A Lipid Peroxidative Mechanism for Posterior Subcapsular Cataract Formation in the Rabbit: A Possible Model for Cataract Formation in Tapetoretinal Diseases

John D. Goosey, Wei Ming Tuan, and Charles A. Garcia

Posterior subcapsular cataracts developed in rabbits following injection of docosahexenoic acid. Autoxidation of docosahexenoic acid in rabbit vitreous was demonstrated by the formation of malondialdehyde and vitreous fluorescence. The onset of posterior subcapsular cataract formation was temporally correlated with declining levels of vitreous malondialdehyde. Invest Ophthalmol Vis Sci 25:608-612, 1984

Posterior subcapsular cataracts (PSC) are commonly associated with retinitis pigmentosa and other hereditary tapetoretinal degenerations.1,2 The association of PSC with retinal and choroidal degenerations, which have various modes of inheritance and different basic metabolic abnormalities,3 supports the hypothesis that posterior subcapsular cataract formation is a secondary phenomenon in the pathogenesis of these diseases. In 1944, Berliner postulated a secondary mechanism for vitreous degeneration and posterior subcapsular cataract formation in retinitis pigmentosa.4 He proposed that unknown “toxic substances” formed during the process of retinal degeneration diffuse anteriorly through the vitreous body, resulting in vitreous opacities and complicated cataracts.

Degeneration of rod outer segment membranes is an early histopathologic change in retinitis pigmentosa. Rod outer segment membrane deterioration also occurs at varying stages in the progression of other tapetoretinal degenerations. Outer segment membranes contain an extraordinarily high amount of docosahexenoic acid.5 This polyunsaturated fatty acid is extremely labile and in the presence of oxygen it undergoes peroxidation and subsequent free radical decomposition to yield toxic aldehydes and lipid radicals.6 Thus, autoxidation of docosahexenoic acid within outer segment membranes may yield toxic products, which diffuse through the vitreous and cause the posterior subcapsular cataracts so commonly seen in retinitis pigmentosa and other tapetoretinal degenerations.

One of the potentially toxic autooxidation products of docosahexenoic acid is malondialdehyde. Because malondialdehyde formation indicates that docosahexenoic acid oxidation has occurred, the present study evaluated malondialdehyde levels following intravitreal injection of docosahexenoic acid in the rabbit and correlated malondialdehyde generation with the onset of posterior subcapsular cataract formation. In addition, a spectrofluorometric analysis of vitreous tissue was conducted in order to demonstrate the formation of fluorescent cross-links, which result from the reaction of malondialdehyde with primary amino groups.7

Materials and Methods. Eleven, 4-month-old rabbits were anesthetized with an intramuscular injection (1 ml/kg) of a solution containing ketamine (1.66 mg/ml), acepromazine (3.33 mg/ml), and xylazine (1.66 mg/ml). After inducing anesthesia, a Glenco microsyringe (Houston, TX) with a 26-gauge needle was used to inject 10 μl (= 10 mg) of docosahexenoic (22:6) acid (grade I, Sigma Chemical Company; St. Louis, MO) into the posterior vitreous of rabbit eyes. Indirect ophthalmoscopy was used to guide the 26-gauge needle through the vitreous and avoid traumatizing the rabbit lens. At eleven different time intervals up to 24 hr following injection of 22:6, rabbits were killed and their eyes were then examined by slit-lamp biomicroscopy prior to enucleation. The enucleated eyes were immediately frozen and stored at −70°C. At each interval, the frozen vitreous was removed and homogenized without the addition of buffer. The homogenized vitreous was centrifuged for 10 min at 100,000 X g. A 150 μl aliquot was removed from the supernatant of each sample and MDA levels were assayed by the thiobarbituric acid (TBA) test. Prior to TBA analysis a chloroform-methanol (2:1) extraction was done to insure removal of any unoxidized 22:6. The TBA assay was then conducted according to the method of Chio7 using an extinction coefficient of 158,000 for malondialdehyde.

Fluorescent lipid peroxidation products were extracted from vitreous tissue according to the method of Fletcher et al.8 The wet weight of the vitreous tissue in each 100,000 g pellet was measured to the nearest 0.1 milligram. Chloroform-methanol, 2:1 (V:V), was added to each pellet in a volume-to-weight ratio of 20:1. Each pellet was then homogenized for 1.5 min in a 45°C water bath. After homogenization an equal volume of water was added to each chloroform extract which was mixed thoroughly by vortexing and then centrifuged at 10,000 g X 2 min. A 250 μl aliquot of the chloroform rich layer was placed in a 0.2 cm² quartz cuvette and fluorescent spectra were obtained.
on an SPF-500 Aminco Spectrofluorometer (Silver Spring, MD).

