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RESEARCH NOTE

PHOTOPEROXIDATION OF LENS LIPIDS: INHIBITION BY ASPIRIN

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Abstract—Rat lenses have been organ cultured in light and dark, and peroxidative degradation of lipids studied in the presence and absence of aspirin. The extent of peroxidative degradation has been assessed by measurement of malonaldehyde. The light-stimulated peroxidative degradation of the tissue lipids has been found to be preventable by aspirin. Aspirin thus appears to be a potential agent against light damage to the tissue.

INTRODUCTION

It has been observed previously that light of visible frequency can initiate peroxidative degradation of rat lens lipids *in vitro* (Varma *et al.*, 1982a,b). This degradation has been attributed to a photochemical generation of superoxide and its derivatization to other active species of oxygen such as hydrogen peroxide and the hydroxyl radical. These oxygen derivatives are considered potent oxidants. Their generation *in vivo* has been proposed to inflict an oxidative stress on various tissues (Fridovich, 1978). Many systemic age-dependent diseases such as arthritis and coronary arteriosclerosis, have now been suggested to arise as a result of such continued oxidative stress (Herman *et al.*, 1968). The age-dependent formation of cataracts has also been proposed to follow oxidation of various lens components by the active species of oxygen (Bhuyan, 1977; Varma, 1977, 1979; Goosey, 1981).

Aspirin is one of the common drugs used as an anti-inflammatory agent. The anti-inflammatory effect of this compound involves its action as an inhibitor of prostaglandin synthesis (Flower, 1974). However, since the drug *in vivo* is deacetylated to the phenolic form (salicylic acid), it is possible that it may also act as an antioxidant. Many phenols are known to act as antioxidants. This possibility, coupled with the report that the incidence of cataracts is supposedly lowered by aspirin (Cotlier, 1981), prompted us to determine if the drug can prevent peroxidative degradation of lens lipids *in vitro*. The results indicate that aspirin is capable of doing so.

MATERIALS AND METHODS

In these studies male rats of the Sprague–Dawley strain were utilized. Animals weighed between 130 and 150 g. All chemicals, except aspirin (acetylsalicylic acid), were obtained from Sigma Chemical Co. (St. Louis, MO). Aspirin was obtained from Aldrich Chemical Co. (Milwaukee, WI). Eyes from these rats were enucleated and the intact lenses dissected out. Each lens was then immediately transferred to a sterile 35 × 10 mm glass Petri dish containing 4 mℓ of modified medium 199 (Merola *et al.*, 1960). The medium contained 9.2 units of ascorbate oxidase/4 mℓ. This was done to eliminate the protective effect of ascorbate that may leach out of the lens during incubation and mask the aspirin effect. Dishes containing the media were prewarmed to 37°C in an incubator gassed with 95% air and 5% carbon dioxide for 30 min prior to lens transfer. Tonicity of the incubation medium was 295 mOsmol/kg. Soon after the lenses were transferred in the medium, the light was turned on; the contralateral pair being incubated in the dark. A fluorescent daylight tube, maintaining an intensity of 150 ft candle (≈ 1600 lux) at the surface of the Petri dish, was used as the source of light. Following culture for 20 h, the lenses in groups of two were transferred to 0.5 mℓ of 0.1 *M* phosphate buffer, pH 7.4, and homogenized. Subsequently 0.5 mℓ of a 20% trichloroacetic acid (TCA) solution was added to each homogenizer and a supernatant obtained by homogenization and centrifugation. An aliquot of the above extract was mixed with an equal volume of 0.67% 2-thiobarbituric acid and heated in a boiling water bath for 15 min. This was followed by chilling in ice water. Malonaldehyde (MDA) values were obtained by reading the absorbance of the reaction mixture at 532 nm (Barber and Bernheim, 1967). Tetramethoxypropane, simultaneously processed, was utilized as a reference standard.

RESULTS AND DISCUSSION

Malonaldehyde (MDA) was used as an index of the peroxidative degradation of lipids. The content of this aldehyde in the lenses as prevalent before and after incubation under various conditions has been described in Table 1. The basal level of MDA, i.e. the values in freshly dissected rat lenses, was approximately 5 nmol/g wet weight of the tissue. Incubation in the dark raised this value only slightly; the value being approximately 6 nmol/g wet weight of the lens. If the incubation was conducted in the

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Table 1. Malonaldehyde content in the lens

			n	P values
A	Freshly dissected	5.2 ± 1	6	
B	Incubated in dark	6.21 ± 1.15	8	
C	Incubated in light	25.93 ± 5.89	10	Between A&C < 0.001
D	Incubated in light c̄ aspirin 10^{-3} M	4.25 ± 1.45	12	Between C&D < 0.001
E	Incubated in light c̄ aspirin 10^{-5} M	6.06 ± 0.79	8	Between C&E < 0.001
F	Incubated in light c̄ aspirin 10^{-6} M	7.56 ± 1.56	10	Between C&F < 0.001
G	Incubated in dark c̄ aspirin 10^{-3} M	5.08 ± 0.56	10	Between C&G < 0.001

Paired lenses after incubation were homogenized with 0.5 mℓ of 20% tricholoracetic acid and 0.5 mℓ of 0.1 M PO_4^- buffer, pH 7.4, and MDA determined on the supernatant as described in the text. n is number of experiments in each case. Values are expressed as nmol/g wet weight of the tissue ± S.D.

presence of the fluorescent daylight, there was a substantial rise in the MDA content. After 21 h of incubation, the level of this aldehyde was approximately 25 nmol/g wet weight of the tissue, reflecting a 3- to 5-fold increase over the basal value. The large variation in light values of MDA probably reflects the physiological variability within the lenses. Incorporation of aspirin at concentrations of 10^{-3}–10^{-6} M prevented photo-acceleration of the peroxidative degradation. The compound was thus effective at micromolar levels. The addition of aspirin under dark conditions, even to 10^{-3} M, was without any effect on the amount of MDA produced. These results thus clearly indicate that aspirin can act as an antioxidant, especially with regard to the peroxidation of lipids. Previous studies from this laboratory have demonstrated that in the case of the lens, photocatalyzed formation of MDA can be inhibited by superoxide dismutase (SOD), catalase, ascorbate, and vitamin E. Since catalase and SOD will not penetrate the cell membranes of a nonphagocytic tissue such as the lens, the oxidation of lipids appears to take place at the cell membrane region. The cytosolar region of the cells is well fortified with superoxide dismutase, catalase, peroxidase and the enzymes of the hexose monophosphate shunt. These are all capable of preventing the aberrant oxidation intracellularly. In view of these earlier findings, it is possible that aspirin is also acting at the membrane level. Other possible sites of its action, however, cannot be ruled out entirely. Nevertheless, the results do indicate that aspirin can exert an antioxidant effect at least in the lens. The physiological implication of this mode of aspirin action remains to be realized.

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