5-4. Reactive Oxygen, Nitrogen and Quinoid Species (“ROS, “RNS”, “RQS”)

Exp Eye Res. 2003 Apr;76(4):397-403.

Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration.

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Oxidative stress is believed to contribute to the pathogenesis of many diseases, including age-related macular degeneration (AMD). Although the vision loss of AMD results from photoreceptor damage in the central retina, the initial pathogenesis involves degeneration of RPE cells. Evidence from a variety of studies suggests that RPE cells are susceptible to oxidative damage. Mitochondrial DNA (mtDNA) is particularly prone to oxidative damage compared to nuclear DNA (nDNA). Using the quantitative PCR assay, a powerful tool to measure oxidative DNA damage and repair, we have shown that human RPE cells treated with H(2)O(2) or rod outer segments resulted in preferential damage to mtDNA, but not nDNA; and damaged mtDNA is not efficiently repaired, leading to compromised mitochondrial redox function as indicated by the MTT assay. Thus, the susceptibility of mtDNA to oxidative damage in human RPE cells, together with the age-related decrease of cellular anti-oxidant system, provides the rationale for a mitochondria-based model of AMD.

Publication Types:

- Review
- Review, Tutorial

PMID: 12634104 [PubMed - indexed for MEDLINE]

Photochem Photobiol. 2004 May;79(5):470-5.

Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells.

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Throughout the lifetime of an individual, light is focused onto the retina. The resulting photooxidative stress can cause acute or chronic retinal damage. The pathogenesis of age-related macular degeneration (AMD), the leading cause of legal blindness in the developed world, involves oxidative stress and death of the retinal pigment epithelium (RPE) followed by death of the overlying photoreceptors. Evidence suggests that damage due to exposure to light plays a role in AMD and other age-related eye diseases. In this work a system for light-induced damage and death of the RPE, based on the human ARPE-19 cell line, was used. Induction of mitochondria-derived reactive oxygen species (ROS) is shown to play a critical role in the death of cells exposed to short-wavelength blue light (425 +/- 20 nm). ROS and cell death are blocked either by inhibiting the mitochondrial electron transport chain or by mitochondria-specific antioxidants. These results show that mitochondria are an important source of toxic oxygen radicals in blue light-exposed RPE cells and may indicate new approaches for treating AMD using mitochondria-targeted antioxidants.

PMID: 15191057 [PubMed - indexed for MEDLINE]

Exp Eye Res. 2005 Jan;80(1):113-9.

Enhanced expression of glutathione-S-transferase A1-1 protects against oxidative stress in human retinal pigment epithelial cells.

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Glutathione-S-transferases (GSTs) play an important role in protection mechanisms against oxidative stress. We sought to determine whether over-expression of human GSTA1-1 in RPE cells is able to attenuate H(2)O(2)-induced oxidative stress. SV40-transformed human fetal RPE cells were stably transfected with pRC/hGSTA1-1 vector which carries a full-length of human GSTA1-1 cDNA. The control RPE cells were either non-transfected or transfected with control vector pRC. Expression of hGSTA1-1 protein in these cells was confirmed by Western blot and immunocytochemical analyses. The protective effects of hGSTA1-1 on cell viability and mitochondrial DNA (mtDNA) damage caused by H(2)O(2) were examined with MTT assay and quantitative PCR (QPCR), respectively. The hGSTA1-1 transfected RPE cells exhibited a similar morphology and growth rate as control RPE cells. Immunocytochemical analysis showed robust expression hGSTA1-1 in hGSTA1-1 transfected cells versus background staining in control cells. Western blotting of protein extracts from cells transfected with hGSTA1-1 revealed a 26 kDa protein band which corresponds to the size of recombinant mature hGSTA1-1. The active GST present in the hGSTA1-1 transfected cells was approximately three times higher than in control cells. The MTT assay showed a significantly greater viability of hGSTA1-1 cells in response to H(2)O(2) (100 and 200 microm) compared to control cells ($p<0.05$). QPCR indicated that mtDNA damage was significantly decreased in hGSTA1-1 cells than in control cells ($p<0.05$). Human GSTA1-1 transfection protect against RPE cell death and mtDNA damage caused by H(2)O(2), suggesting an important role of GST in protection against oxidative stress in RPE cells.

PMID: 15652532 [PubMed - indexed for MEDLINE]

Biochemistry. 2005 Jan 18;44(2):480-9.