The investigations utilizing animals, as described in this manuscript, conform to the ARVO Resolution on the Use of Animals in Research.

Results. The thiobarbituric acid (TBA) test is a very sensitive measure of lipid peroxidation. Autoxidation of unsaturated fatty acids with three or more double bonds, such as 22:6, results in the formation of malondialdehyde, which complexes with 2 TBA molecules to form a chromogen with an absorption maximum of 532 nm. In the present study, the TBA test was used to assay the autoxidation of 22:6 after 22:6 was injected into the rabbit vitreous. As shown in Figure 1, vitreous malondialdehyde (MDA) levels rapidly increase following intravitreal injection of 22:6. The MDA concentration reached a maximum of 7.3 micromoles 2 hr after injection of 22:6 and then rapidly declined over the next 6 hr. This was followed by a gradual return of MDA concentration to blank control levels 24 hr post 22:6 injection.

Although the TBA test is very sensitive, its validity depends on its correlation with other tests for measuring lipid peroxidation. Therefore, fluorescent lipid peroxidation products were measured in the vitreous following injection of 22:6. These fluorescent products are thought to be derived from the reaction of MDA with primary amino groups to form conjugated Schiff base fluorochromes. Vitreous fluorescence profiles following injection of 22:6 are shown in Figure 2. These emission spectra have a 430 nm maximum with excitation at 355 nm that increase in intensity relative to time after 22:6 injection. The formation of these fluorescent products indicates that some of the MDA formed during autoxidation of intravitreal 22:6 is reacting with vitreous tissue.

Posterior subcapsular cataracts (PSC) were observed in 9 of the 22 rabbit eyes injected with 22:6 (Fig. 3). No cataracts were observed during the first 4 hr following intravitreal injection of 22:6. Nine of the ten eyes that received intravitreal 22:6 for 6–24 hr developed posterior subcapsular cataracts. The distribution of PSC at these later times are as follows: 6 hr–1 PSC; 8 hr–2 PSC; 10 hr–1 PSC, 12 hr–2 PSC; 14 hr–1 PSC; and 24 hr–2 PSC.

Discussion. There are two existing schools of thought regarding the etiology of posterior subcapsular cataracts in tapetoretinal degenerations. One theory states that lenticular changes are a direct manifestation of gene expression, viz, polygenic inheritance. A second theory, proposed by Berliner in 1949, states that posterior subcapsular cataracts are a secondary manifestation of the underlying retinal pathology in tapetoretinal diseases, which results in the formation of substances toxic to the crystalline lens. In a recent report, Heckenlively stated that the frequent occurrence of posterior subcapsular cataracts in tapetoretinal degenerations, which have diverse Mendalian inheritance patterns, supports the hypothesis that posterior subcapsular cataracts may occur as a secondary process.
The primary metabolic abnormalities of tapetoretinal degenerations are thought to occur at the level of the photoreceptor-pigment epithelial complex. Photoreceptor outer segments are continually renewed and the terminal outer segment discs are removed by the pigment epithelium. The dynamic metabolism of this system is exemplified in the monkey eye where each retinal rod produces 80–90 discs per day, the entire complement of outer segment discs is replaced every 9–13 days, and each pigment epithelial cell engulfs about 3,000 discs per day. It is evident that any metabolic disorder that compromises the interaction of photoreceptor cells with the pigment epithelium would lead to the accumulation and deterioration of outer segment membranes. Outer segment membranes contain a high amount of docosahexenoic acid that would be very susceptible to peroxidation if left unprotected in the dynamic, oxidative milieu of the retina. Thus, the anterior diffusion of toxic aldehydes and lipid radicals from docosahexenoic acid oxidation conceivably could cause the vitreous changes and posterior subcapsular cataracts observed in many tapetoretinal degenerations.

Peroxidation of intravitreal docosahexenoic acid was demonstrated in the present study by the formation of malondialdehyde and vitreous fluorescence. Rabbit vitreous that has not been injected with 22:6 does not contain TBA reactive material or fluorescent chromophores which excite at 355 nm. The baseline levels of vitreous MDA and fluorescent material at the zero time points in these studies may be due to the injection of partially oxidized 22:6 and/or lipid peroxidation that occurred during tissue preparation for the TBA assay and fluorescent studies.

