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INDUCTION OF LIPID PEROXIDATION BY THE LENS

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The lens tissue is avascular and its metabolism is entirely dependent on the aqueous humor. The possibility therefore cannot be ruled out that the causes of opacity of the lens during cataract development may be connected with disturbance of the normal composition of the aqueous humor [2]. Because of the role of lipid peroxidation (LPO) in the genesis of opacity of the lens [1], it was decided to study whether LPO products may not appear in the aqueous humor. The presence of high concentrations of hydrogen peroxide (H_2O_2) in the aqueous humor of some cataract patients was demonstrated previously [10]. Meanwhile, we know that the aqueous contains about 4 μg/ml of high-density lipoproteins, which evidently take part in the renewal of the lipid composition of the lens [4], and, in principle, they may undergo oxidation.

In the investigation described below two problems were studied: 1) Does the concentration of LPO products in the aqueous of the lens change in cataracts; 2) Can the isolated lens affect the concentration of LPO products in the surrounding medium.

EXPERIMENTAL METHOD

The aqueous extracted from the anterior chamber of 27 eyes from cataract patients during operations for intracapsular cryoextraction was used as the test material. The aqueous from 10 eyes from donors, supplied from a corneal transplant bank, was used as the control. The average age of the patients was 65 ± 10 years. Immediately after the material had been obtained, lipids were extracted by the method described previously [1]. The relative concentration of lipids in the extract was determined by their absorption at 206 nm (D_{206}). The concentration of primary (diene conjugates) and secondary (ketodienes) LPO products was estimated spectrophotometrically from their characteristic absorption in the region of the maxima at 232 nm (D_{232}) and 280 nm (D_{280}) on a Hitachi-557 spectrophotometer, and expressed

TABLE 1. Concentrations of LPO Products in Aqueous Humor of Cataract Patients (M ± m)

Parameter tested	Control (10)	Cataract (27)
D_{232}/D_{206}	0,331±0,030	0,448±0,027*
D_{280}/D_{206}	0,122±0,006	0,170±0,1*
Fluorescent products, relative units	47,4±18,3	231,5±61,9*

Legend. Number of experiments in parentheses. *P < 0.01 compared with control.

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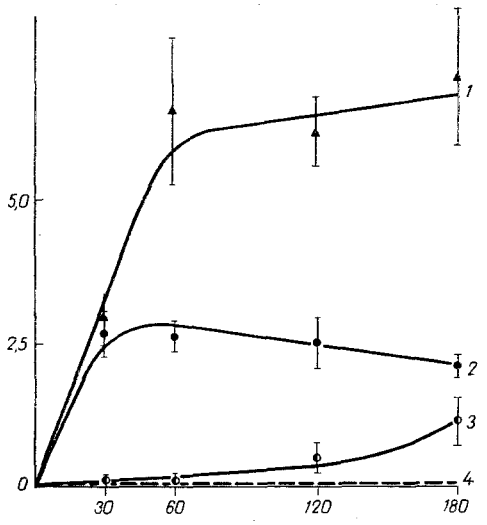

Fig. 1. Kinetics of MDA accumulation in suspension of liposomes during incubation of lenses. Abscissa, incubation time (in min); ordinate, MDA level (in nmoles/μmole of phospholipids). 1) Transparent rabbit lenses; 2) transparent human lenses, initial cataract; 3) human lenses with ripe cataract; 4) control (liposomes).

as the ratio between the corresponding absorptions D_{232}/D_{206} and D_{280}/D_{206} [1]. Fluorescent end products of LPO were determined in the lipid extract in the region of the maximum at 420-440 nm, with excitation wavelength of 365 nm, on a Hitachi MPF-4 spectrofluorometer, the concentrations of lipids in the samples being normalized relative to D_{206}.

Twelve human lenses from patients with different degrees of opacity, and also 12 transparent rabbit lenses were used. The lenses were incubated in 3 ml of medium (0.14 M NaCl, 0.01M Tris-HCl, pH 7.4) containing liposomes (0.5 ml/ml) made from a total fraction of phospholipids from hens' egg yolks, by the phase reversal method [3]. The final osmolarity of the medium was 290-300 milliosmoles. Incubation was carried out at room temperature. To record the kinetics of oxidation of the liposomes, samples of 0.5 ml were taken 0, 30, 60, 120, and 180 min after the beginning of incubation. LPO products were determined as the accumulation of malonic dialdehyde (MDA), using the test with 2-thiobarbituric acid [9]. The following enzymes were used: superoxide dismutase — SOD (from Sigma, USA), catalase (from Serva, West Germany), and reduced glutathione (Calbiochem-Behring, USA). The remaining chemical reagents were of the analytically pure grade.