Microsomal glutathione S-transferase 1 in the retinal pigment epithelium: protection against oxidative stress and a potential role in aging.

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High oxygen tension, exposure to light, and the biochemical events of vision generate significant oxidative stress in the retina and the retinal pigment epithelium (RPE). Understanding the mechanisms and basis of susceptibility to progressive retinal diseases involving oxidative damage such as age-related macular degeneration (AMD) remains a major challenge. Here microsomal glutathione S-transferase (MGST1) is shown to be a dominant, highly expressed enzyme in bovine and mouse RPE microsomes that displays significant reduction activity toward synthetic peroxides, oxidized RPE lipids, and oxidized retinoids. This enzymatic reduction activity (GPx) can be partially neutralized with a monoclonal anti-MGST1 antibody developed in this study. MGST1-transfected HEK293 cells exhibited greater viability (70 +/- 4% survival) compared with untransfected control cells (46 +/- 4% survival) when challenged with 20 microM H(2)O(2), and greater viability of MGST1-transfected cells following challenge with oxidized docosahexaenoic acid was also observed. Cultured ARPE19 cells transfected with silencing MGST1 siRNAs exhibited lower expression of MGST1 (12% and 26% of the controls) and significantly lower GPx activity (44 +/- 13%) and, thus, were more susceptible to oxidative damage. Immunoblotting revealed that the in vivo expression of MGST1 in mouse RPE decreases 3-4-fold with age, to trace levels in 18-month-old mice. GPx activity in the RPE was also found to be reduced in 12-month-old mice to approximately 67%. These results support an important protective function for MGST1 against oxidative insult in the RPE that decreases with age and suggest that this enzyme may play a role in the development of age-related diseases such as AMD.

PMID: 15641772 [PubMed - indexed for MEDLINE]

Invest Ophthalmol Vis Sci. 2004 Oct;45(10):3767-77.

Survival of retinal pigment epithelium after exposure to prolonged oxidative injury: a detailed gene expression and cellular analysis.

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PURPOSE: To detail, by DNA microarrays and cellular structure labeling, the in vitro responses of retinal pigment epithelial (RPE) cells to a nonlethal dose of the oxidant agent hydroquinone (HQ). METHODS: The viability of growth-quiescent ARPE-19 cells after treatment with HQ was measured by XTT conversion, (3)H-leucine incorporation, trypan blue exclusion, and the presence of DNA laddering. The effect of a nonlethal dose of HQ on the localization of apoptosis-induced factor (AIF) and phosphorylation of stress-activated kinase-2/p38 (SAPK2/p38) was detected by immunocytochemistry. Actin structures were visualized by phalloidin staining. Cell membrane blebbing was detected using GFP-membrane-labeled RPE cells (ARPE-GFP-c'-rRas). Changes in gene expression patterns of RPE cells within 48 hours of prolonged treatment with a nonlethal dose of HQ were evaluated by microarray analysis and confirmed by Northern blotting. RESULTS: The viability of RPE after a prolonged sublethal injury dose of HQ was determined by multiple assays and confirmed by the absence of AIF translocation or DNA laddering. Prolonged exposure (16 hours) of RPE cells to a nonlethal dose of HQ resulted in actin rearrangement into globular aggregates and cell membrane blebbing. Kinetic microarray analysis at several time points over a 48-hour recovery period revealed significant upregulation of genes involved in ameliorating the oxidative stress, chaperone proteins, anti-apoptotic factors, and DNA repair factors, and downregulation of pro-apoptotic genes. Genes involved in extracellular matrix functions were also dysregulated. Recovery of RPE cells after the injury was confirmed by the normalization of gene expression dysregulation back to baseline levels within 48 hours. CONCLUSIONS: RPE cells avoided cell death and recovered from prolonged oxidative injury by activating a host of defense mechanisms while simultaneously triggering genes and cellular responses that may be involved in RPE disease development.

PMID: 15452088 [PubMed - indexed for MEDLINE]

Eur J Pharmacol. 2002 Aug 9;449(3):213-20.

Induction of vascular endothelial growth factor by 4-hydroxynonenal and its prevention by glutathione precursors in retinal pigment epithelial cells.