The rapid rise and fall of MDA levels during the first 8 hr after 22:6 injection is probably due to diffusion of MDA out of the vitreous and/or reaction of MDA with vitreous tissue. The presence of vitreous fluorescence with an emission maximum of 430 nm and an excitation maximum of 355 nm is consistent with the formation of iminopropene cross-links. These cross-links are formed when MDA reacts with primary amino groups of proteins, nucleic acids or phospholipids. Once MDA reacts with an amino group to form a fluorescent cross-link it can no longer complex two TBA molecules, which is necessary for a positive TBA reaction. Thus, the decline in vitreous MDA levels and accompanying increase in vitreous fluorescence indicate that MDA is reacting with vitreous tissue.

The onset of posterior subcapsular cataract formation is temporally correlated with the rapid decline in vitreous MDA levels. This suggests, but does not prove, that MDA or some other toxic product of lipid peroxidation may have diffused anteriorly and reacted with the posterior lens surface, resulting in posterior subcapsular cataracts.

In conclusion, the present studies demonstrate that intravitreal oxidation of 22:6 in the rabbit results in the formation of posterior subcapsular cataracts. These results support the hypothesis that a lipid peroxidative mechanism may be responsible for the lenticular changes observed in tapetoretinal degenerations. Additional support for this hypothesis is provided by the recent studies of Zigler et al. They demonstrated that the oxidation of free 22:6 and bovine rod outer segment membranes (30% of free fatty acid content is 22:6) is toxic to transport systems of rat lenses in organ culture. Thus, the release of toxic aldehydes and lipid radicals from outer segment membranes following oxidation of 22:6 may represent a common pathologic pathway for the formation of posterior subcapsular cataracts in tapetoretinal diseases.
Fig. 3. A, Slit-lamp photograph of a rabbit lens depicting posterior subcapsular cataract formation 24 hr after intravitreal injection of 10 μl of docosahexenoic acid. B, Retroilluminated picture of Figure 3A.
Key words: lipid peroxidation, docosahexenoic acid, posterior subcapsular cataract, tapetoretinal degeneration

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References

Aniridia: Enzyme Studies in an 11p− Chromosomal Deletion

J. Bronwyn Bateman,* Maryellen C. Sparkes,† and Robert S. Sparkes‡

A patient with aniridia and an interstitial deletion of the bands p13–p14 of the short arm of chromosome 11 was studied to determine the relative locations of the gene(s) encoding for the aniridia-Wilms' tumor association with other genes on the same chromosome. Quantitative analysis was performed on the red blood cell enzymes lactic acid dehydrogenase-A (LDH-A) and catalase, the genes for which are located on the short arm of chromosome 11. The activity of LDH-A was normal; the activity of catalase was reduced to approximately half normal. This evidence supports loci for the genes encoding for both catalase and the aniridia-Wilms' tumor association within the bands p13–p14 of the short arm of chromosome 11; the normal activity of LDH-A supports a locus outside this region. Invest Ophthalmol Vis Sci 25:612–616, 1984

The association of aniridia, genitourinary malformations, developmental delay, and Wilms' tumor with interstitial deletions of the short arm of chromosome 11 initially was recognized by Smith and co-workers.1 Although neither the smallest possible deletion resulting in this association nor the number of genes involved is established, a patient with aniridia, Wilms' tumor, and no demonstrable chromosomal deletion was reported recently.2 As Wilms' tumor is life-threatening, methods for identifying those patients with aniridia who are at higher risk for this malignancy would be clinically useful. Regional gene mapping by correlating cytogenetic break points with enzymatic activities of genes known to be located on a given chromosome may provide such clues. We selected two enzymes, the genes for which are known to be on the short arm of chromosome 11, for study in a patient with aniridia and an interstitial deletion of the same chromosome; quantitative analyses of the red cell enzymes catalase and lactic acid dehydrogenase-A (LDH-A) were performed to determine activity; both of these enzymes have been shown to exhibit a gene-dose relationship.

Materials and Methods. A Caucasian girl was identified as having absent pupils at 6 months of age; a diagnosis of aniridia was made, and she was referred to the UCLA Genetics Clinic. The full clinical features will be described in a separate report. Informed human consent was obtained prior to undertaking the study.

Giemsa and reverse (R) banding of cultured, synchronized lymphocytes of the patient and of her parents in late prophase or early metaphase were performed; three full karyotypes and six partial karyotypes of pair 11 of the patient were reviewed.

Quantitative analysis of red cell catalase was performed twice on the proband and three unrelated controls by the technique of Beutler3; for each analysis, the degradation of H2O2 was monitored spectrophotometrically twice on the Gilford 2000 (Gilford Instrument Laboratories; Oberlin, OH).

Red cell LDH-A was assayed twice on the proband and three unrelated controls by electrophoresing a sa-