

Role of lipid peroxidation in the pathogenesis of myopic and senile cataract

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Abstract

Aims/background—Increased production of free radicals, consumption of antioxidant, and oxidation of unsaturated lipids have been observed recently in cataractous lenses and active participation of the retina in human cataractogenesis has been proposed. To verify this hypothesis, the total (GSH) and oxidised (GSSG) glutathione concentrations were assayed in the lens and the malondialdehyde (MDA) levels assayed in the vitreous and in the lens of normal controls and patients with senile or myopic cataract.

Methods—The study was conducted on 34 lenses (nucleus and epinucleus) (nine clear lenses, 14 lenses with idiopathic senile cataract, and 11 lenses affected by severe myopic cataract) and vitreous of 19 (seven non-myopic, seven myopic, and five control) subjects. Glutathione determination was performed following the method of Reed, while malondialdehyde was assayed using a modification of the method of Dahle.

Results—Cataractous lenses showed a decreased content of GSH and increased concentration of GSSG compared with clear lenses. A higher oxidative consumption of GSH was found in myopic cataracts compared with senile ones. Also, increased levels of MDA were observed both in cataractous lenses and in the vitreous of myopic patients compared with the control and the senile ones.

Conclusion—The observed alterations strongly suggest that retinal lipid peroxidation might play a key role in human cataractogenesis, especially in the myopic type.

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Recent studies have shown that lipid peroxidation, an event caused by imbalance between free radical production and antioxidant defence, may play a role in the genesis of the cataract.¹⁻³ Higher levels of malondialdehyde (MDA), a final product of the lipid peroxidation process, have been observed in diabetic and myopic cataracts¹⁻³ compared with senile cataracts. In fact, the myopic form is differentiated from the senile both because of age of onset and morphological features and not much is known about retinal participation in the development of human cataract *in vivo*.⁴ Indeed, a key role played by retinal lipid peroxidation could be hypothesised on the basis of the observation that the injection of

peroxidative products in the vitreous caused posterior subcapsular cataract in the rabbit.⁵

Moreover, the activities of glutathione reductase and glutathione peroxidase, known to play a key role in the protection against oxidative damage, have been reported to be decreased in cataractous lenses.⁶⁻⁷ This may be the consequence of decreased availability of reduced substrates (GSH) or of functional inactivation of the enzyme molecules as a result of structural changes determined by lipid peroxidation itself. Recently, decreased levels of glutathione have been noticed in human cataract, especially in patients affected by diabetes.⁸ Moreover, it has been observed that administration of buthionine sulphoximine, a specific inhibitor of glutathione synthesis, caused cataract in mice,⁹ while decreased non-enzymatic glycation of lens proteins has been noticed in the presence of glutathione.¹⁰⁻¹¹ However, the mechanisms underlying the onset of lens opacity are not completely understood.

The purpose of this study, therefore, was to investigate the role and source of lipid peroxidation in human myopic and senile cataractogenesis, and to see if MDA levels in the vitreous of the myopic eye may account for the increased levels of lens MDA, through the measurement of MDA levels in lenses and vitreous and its relation with the glutathione redox state.

Patients and methods

This study was performed on lenses and vitreous from patients aged 49-74 years, admitted to the Institute of Ophthalmology from December 1993 to December 1994. They were divided as follows: nine clear lenses (from seven men and two women; mean age 57 years) were obtained following extracapsular lens extraction in eyes undergoing vitrectomy for giant retinal tears; 14 lenses from six men and eight women (mean age 67 years) with idiopathic senile cataract; and 11 lenses from seven men and four women (mean age 56 years) with myopic cataract were removed by extracapsular cataract extraction. Vitreous was removed from five patients before enucleation for intraocular malignant neoplasm; from seven myopic patients (four males and three females); and from seven (three males and four females) non-myopic patients after vitrectomy performed for retinal detachment.

All subjects with chronic liver or kidney disease, hyperlipidaemia, alcohol abuse, diabetes, or patients affected by other local or systemic pathologies that may influence the

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redox state of the lens were excluded from the study. After extracapsular cataract extraction, the lenses (nucleus and epinucleus) were weighed, immersed in a 0.9% sodium chloride solution, divided in two parts and immediately processed for the measurement of GSH, GSSG, and MDA, in order to avoid modifications that could occur with long term storage. Immediately after extraction the vitreous was centrifuged (3000 g for 8 minutes) and the supernatant frozen for MDA determination.

A blood sample for plasma MDA evaluation was drawn by venepuncture from all patients on the same day as the eye operation.

GLUTATHIONE MEASUREMENTS

The lenses were homogenised in ice cold Krebs buffer (pH 7.4), treated with 5% (v/v) concentrated perchloric acid for protein precipitation, and centrifuged at 10 000 g for 10 minutes at 2°C. The supernatant was processed for high performance liquid chromatography (HPLC) analysis of GSH and GSSG, according to the method of Reed *et al.*¹² Results, expressed in nmol/g of wet weight, were obtained by comparing the peak areas of the samples with those of freshly prepared standards. The recovery of GSH and GSSG added to the lens homogenate was 93–101% and 95–102%, respectively.