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Although 4-hydroxynonenal, a highly reactive lipid peroxidation product, is implicated in several age-related disorders such as Alzheimer's and Parkinson's diseases, its role in age-related macular degeneration is not known. The purpose of this study was to determine whether 4-hydroxynonenal increases vascular endothelial growth factor (VEGF) expression in human retinal pigment epithelial cells (ARPE-19), a source of VEGF in choroidal neovascularization observed in age-related macular degeneration. In addition, it was the purpose of this study to assess whether glutathione (GSH) and GSH precursors can inhibit the effects of 4-hydroxynonenal. At 1 micro M, 4-hydroxynonenal did not alter cell viability, but elevated VEGF secretion and mRNA expression by 35% ($p<0.05$) and 1.9-fold ($p<0.05$), respectively. However, at concentrations 5 microM and above, 4-hydroxynonenal reduced VEGF secretion as well as cell viability. At 1 and 10 microM, 4-hydroxynonenal did not induce apoptosis in ARPE-19 cells. 4-Hydroxynonenal (1 microM) reduced intracellular GSH by 25% ($p<0.05$) and increased oxidative stress by 50% ($p<0.05$). GSH precursor pretreatment for 1 h, which increased intracellular GSH levels by 50% ($p<0.05$), as well as GSH co-treatment, inhibited the VEGF-inductive and cytotoxic effects of 4-hydroxynonenal. Thus, 4-hydroxynonenal (1 microM) induces VEGF expression and secretion in ARPE-19 cells. This effect is likely due to GSH depletion and an associated increase in intracellular oxidative stress, resulting in increased VEGF mRNA levels. 4-Hydroxynonenal-mediated VEGF secretion as well as cytotoxicity can be reversed with GSH precursor pretreatment or GSH co-treatment.

PMID: 12167462 [PubMed - indexed for MEDLINE]

J Clin Invest. 1996 Oct 1;98(7):1667-75.

Reactive oxygen intermediates increase vascular endothelial growth factor expression in vitro and in vivo.

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Elevated vascular endothelial growth factor (VEGF) levels are required for ocular and tumor angiogenesis in animal models. Ischemic hypoxia is strongly correlated with increased VEGF expression in these systems and is considered a physiologically relevant stimulus. Because ischemic hypoxia is often followed by reperfusion and reactive oxygen intermediate (ROI) generation, we examined the potential role of ROI in the control of VEGF gene expression. Human retinal pigment epithelial cells exposed to superoxide or hydrogen peroxide rapidly increased VEGF mRNA levels. Superoxide-associated mRNA increases were dose dependent, blocked by antioxidants, and associated with elevated VEGF protein levels in conditioned media. Increases in VEGF mRNA levels were also observed in cultured human melanoma and rat glioblastoma cells with superoxide or hydrogen peroxide. Cycloheximide prevented the ROI-associated increases in VEGF mRNA. Transcriptional inhibition with actinomycin D revealed an inducible increase in VEGF mRNA half-life, but nuclear run-on experiments showed no increase in VEGF transcriptional rate. Reoxygenation of human retinal pigment epithelial cells in vitro and ocular reperfusion in vivo increased retinal VEGF mRNA levels. Antioxidants prevented the reperfusion-associated VEGF mRNA increases in retina. We conclude that ROIs increase VEGF gene expression in vitro and during the reperfusion of ischemic retina in vivo. The ROI-associated increases are mediated largely through increases in VEGF mRNA stability.

PMID: 8833917 [PubMed - indexed for MEDLINE]

J Cell Physiol. 2001 Dec;189(3):323-33.

Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF.

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We investigated gene expression profiles of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) in differentiated and non-differentiated retinal pigment epithelial (RPE) cells during oxidative stress. Human RPE cells were grown in culture on laminin-coated flasks to obtain differentiated features. Cells cultured on plastic were used as non-differentiated controls. After confluence, hydrogen peroxide (H2O2) was added for 48 h, then, total RNA was extracted and used for RT-PCR and Northern blot analysis. Medium conditioned by RPE was used for ELISA, Western blotting, and in vitro angiogenesis assay. As a result, differentiated RPE cells expressed significantly higher levels of VEGF protein, as compared to their non-differentiated counterparts. The expression pattern remained consistent even after cellular exposure to H2O2. Conversely, while elevated levels of PEDF transcript and protein were seen in differentiated RPE cells, compared to non-differentiated cells, a marked decrease at both PEDF mRNA and protein levels was seen after treatment with H2O2. Moreover, this decrease in PEDF expression was dosage dependent. In in vitro angiogenesis assay, conditioned medium from differentiated human RPE cells after exposure to H2O2 showed a dramatic increase in tubular formation and migratory activity of microvascular endothelial cells. These data suggest that, in physiological conditions, a critical balance between PEDF and VEGF exists, and PEDF may counteract the angiogenic potential of VEGF. Under oxidative stress, PEDF decreases disrupting this balance. This equilibrium shift may be significant in promoting a pathological condition of RPE cells and contributing to choroidal neovascularization in age-related macular degeneration.
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Change of redox status and modulation by thiol replenishment in retinal photooxidative damage.

