing leakage of lytic enzymes and eventually cell death. We suggest that photo-oxidation of lysosomal membranes surrounding accumulated lipofuscin may be of importance for the development of age-related macular degeneration.

PMID: 8821332 [PubMed - indexed for MEDLINE]