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Region and Age-Dependent Variation in Susceptibility of the Human Retina to Lipid Peroxidation

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Trehpined buttons from the posterior central (including the macula) and four peripheral regions of human retina were subjected to in vitro lipid peroxidation initiated by Fe$^{2+}$. There was an age-related increase in peroxidation in the retinal tissue from the posterior region ($P = 0.0019$), but not in tissues from any of the four peripheral retinal quadrants (collective $P = 0.24$). These results suggest that the posterior region of the human retina is susceptible to lipid peroxidation and that age is a factor. Invest Ophthalmol Vis Sci 33:3497–3499, 1992

Lipid peroxidation is a complex process that has been implicated in the pathogenesis of numerous ocular diseases. It has been suggested that peroxidative damage is involved in the pathogenesis of age-related macular degeneration (ARMD), a complex disease relatively selective for the macula that is the leading cause of visual loss in the United States in patients over 50 years old. Outer segment membranes of photoreceptor cells are particularly susceptible to lipid peroxidation because of their high polyunsaturated fatty acid content. Minimal scientific investigation has been performed to elucidate whether the human retina has an age-dependent or regional susceptibility to oxidative processes. Based on an in vitro system we developed to study peroxidation of bovine rod outer segment lipids, we provide evidence that suggests there is an age-dependent increase in susceptibility of the posterior region of the human retina to lipid peroxidation that is not noted in the peripheral retina.

Materials and Methods

Human retinal tissue was obtained from 15 pairs of cadaver whole globes that had been stored in moist media at 4°C. Post-mortem times ranged from 12 hr to 4 days. Causes of death included myocardial infarction in nine patients, cerebrovascular accident in one patient, and sepsis in one patient. Eight patients were either septic, febrile, or on intravenous antibiotics at the time of their deaths. With a 7 mm trephine, samples of retina were obtained from the posterior pole centered over the macula and from the superior, inferior, nasal, and temporal peripheral quadrants centered between the equator and the ciliary body. The trephined retinal tissues were carefully dissected from the underlying retinal pigment epithelium and choroid, and any adhering vitreous was removed.

Lipid peroxidation was estimated by the thiobarbituric acid assay. Each retina sample was combined with 400 µl HEPES buffer and homogenized at 4°C. A 300 µl aliquot of this solution was combined with 150 µl HEPES buffer. Lipid peroxidation was initiated by incubating the solution for 10 min at 37°C with 50 µl Fe$^{2+}$, such that the final concentration was 100 µmol/l. The incubation was stopped by adding 500 µl of HEPES that contained the chelating agent diethylene-triamine pentaacetic acid in a final concentration of 2.5 mmol/l. One globe from each pair of eyes was randomly placed in a control group, and retinal tissue from this eye was incubated exactly as described above without Fe$^{2+}$.

Lipid peroxidation was estimated by the thiobarbituric acid assay. The absorbance of each reaction mixture was measured at 535 nm on a Gilford 250 spectrophotometer against a blank that contained the reagents minus the retina homogenate. We assumed that the same weight of retina was present in each regional sample. Because this assumption may not have been valid for the different regions, comparisons were made only within the same region.

Results

For each pair of globes, the difference between the Fe$^{2+}$-stimulated and control absorbance values was
determined for each location in the retina. This difference was determined to control for the presence of substances in the homogenate that absorb at 545 nm and for peroxidation reactions that occur in the absence of added Fe$^{2+}$. The adjusted absorbance values measure the relative susceptibility of the retinal region to lipid peroxidation. These values were compared to the ages of the patients at the time of death.

The adjusted absorbances of the posterior region are plotted against the ages of the patients in Figure 1. Using analysis of variance, an age-dependent increase in susceptibility of the posterior region to lipid peroxidation was noted ($P = 0.0019$, $R^2 = 0.537$). The increase in susceptibility persisted even when the data from the 93-yr-old patient (adjusted absorbance = 0.280) was removed from the analysis ($P = 0.0277$, $R^2 = 0.343$), although there would be no reason for ignoring this data point. The adjusted absorbances from the posterior region in patients who were septic, febrile, or on intravenous antibiotics were not higher than those from the other patients. Using multivariate analysis with oxidation as dependent variable and age and postmortem time as independent variables, postmortem time had no effect ($P = 0.3017$) on the susceptibility of the posterior retina to lipid peroxidation.

In Figure 2, adjusted absorbances from the four peripheral locations are plotted against patient age. Increasing age was not associated with greater susceptibility of the peripheral retina to lipid peroxidation.

**Discussion**

The etiology of age-related macular degeneration is not known. Although unproven, it has been suggested that light may be involved in the disease process.$^8$$^9$
We have observed that retinas of rats raised under bright cyclic light have lower levels of 22:6n-3—the major fatty acid in the retinas of mammals and the one most susceptible to lipid peroxidation—and have higher levels of antioxidants and antioxidant enzymes compared to rats raised under dim cyclic light. Presumably, the bright lights were a greater risk for lipid peroxidation and the retinas responded to this oxidant challenge by decreasing their levels of substrate (22:6n-3) and increasing their antioxidant defenses. Penn and Anderson called this phenomenon "biochemical adaptation."

It is interesting that the level of 22:6n-3 in the human macular region is less than in the peripheral retina, suggesting that the oxidant challenge to this area may be greater than that to the peripheral tissue. With increasing age, the human macula may lose its ability to adapt and thus becomes more susceptible to lipid peroxidation. If this is true, then changes in antioxidants such as vitamin E, ascorbic acid, and glutathione, or changes in antioxidant enzymes such as glutathione peroxidase, glutathione S-transferase, glutathione reductase, superoxide dismutase, and catalase as a function of age may partly determine the development of ARMD. None of these has been measured in peripheral and posterior retina as a function of age.

The evidence in this report suggests that the oxidative potential of the posterior region (including the macula) of the human retina increases with age. The age-dependent increase in oxidative potential was not noted in the peripheral regions. These findings suggest that age-related changes in the antioxidant systems may make the macula more susceptible to oxidative damage and that lipid peroxidation may be an important factor in ARMD.

Key words: age-related macular degeneration, antioxidants, lipid peroxidation, macular degeneration, retinal degeneration.

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References