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PURPOSE: Cellular or tissue reduction-oxidation (redox) is crucial in various diseases. The present study was conducted to analyze how tissue redox status is affected by photooxidative stress and whether the exogenous thiol antioxidant N-acetylcysteine (NAC) affects photooxidative stress-induced retinal damage. METHODS: Mice were intraperitoneally injected with either NAC (250 mg/kg) or phosphate-buffered saline (PBS) and exposed to white fluorescent light (8000 lux) for 2 hours. Levels of thioredoxin (TRX), glutaredoxin (GRX), and glutathione (GSH), endogenous regulators of redox; 4-hydroxy-2-nonenal (HNE)-modified protein, a marker of lipid peroxidation; and nuclear factor (NF)-kappaB, a redox-sensitive transcription factor in retinal samples, was measured by immunohistochemistry and Western blot or enzymatic recycling assay. Light-induced retinal damage estimated by electroretinography and quantitative immunohistochemistry for 8-hydroxy-2-deoxyguanosine (8OHdG index), a marker of oxidative stress-induced DNA damage, was compared in NAC- and PBS-treated mice. RESULTS: Upregulation of TRX and HNE-modified protein, decrease of GSH, and nuclear translocation of NF-kappaB were noted after light exposure in PBS-treated mice. These changes were suppressed in NAC-treated mice compared with PBS-treated mice. GRX was not upregulated after light exposure in any mice. The a- and b-wave amplitudes were significantly higher, and the 8OHdG index was significantly lower after light exposure in NAC-treated mice than in PBS-treated mice. CONCLUSIONS: Retinal redox status is altered by intense light and is normalized partially by the effect of NAC on TRX and GSH tissue levels. Manipulation of the tissue redox state by exogenous thiol replenishment may be a useful strategy to prevent retinal photooxidative damage.

PMID: 12091442 [PubMed - indexed for MEDLINE]

Biochimie. 2004 Nov;86(11):825-31.

Lipids and lipid peroxidation products in the pathogenesis of age-related macular degeneration.

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In people over 50, age-related macular degeneration (ARMD) has become the most common cause for severe visual loss and legal blindness in all industrialized nations. Currently, there is no effective treatment for the majority of patients. To develop new and effective modes of therapy, understanding of the molecular basis of the disease in mandatory. However, the pathogenesis of ARMD is still poorly understood. Several lines of evidence suggest that aging changes of the retinal pigment epithelium (RPE), in particular the accumulation of autofluorescent lipofuscin granules in the lysosomal compartment of postmitotic RPE cells, play a key role in the pathogenesis of the disease. Recent studies indicate that lipidic compounds of lipofuscin, represented by the retinoid A2-E, and protein damage by lipid peroxidation products, in particular malondialdehyde and 4-hydroxynonenal, induce lysosomal dysfunction and lipofuscinogenesis in the RPE. The possible mechanisms underlying this lysosomal dysfunction and the resulting adverse effects on overall RPE function are discussed.

PMID: 15589692 [PubMed - in process]

Biochim Biophys Acta. 2004 May 24;1689(1):33-41.

Products of lipid peroxidation induce missorting of the principal lysosomal protease in retinal pigment epithelium.

