ACCUMULATION OF LIPID PEROXIDATION PRODUCTS IN THE LENS IN CATARACT

M. A. Babizhaev, A. A. Shvedova,
Yu. V. Arkhipenko, and V. E. Kagan

UDC 617.741-004.1-07:
617.741-008.939.15-39

KEY WORDS: human lens; cataract; lipid peroxidation.

The development of senile cataract is accompanied by disintegration of the membranous structures of the lens [8]. Among endogenous processes which can cause injury to membranous structures of cells and tissues one of the most important is lipid peroxidation (LPO). During aging and, in particular, during the development of senile cataract, activity of enzymic (superoxide dismutase, glutathione peroxidase, catalase) and nonenzymic (ascorbate, cysteine, glutathione) antioxidant systems in the lens and aqueous humor is reduced [6, 11, 14]. Accumulation of LPO products in the lens may be facilitated by the presence of compounds in the lens which are photosensitizers of free-radical oxidation reactions of the 3-hydroxykynurenin or N-formylkynurenin type, which absorb light in the near UV-region of the spectrum (360-400 nm). These photo-oxidation products of tryptophan, under the influence of light, can generate active forms of oxygen and products of its successive single-electron reduction (singlet oxygen, superoxide anion-radicals, hydrogen peroxide, hydroxyl radicals), which can be found in the lens tissue and also in the aqueous humor [13].

Several varieties of cataract have been described in the literature, whose mechanisms of development have been linked with the generation of active forms of oxygen. They include psoraline cataract, cataract induced by the action of hyperbaric oxygenation, and cataract arising in animals fed with the catalase inhibitor 3-amino-1,2,4-triazole [2, 10, 12].

On the basis of these facts it has been postulated that LPO may play a role in the etiology and pathogenesis of cataract [3, 9]. However, no direct proof of LPO activation has yet been obtained in cataract. The results of determination of the content of LPO products, interacting with 2-thiobarbituric acid (such as malonyl dialdehyde) [3, 9], cannot be regarded as proof because this test is not suitable for quantitative analysis of LPO products *in vivo* [1].

In view of the considerations mentioned above it was decided to investigate the content of LPO products in lenses extracted from patients during operations, in mice with an inherited form of cataract, and also in transparent human and mouse lenses.

EXPERIMENTAL METHOD

The test material consisted of opaque lenses obtained during operation by intracapsular cryoextraction: ripe senile cataract (n = 27), total traumatic cataract (n = 5), and complicated cataract (n = 11). Transparent lenses were removed from the enucleated eyes of young patients (n = 18) with small neoplasms of the internal membranes in the posterior chamber of the eye. Opaque mouse lenses were taken from the mutant dominant cataract (Fr) line, and control transparent lenses were taken from mice of inbred line A.

Immediately after the lens material had been obtained lipids were extracted [7] by the addition of 20 volumes of a chloroform–methanol mixture (2:1 by volume) to the lens homogenate. To prevent the formation of LPO products *in vitro*, the antioxidant 4-methyl-2,6-di-tert-butylphenol was added (0.5 mg/100 ml) to the chloroform–methanol mixture and the samples were ventilated with argon. After separation of the phases and removal of the aqueous-methanol layer 0.3 ml of the chloroform extract was evaporated on a rotary evaporator, and the lipids thus

Helmholtz Moscow Research Institute of Eye Diseases and Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. Institute of Physiology, Bulgarian Academy of Sciences, Sofia. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 9, pp. 299-301, September, 1985. Original article submitted March 15, 1985.

obtained were dissolved in 4 ml of methanol—heptane mixture (5:1 by volume) and used for UV spectrophotometry. The content of total lipids in the chloroform extract was determined gravimetrically. The concentration of LPO products was estimated spectrophotometrically from absorbents of diene conjugates in the UV region (at 230 nm) and absorbence of conjugated trienes at 274 nm [4]. UV absorption spectra of the lipids were recorded on a Hitachi-557 spectrophotometer (Japan). The content of end products of LPO (Schiff bases) was determined from fluorescence of chloroform extracts [5], measured on a Hitachi MPF-4 spectrofluorometer. The fluorometer was calibrated against a solution of quinine sulfate.

EXPERIMENTAL RESULTS

Typical UV absorption spectra of lipids extracted from lenses are given in Fig. 1. The maximum in the 206 nm regions corresponds to absorption of isolated double bonds of hydrocarbon chains. In absorption spectra of lipid extracts from lenses with a ripe cataract there were two additional maxima, at 230 and 274 nm. The first of them corresponds to absorbence of diene conjugated structures. The maximum at 274 nm corresponds to triene conjugates — secondary molecular LPO products. The maximum at 230 nm was virtually absent in lipids extracted from transparent lenses, and the maximum at 274 nm had significantly smaller amplitude than in lipids from opaque lenses.