MALONDIALDEHYDE DETERMINATION

Lens, vitreous, and plasma MDA concentrations were measured as the product of the reaction with thiobarbituric acid (TBA) using a modification of the method of Dahle *et al.*¹³ The lenses were homogenised in phosphate buffer (pH 7.4) and treated with 20% trichloroacetic acid (TCA); butylated hydroxytoluene (BHT) was also added to the system in order to minimise or inhibit spontaneous lipid autoxidation. An aliquot of TCA supernatant was condensed after the reaction with TBA. The absorbance of the pink coloured product was measured spectrophotometrically at 532 nm. The same procedure was used for plasma and vitreous samples. Results, expressed in nmol/g of wet weight, were obtained by

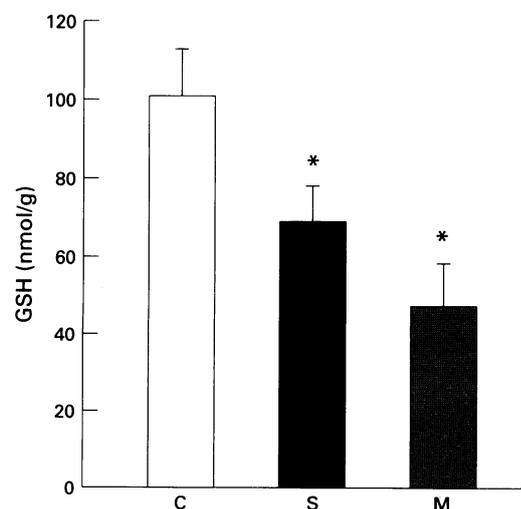


Figure 1 Total glutathione (GSH) content in clear lenses (C) and in senile (S) and myopic cataract (M). * $p < 0.001$ compared with clear lenses.

comparing the absorbance of the samples with those of freshly prepared standards by different dilutions of 3,3,5,5-tetraethoxypropane.

STATISTICAL ANALYSIS

The results are expressed as mean (SD). The data were analysed using the Student's *t* test for unpaired data and by one way ANOVA analysis of variance. The minimum level of significance was considered as $p < 0.05$.

Results

Figures 1 and 2 report the total GSH and oxidised (GSSG) glutathione concentrations, respectively, in the clear lenses, and in those with senile or myopic cataract. As shown, in each group of cataractous lenses, the values of GSH were lower than those found in healthy controls (69 (14), 47 (16), and 101 (19) nmol/g, respectively). By contrast, the values of GSSG were significantly higher than those of control subjects (17 (7), 12 (7), and 7 (3) respectively, expressed as a percentage of total glutathione). In particular, among cataracts the myopic type showed more evident changes in the glutathione content and status.

Figure 3 indicates the levels of MDA in the clear lenses (1.6 (0.3) nmol/g) as well as in those with cataract (2.8 (0.7) and 7.6 (0.5) nmol/g). A significant increase in the concentration of MDA was observed in cataractous lenses with respect to the controls, especially in those affected by the myopic form ($p < 0.001$).

Furthermore, the concentrations of MDA in cataractous lenses were inversely related to the levels of GSH ($r = -0.8$, $p < 0.001$) and directly related to the values of GSSG ($r = 0.76$, $p < 0.001$).

In Table 1 the vitreal concentrations of MDA are reported. As shown, MDA levels were significantly higher in patients with myopic cataract compared with non-myopic cataract and controls ($p < 0.02$).

Besides, there was no statistical difference among the study groups in the plasma levels of MDA and in the ratios triglycerides/MDA and

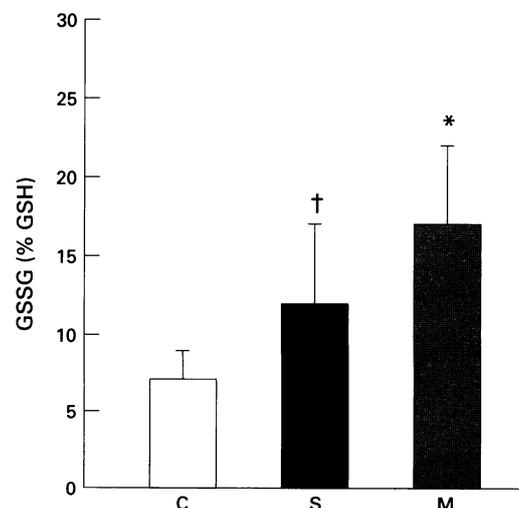


Figure 2 Oxidised glutathione (GSSG) concentration in clear lenses (C) and in cataractous lenses: senile (S) and myopic (M). GSSG is expressed as percentage of total glutathione (GSH). † $p < 0.01$ and * $p < 0.001$ compared with clear lenses.