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Phagocytosis of photoreceptor outer segments (OS) by retinal pigment epithelium (RPE) is essential for OS renewal and survival of photoreceptors. Internalized, oxidatively modified macromolecules perturb the lysosomal function of the RPE and can lead to impaired processing of photoreceptor outer segments. In this study, we sought to investigate the impact of intracellular accumulation of oxidatively damaged lipid-protein complexes on maturation and distribution of cathepsin D, the major lysosomal protease in the RPE. Primary cultures of human RPE cells were treated with copper-oxidized low density lipoprotein (LDL) and then challenged with serum-coated latex beads to stimulate phagocytosis. Three observations were noted to occur in this experimental system. First, immature forms of cathepsin D (52 and 46 kDa) were exclusively associated with latex-containing phagosomes. Second, maturation of cathepsin D was severely impaired in RPE cells loaded with oxidized LDL (oxLDL) prior to the phagocytic challenge. Third, pre-treatment with oxLDL caused sustained secretion of pro-cathepsin D and the latent form of gelatinase A into the extracellular space in a dose-dependent manner. These data stimulate the hypothesis that intracellular accumulation of poorly degradable, oxidized lipid-protein cross-links, may alter the turnover of cathepsin D, causing its mistargeting into the extracellular space together with the enhanced secretion of a gelatinase.

PMID: 15158911 [PubMed - indexed for MEDLINE]

Doc Ophthalmol. 2003 Jan;106(1):13-6.

Aging of cultured retinal pigment epithelial cells: oxidative reactions, lipofuscin formation and blue light damage.

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This report reviews our experimental work on cultured retinal pigment epithelial (RPE) cells, fed native or UV-irradiated photoreceptor outer segments (POS). We showed that significantly more lipofuscin (LF) was formed in cells cultured in 40% oxygen than in cells cultured in 8% oxygen, indicating an involvement of oxidative mechanisms in LF formation. The antioxidants alpha-tocopherol, lycopene, zeaxanthin and lutein significantly reduced LF formation. RPE cells high in melanin content exhibited significantly less formation of LF than cells low in or devoid of melanin, suggesting that melanin acts as an effective antioxidant. The phagocytic capacity of LF-loaded RPE cells was significantly reduced compared to that of unloaded control cells, indicating that LF-loaded RPE cells may be unable to serve the photoreceptors sufficiently regarding phagocytosis of shed outer segment tips. Blue light irradiation destabilized lysosomal membranes in LF-loaded RPE cells and significantly reduced the viability of such cells compared to unloaded, irradiated control cells. These results may be of significance in relation to the development of age-related macular degeneration (AMD).

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Invest Ophthalmol Vis Sci. 2002 Aug;43(8):2546-53.

Increased oxidant-induced apoptosis in cultured nondividing human retinal pigment epithelial cells.

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PURPOSE: To determine whether long-term cultured nondividing human retinal pigment epithelial (hRPE) cells are sensitive to oxidant-induced apoptosis and whether the Fas pathway is involved in the process. METHODS: Confluent hRPE cells were maintained for 2 to 3 months in the basal medium (DMEM containing 2% fetal bovine serum) with one medium change per week. DNA synthesis was measured by incorporation of bromodeoxyuridine (BrdU) and the cell cycle was analyzed by flow cytometry. Intracellular glutathione (GSH) and glutathione disulfide (GSSG) were measured by HPLC. Apoptosis was triggered with the oxidant tert-butylhydroperoxide (tBH), recombinant soluble Fas ligand (sFasL), or agonistic anti-Fas antibody (CH-11). Cell viability was assessed by tetrazolium salt (WST-1) assay, and apoptosis was determined by measuring DNA cleavage or phosphatidylserine exposure. FasL and Fas proteins were detected by flow cytometry and Western blot. FasL and Fas transcripts were analyzed by RT-PCR. RESULTS: After incubation in basal medium for more than 2 months, hRPE cells were largely nondividing and accumulated autofluorescent granules identified by electron microscopy to be lysosomes. Compared with proliferating hRPE cells, the nondividing cells had lower intracellular GSH, GSSG, and GSH/GSSG and a more oxidized redox potential (E(h)). Downregulation of Fas but upregulation of FasL was observed in these cells. The nondividing hRPE cells appeared more susceptible to tBH-induced apoptosis. Similar to proliferating hRPE cells, the apoptosis induced by tBH was preceded by induction of FasL, and antioxidants inhibited both FasL increase and apoptosis. Apoptosis was also inhibited with the antagonistic anti-Fas antibody ZB4. However, the nondividing hRPE cells had decreased sensitivity to apoptosis triggered by sFasL or CH-11. CONCLUSIONS: Long-term hRPE culture created cells that were nondividing and accumulated autofluorescent granules. The increased sensitivity to tBH-induced apoptosis in these cells was associated with intracellular oxidation and upregulation of FasL. These results suggest that an increase in FasL may contribute to the vulnerability of nondividing hRPE cells to oxidant-induced apoptosis.