Determination of the content of Schiff bases revealed an increase in the intensity of fluorescence of the chloroform lipid extracts from lenses with a ripe cataract (Table 1). Since no differences in the content of LPO products were observed between the types of cataract — ripe senile and total (traumatic and complicated), they were united into one clinical group of "ripe cataract." The absence of differences between these types of cataracts may indicate a universal role of LPO in lenticular opacity. In the series starting with transparent lenses and going on to unripe, ripe, and overripe cataracts, a tendency was observed for the content of primary and secondary LPO products to pass through a maximum. In the same series the content of end products of LPO rose steadily. This trend of accumulation of LPO products in the course of cataract development agrees well with known ideas on the temporary increase in hydroperoxide formation and continuous accumulation of end products of LPO during auto-oxidation of lipids in biological membranes. It must be emphasized that accumulation of fluorescent LPO products was found also in lenses of mice with hereditary cataract (Table 1). Taken as a whole, the results are direct experimental proof of an increased content of LPO products in opaque lenses in patients and in mice with the hereditary form of this disease, evidence that LPO may perhaps play a role in the pathogenesis of cataract. Other results of accumulation of LPO products in the lens may be disturbance of the barrier function of the

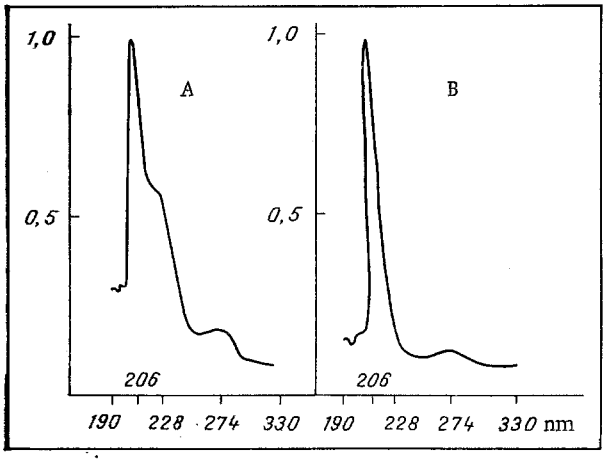

Fig. 1. UV absorption spectra of lipid extracts from human lenses in methanol-heptane. Abscissa, wavelength, in nm; ordinate, optical density units. A) Ripe cataract, B) transparent lens. Abscissa, optical density, in optical density units; ordinate, wavelength, in nm.

TABLE 1. Content of LPO Products in Lipids Extracts of Lenses (M ± m)

Test parameter	Control	Cataracts
Diene conjugates	1,87±0,17	2,34±0,15*
Triene conjugates	0,45±0,06	0,66±0,06†
Schiff bases		
human	18,0±4,7	67,0±13,2‡
mouse	14,0±3,1	26,5±3,8*

Legend. Content of diene and triene conjugates expressed in optical density units of lipid extracts at 230 and 274 nm, respectively, calculated per kilogram of lipids; content of Schiff bases expressed in relative units. Content of original material in samples standardized according to phospholipid content. Number of lenses shown in parentheses. *$P < 0.05$, †$P < 0.02$, ‡$P < 0.01$ compared with control.

membranes of the tissue structures of the lens, inhibition of membrane-bound enzymes, and oligomerization of lens proteins, leading ultimately to a change in the optical properties of the lens.

The results provide a basis for the undertaking of clinical investigations in order to determine whether antioxidants may be used for the prevention and treatment of cataract.

LITERATURE CITED

1. Yu. P. Kozlov, V. E. Kagan, S. M. Danilov, and M. M. Sitkovskii, Free-radical Oxidation of Lipids in Biomembranes [in Russian], Moscow (1972).
2. K. C. Bhuyan, D. K. Bhuyan, and H. M. Katzin, Ophthalmol. Res., 5, 236 (1973).
3. K. C. Bhuyan, D. K. Bhuyan, and S. M. Podos, Invest. Ophthalmol., 18, Suppl., 97 (1979).
4. J. L. Bolland and H. P. Koch, J. Chem. Soc., 7, 445 (1945).
5. K. S. Chio and A. L. Tappel, Biochemistry (Washington), 8, 2821 (1969).
6. J. V. Fecondo and R. C. Augusteyn, Exp. Eye Res., 36, 15 (1983).
7. J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
8. M. H. Garner, Proc. Natl. Acad. Sci. USA, 78, 1892 (1981).
9. H. Kobayashi, A. Majima, T. Aritake, et al., Acta Soc. Ophthalmol. Jpn., 85, 59 (1981).
10. S. Lerman and J. Megaw, Invest. Ophthalmol., 23, 801 (1982).
11. R. J. Olson, R. Sherman, and R. Dickman, Invest. Ophthalmol., 20, Suppl. No. 3, 218 (1981).
12. S. S. Shocket, J. Esterson, and B. Bradford, Israel J. Med. Sci., 8, 1596 (1972).
13. A. Spector and W. H. Garner, Exp. Eye Res., 33, 673 (1981).
14. S. D. Varma, V. K. Srivastava, and R. D. Richards, Ophthalmol. Res., 14, 167 (1982).