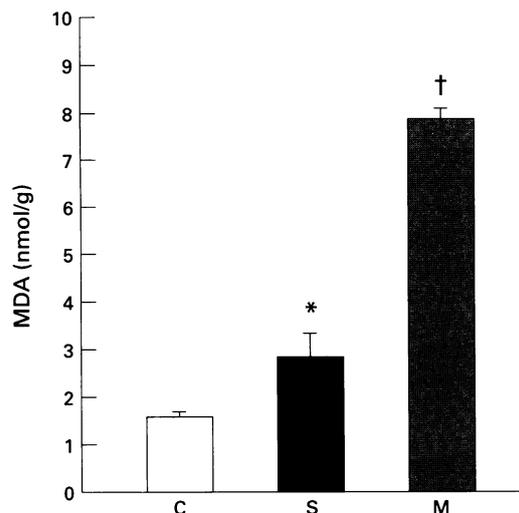


Figure 3 Malondialdehyde concentrations in clear lenses (C) and in senile (S) and myopic (M) cataract. * $p < 0.001$ compared with clear lenses; † $p < 0.001$ compared with clear lenses and senile cataract.

cholesterol/MDA; no correlation was observed between the lens and plasma concentrations of MDA.

Discussion

The first outcome of this study confirms that an alteration of the antioxidant defence system is present in lenses affected by cataract,^{7, 14} and especially in those with myopic cataract. In fact, low levels of GSH (which influences the activity of other antioxidants such as catalase, vitamin E, and superoxide dismutase) have been observed in cataractous lenses. This seems to be of great importance because GSH and other sulphhydryls are effective in preventing autoxidation of lens lipids and proteins.¹⁵ The fall in the GSH concentrations, observed in lenses affected by cataract, could be the result of various events such as: decrease of GSH synthesis that may occur in parapsychological conditions as a consequence of deficient availability of substrates (senile cataract); increased GSH catabolism which follows the contrasting activity against toxic compounds; and protective binding to lens proteins.⁶

The increased levels of GSSG, found in cataractous lenses, seem to support the hypothesis of an oxidative consumption of GSH; however, since the rate of GSSG formation did not completely account for the extent of total GSH depletion, this may probably be ascribed also to a deficient synthesis. It is well known that the oxidised form of glutathione is inactive,¹⁶ thus excess of it can cause imbalance of the glutathione related enzyme system that are involved in lens protection from oxidative damage.⁷ Therefore, it is likely that the decreased activity of the glutathione reductase and glutathione peroxidase observed in cata-

ractous lenses may be the consequence of a chronic deficiency in reduced glutathione.

Of interest, the correlations found among GSH, GSSG, and MDA levels indirectly suggest that in the lens GSH acts in order to preserve the unsaturated lipids from oxidative alterations.

The increased levels of MDA in lenses with cataract and the absence of a systemic imbalance of the lipid redox status in these patients suggest an ocular origin of the lipid peroxidative products. The high level of MDA in cataractous lenses may, in turn, be the result of lipid peroxidation of the lens cell membranes produced locally or may be a consequence of migration of products from the retina.¹⁷ The latter hypothesis may also account for the higher MDA levels observed in the vitreous and lenses of myopic subjects in our study. In fact, in these patients the retina, which is very rich in polyunsaturated fatty acids especially at the rod outer segment, could be an elective place for lipid peroxidation because of chronic hypoxia due to choroidal thinness and to photic oxidative injury. Accordingly, the MDA produced could easily diffuse through the synergetic vitreous and spread the damage to the lens.¹⁸ This could account for the opacities in the posterior or equatorial area of the lens, typical of myopia.

Moreover, our results confirm in a clinical setting the experimental observation of Goosey *et al.*,⁵ where injection of peroxidative products in the vitreous caused posterior subcapsular cataract in the animal, and also what has been already observed in the cataract secondary to retinal degeneration.^{11, 18} In such conditions, and in the presence of deficient antioxidant activity, the high concentrations of lipid peroxidation products may also promote oxidation, aggregation, and conversion of soluble to insoluble proteins in the nucleus of the lens,^{19, 20} and chain events such as the activation of transition metals from the storage pool, with a final fragmentation of the lens structure. The fall of lens GSH occurring usually earlier in myopic cataract than in senile idiopathic cataract suggests an extrinsic origin of prooxidant molecules, able progressively to damage the defence system of the lens; if this is the case, retinal degeneration is what really differentiates the myopic from the senile cataract.

In conclusion, we can confirm that the degree of the lens opacity is related to the local level of peroxidative products, especially in the presence of myopia, as well as with the oxidation of sulphhydryl groups. The higher concentration of MDA in the vitreous of myopic subjects strongly suggests a retinal involvement in the genesis of the human myopic cataract. These events may also be facilitated by a decreased availability of reduced glutathione, as already observed in diabetic subjects.⁸

Table 1 Malondialdehyde (MDA) concentrations (SD) in human vitreous obtained from myopic, non-myopic, or control subjects

	Myopic subjects (n=7)	Non-myopic subjects (n=7)	Control subjects (n=5)
MDA (nmol/mg protein)	8.25 (4.02)*	10.74 (4.3)	8.24 (3.53)

* $p < 0.02$ compared with non-myopic and control subjects.

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