PMID: 12147583 [PubMed - indexed for MEDLINE]

Prog Retin Eye Res. 2000 Mar;19(2):205-21.

Oxidative damage and protection of the RPE.

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This review provides a model for the role of oxidative stress in the etiology of age-related macular degeneration (AMD). Epidemiological studies of diet, environmental and behavioral risk factors suggest that oxidative stress is a contributing factor of AMD. Pathological studies indicate that damage to the retinal pigment epithelium (RPE) is an early event in AMD. In vitro studies show that oxidant treated RPE cells undergo apoptosis, a possible mechanism by which RPE cells are lost during early phase of AMD. The main target of oxidative injury seems to be mitochondria, an organelle known to accumulate genomic damages in other postmitotic tissues during aging. The thiol antioxidant GSH and its amino acid precursors protect RPE cells from oxidant-induced apoptosis. Similar protection occurs with dietary enzyme inducers which increase GSH synthesis. These results indicate that therapeutic or nutritional intervention to enhance the GSH antioxidant capacity of RPE may provide an effective way to prevent or treat AMD.

Publication Types:

- Review
- Review, Academic

PMID: 10674708 [PubMed - indexed for MEDLINE]

Surv Ophthalmol. 2000 Sep-Oct;45(2):115-34.

The role of oxidative stress in the pathogenesis of age-related macular degeneration.

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Age-related macular degeneration (AMD) is the leading cause of blind registration in the developed world, and yet its pathogenesis remains poorly understood. Oxidative stress, which refers to cellular damage caused by reactive oxygen intermediates (ROI), has been implicated in many disease processes, especially age-related disorders. ROIs include free radicals, hydrogen peroxide, and singlet oxygen, and they are often the byproducts of oxygen metabolism. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, its high proportion of polyunsaturated fatty acids, and its exposure to visible light. In vitro studies have consistently shown that photochemical retinal injury is attributable to oxidative stress and that the antioxidant vitamins A, C, and E protect against this type of injury. Furthermore, there is strong evidence suggesting that lipofuscin is derived, at least in part, from oxidatively damaged photoreceptor outer segments and that it is itself a photoreactive substance. However, the relationships between dietary and serum levels of the antioxidant vitamins and age-related macular disease are less clear, although a protective effect of high plasma concentrations of alpha-tocopherol has been convincingly demonstrated. Macular pigment is also believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching ROIs. Many putative risk-factors for AMD have been linked to a lack of macular pigment, including female gender, lens density, tobacco use, light iris color, and reduced visual sensitivity. Moreover, the Eye Disease Case-Control Study found that high plasma levels of lutein and zeaxanthin were associated with reduced risk of neovascular AMD. The concept that AMD can be attributed to cumulative oxidative stress is enticing, but remains unproven. With a view to reducing oxidative damage, the effect of nutritional antioxidant supplements on the onset and natural course of age-related macular disease is currently being evaluated.

Publication Types:

- Review
- Review, Academic

PMID: 11033038 [PubMed - indexed for MEDLINE]

Mol Vis. 1999 Nov 3;5:32.

Oxidative damage and age-related macular degeneration.

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This article provides current information on the potential role of oxidation in relation to age-related macular degeneration (AMD). The emphasis is placed on the generation of oxidants and free radicals and the protective effects of antioxidants in the outer retina, with specific emphasis on the photoreceptor cells, the retinal pigment epithelium and the choriocapillaris. The starting points include a discussion and a definition of what radicals are, their endogenous sources, how they react, and what damage they may cause. The photoreceptor/pigment epithelium complex is exposed to sunlight, is bathed in a near-arterial level of oxygen, and membranes in this complex contain high concentrations of polyunsaturated fatty acids, all considered to be potential factors leading to oxidative damage. Actions of antioxidants such as glutathione, vitamin C, superoxide dismutase, catalase, vitamin E and the carotenoids are discussed in terms of their mechanisms of preventing oxidative damage. The phototoxicity of lipofuscin, a group of complex autofluorescent lipid/protein aggregates that accumulate in the retinal pigment epithelium, is described and evidence is presented suggesting that intracellular lipofuscin is toxic to these cells, thus supporting a role for lipofuscin in aging and AMD. The theory that AMD is primarily due to a photosensitizing injury to the choriocapillaris is evaluated. Results are presented showing that when protoporphyric mice are exposed to blue light there is an induction in the synthesis of Type IV collagen synthesis by the choriocapillary endothelium, which leads to a thickened Bruch's membrane and to the appearance of sub-retinal pigment epithelial fibrillogranular deposits, which are similar to basal laminar deposits. The hypothesis that AMD may result from oxidative injury to the retinal pigment epithelium is further evaluated in experiments designed to test the protective effects of glutathione in preventing damage to cultured human pigment epithelial cells exposed to an oxidant. Experiments designed to increase the concentration of glutathione in pigment epithelial cells using dimethylfumarate, a monofunctional inducer, are described in relation to the ability of these cells to survive an oxidative challenge. While all these models provide undisputed evidence of oxidative damage to the retinal pigment epithelium and the choriocapillaris that is both light- and oxygen-dependent, it nevertheless is still unclear at this time what the precise linkage is between oxidation-induced events and the onset and progression of AMD.