EXPERIMENTAL RESULTS

Concentrations of LPO products in aqueous of the anterior chamber from patients with cataracts were found to be increased in all cases irrespective of the genesis of the opacity of the lens (Table 1). The greatest difference was observed in the concentration of fluorescent LPO products. The question arises: what is the source of the peroxides in the aqueous? In principle, the possibility cannot be ruled out that LPO products arise in other tissues, although it is more probable that they are formed in the aqueous in situ. In particular, it has been suggested that peroxides may be generated through oxidation of ascorbic acid actually in the auqeous. This process is intensified by light, and is also catalyzed by riboflavine [8].

To discover whether the lens itself could be an endogenous source of lipid peroxides, human lenses with different degrees of opacity and transparent rabbit lenses were placed in medium containing liposomes, as oxidation substrate, and the kinetics of MDA accumulation was estimated (Fig. 1). In the absence of the lens virtually no oxidation of liposomes took place during 180 min. In the presence of the lens, however, there was a marked increase in MDA concentration (Fig. 1). The rate of MDA accumulation during the first 30 min of incubation in the presence of transparent human lenses, and also of lenses at the initial cataract stage, was 14 times higher than in the presence of lenses with a ripe cataract. The final level of MDA accumulation during incubation of a transparent lens for 3 h was 2.5-4.5 times higher than during incubation of a lens with a ripe cataract. The level of MDA accumulation after incubation of the rabbit lenses for 3 h was 3.5 times higher than in experiments with normal human lenses. As will be clear from the data in Figs. 1 and 2, in some cases a small decrease was observed in the MDA concentration after incubation of lenses for 2 h in a suspension of liposomes. This fact may be connected with utilization of MDA by the lens itself (interaction of MDA with amino group, lowering of the MDA level with the participation of lenticular aldehyde dehydrogenase).

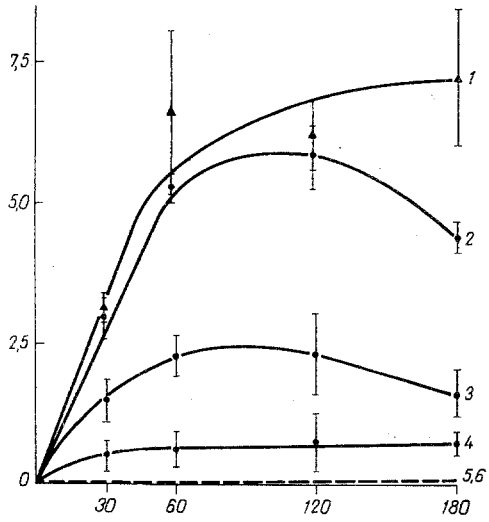

Fig. 2. MDA accumulation in liposomes during incubation of rabbit lens in the presence of additives. 1) Transparent rabbit lenses; 2) $FeCl_3$ (0.1 mM) + ADP (1 mM); 3) catalase; 4) SOD; 5) EDTA (1 mM); 6) control (liposomes). Remainder of legend as in Fig. 1.

What is the mechanism of induction of LPO in this system? Two possible explanations can be suggested: first, lipid peroxides are generated in the course of enzyme reactions in lens, catalyzed by lipoxy- and cyclooxygenase [5, 11]; second, free-radical oxidation of lipids is induced by active forms of oxygen with the participation of Fe ions in the free or chelated state. This second hypothesis is supported by the virtually total inhibition of MDA accumulation in the liposomes and the addition of the chelating agent EDTA (1 mM, Fig. 2). Hydroxyl radicals, formed in Fenton's reaction:

$$H_2O_2 + Fe^{2+} \longrightarrow OH\cdot + Fe^{3+}$$

are evidently the agent inducing oxidation of the liposomes. Moreover, Fe^{++} ions are accelerate LPO, by causing branching of the oxidation chain. Addition of catalase (900 units per sample) to the indubation medium of the lens considerably reduced the rate of accumulation of LPO products in the liposomes (Fig. 2), evidence that H_2O_2 plays a role in the generation of lipid peroxides by the lens. Addition of SOD (114 units per sample) to the incubation medium of the lens also led to a marked reduction of MDA accumulation in the liposomes (Fig. 2), evidence that the lens is able to generate $O_2^{\cdot-}$ in the surrounding medium. Addition of chelated iron to the incubation medium of the lens reduced by 33-50% the amount of MDA accumulated in the liposomes (Fig. 2). This effect can evidently be explained by lowering of the steady-state $O_2^{\cdot-}$ concentration in the system on account of its utilization for reduction of Fe^{+++} into Fe^{++}, as a result of which the probability of conversion of $O_2^{\cdot-}$ into H_2O_2 is reduced.

Taken as a whole the results show that the lens can induce LPO in the surrounding tissues and can act as the endogenous source of active forms of oxygen and of lipid peroxides. Participation of catalytic ferrous ions (Fe^{++}) in this process is essential; under these circumstances active forms of oxygen are generated through $O_2^{\cdot-}$ and H_2O_2.

Incidentally, induction of LPO by the lens may be connected with synthesis of physiologically important compounds such as leukotrienes and prostaglandins in the eye [6, 7].

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