Publication Types:

- Review
- Review, Tutorial

PMID: 10562656 [PubMed - indexed for MEDLINE]

Retina. 1999;19(2):141-7.

Characterization of peroxidized lipids in Bruch's membrane.

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PURPOSE: To determine if peroxidized lipids occur in Bruch's membrane isolates and to characterize the type present in human necropsy specimens. METHODS: Bruch's membrane isolates from eye bank eyes obtained from 13 white donors were homogenized. Measurement of peroxidized lipids was done with the fluorometric thiobarbituric acid assay and high pressure liquid chromatography. RESULTS: Bruch's membrane isolate homogenates contained native unsaturated fatty acids and peroxidized lipids in a ratio of about 200:1. The amount of thiobarbituric acid reacting substances increased exponentially with age. The peroxidized lipids identified in Bruch's membrane isolates were derived from long chain polyunsaturated fatty acids, particularly docosahexaenoic acid and linolenic acid, which are normally found in the photoreceptor outer segments. CONCLUSIONS: Lipids are known to accumulate in Bruch's membrane, an acellular layer with no known intrinsic mechanisms to combat lipid peroxidation. In related studies, lipid peroxides have been shown to induce neovascularization by inducing expression of a cascade of angiogenic cytokines. This is the first study to show that lipid peroxides, biological molecules that have the potential to incite new vessel growth, occur in Bruch's membrane. The increase in amount of peroxidized lipids with age, combined with their vasogenic potential, suggests that peroxidized lipids may play a role in the etiology of age-related macular degeneration, particularly choroidal neovascularization.

PMID: 10213241 [PubMed - indexed for MEDLINE]

Neuroscience. 1999;90(4):1493-9.

Glutathione depletion causes an uncoupling effect on retinal horizontal cells through oxidative stress.

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To investigate a physiological role of glutathione in the horizontal cells of carp retina, the gap junctional intercellular communication between horizontal cells was studied using the techniques of intracellular recording of light-induced responses and coupling of the fluorescence dye Lucifer Yellow. Intravitreal injection of 2.5 micromol L-buthionine sulfoximine, an inhibitor of glutathione synthesis, induced a dramatic reduction (20% of control) of retinal glutathione level two days after treatment. The low level of glutathione continued for a further four to five days, and thereafter gradually recovered to about 40% (20 days after injection) and 70% (50 days after injection) of the control level. The spatial properties of the photopic L-type horizontal cell response were examined by enlarging the diameter of the central spot and peripheral annulus over the recording point. In normal retinas, the response amplitude of horizontal cells was monotonically enhanced as the diameter of the spot increased (0.5-4.0 mm) and correspondingly the dye diffusion area was wide, as the injected Lucifer Yellow normally diffused to several neighboring cells. Treatment with L-buthionine sulfoximine significantly altered the spatial properties of horizontal cells by increasing the response amplitude to central spots and slightly decreasing that to peripheral annuli, which were observed by four days after injection. It also restricted intracellular Lucifer Yellow to one or two cells. Accompanying the recovery of the cellular level of glutathione, the spatial properties and dye coupling of horizontal cells were restored to normal. A time lag (two days) of initiation in retinal glutathione depletion and alteration of spatial or dye coupling properties of horizontal cells is discussed, together with reactive oxygen species accumulation.

PMID: 10338315 [PubMed - indexed for MEDLINE]