Photosensitizer-Induced Lipid Peroxidation in Retinal Homogenates under Illumination

Tadahisa HIRAMITSU,* Yoshifumi MIURA, and Hiroyuki MACHIDA

Department of Ophthalmology, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan

(Received January 17, 1992)

Summary In this study, the effect of photosensitizers on lipid peroxidation in the retina was investigated. Porcine retinal homogenate was exposed to illumination from a fluorescent lamp with or without photosensitizers such as hematoporphyrin, rose bengal, and fluorescein for 60 min. The control, which was exposed to illumination without photosensitizers, did not show a significant increase in lipid peroxidation. However, lipid peroxidation in the homogenates exposed to illumination with photosensitizers increased significantly and in a dose-dependent manner. For concentrations of more than 100 μM of all three photosensitizers, lipid peroxidation in the retina homogenates increased to a level approximately 2–3 times higher than that in the sample before illumination, and to a level 10 times higher than that of the control. α-Tocopherol inhibited hematoporphyrin-induced lipid peroxidation in a dose-dependent manner, but superoxide dismutase did not show an inhibitory effect.

Key Words: photosensitizers, lipid peroxidation, retina, α-tocopherol, superoxide dismutase

It is well known that lipid peroxidation has a deleterious effect on cells and tissues that can lead to many diseases. The retinal photoreceptor membrane phospholipids contain large amounts of long-chain polyunsaturated fatty acids, especially docosahexaenoic acid [1]. Therefore, the retina may be especially susceptible to lipid peroxidation, which could result in retinal degeneration.

We demonstrated earlier that retinal lipid peroxidation occurs after UV irradiation in vitro [2]. Many factors such as iron, oxygen, and X-ray-irradiation can induce lipid peroxidation in the retina in vivo and cause changes in electroretinograms that reflect retinal degeneration [3–5].

*To whom correspondence should be addressed.
Recently, light-induced retinal damage has become of great concern. Though the biochemical mechanism of this damage still remains speculative, there are several studies to suggest the involvement of lipid peroxidation in it [6, 7].

It has been pointed out that photosensitizers can produce singlet oxygen, which would trigger chemical photooxidation. Experimental systems of photooxidation induced by photosensitizers may be considered to give some information about light-induced tissue damage. In addition, some photosensitizers are clinically used in ophthalmology. One of these photosensitizers, sodium fluorescein, is used for fluorescein angiography to detect pathological changes in the choroid and retina. Hematoporphyrin also is used in photodynamic therapy to amplify the effect of laser photoocoagulation on retinoblastomas. However, there has been no study to examine the relationship between photosensitizer and lipid peroxidation in the retina. In this study, the effect of photosensitizers such as hematoporphyrin, rose bengal, and fluorescein on lipid peroxidation in the retina was investigated.

MATERIALS AND METHODS

Fresh porcine eyes were obtained from a local slaughter house, placed on ice, and transported to the laboratory for preparation and analysis. The retinas were removed by the method previously reported [8]. The retinal tissues from 2 eyes were homogenized in 1.0 ml of cold normal saline (0.9%) in a Potter-Elvehjem homogenizer and diluted to 4.0 ml with saline solution. Duplicate aliquots of the retinal homogenates (0.75 ml) were mixed with 0.1 ml of photosensitizer solution of various concentrations (10 µM–1 mM) and 0.15 ml of saline to produce the test condition (final volume, 1.0 ml). The following photosensitizers were used: hematoporphyrin (Sigma Chemical, Co., St. Louis, MO), rose bengal (Katayama Chemical Co., Osaka), and sodium fluorescein (Japan Alcon Co., Tokyo). In experiments to examine inhibitory effects, α-tocopherol (provided from Eisai Pharmaceutical Co., Tokyo) at a final concentration of 1 µM–1 mM or superoxide dismutase (SOD, purified from bovine erythrocytes, Sigma Chemical, Co.) at a final concentration of 1 µM–1 mM was added to the sample. Hematoporphyrin and α-tocopherol were dissolved in ethanol; and the other drugs, in saline. Absolute ethanol and saline without addition of drugs (vehicle only) were used as controls. A 15W fluorescent lamp (with output spectral range from 350 to 710 nm with a peak at 580 nm) was positioned 10 cm above the homogenate, and the sample was illuminated for 60 min. During the light exposure, the test tubes, which were kept in water at 20°C, were shaken gently at 3-min intervals. Samples without light exposure were immediately analyzed (zero time). Lipid peroxide concentration was determined by the method of Ohkawa et al. [9], which measures the amount of thiobarbituric acid-reactive substance (TBARS). TBARS was determined by fluorometry. Total protein was measured by the method of Lowry et al. [10] with bovine serum albumin used as the standard. A calibration curve was made with 1,1,3,3-tetramethoxypropane (Tokyo Kasei, Ind., Co., Tokyo) as the standard, and

the amount of lipid peroxide was expressed per milligram of protein. Three experiments were done for each drug concentration. Since high concentrations of photosensitizers slightly interfere with the fluorescence spectrometry for TBARS measurements, the assay was done with solutions containing only photosensitizer, as a blank (A value), and with the solution containing retina tissue plus photosensitizer (B value). TBARS values were then calculated by subtraction of the A value from the B value.

In separate experiments, we confirmed that SOD (10 \mu M–1 mM) activity in a solution containing 100 \mu M hematoporphyrin (measured by the nitroblue tetrazolium method [11]) was not changed after 60 min illumination with the fluorescence lamp.

RESULTS

Control solutions without photosensitizer exposed to the fluorescent lamp for 60 min showed only a slight increase in their TBA value. However, addition of the photosensitizers hematoporphyrin or fluorescein, at concentrations of more than 10 \mu M, and of rose bengal at concentrations of more than 1 \mu M, significantly increased lipid peroxidation in the retinal homogenates in a dose-dependent manner. At concentrations greater than 100 \mu M of all photosensitizers, lipid peroxidation in the irradiated solutions increased to levels approximately 2–3 times higher than those of samples before illumination, and to values 10 times higher than those of controls without photosensitizers. Rose Bengal was the most effective in increasing lipid peroxidation (Table 1). \alpha-Tocopherol inhibited lipid peroxidation induced by hematoporphyrin in a dose-dependent manner, and had an almost complete inhibitory effect at concentrations of 1 mM. SOD was not inhibitory at any concentration (Table 2).

DISCUSSION

When photosensitizers absorb visible or near-UV light, two systems of electronically excited states are produced: the singlet (Sens) and the triplet (Sens). Two major mechanisms of photodynamic reactions by way of light excitation of a photosensitizer have been proposed: in Type I, the sensitizer interacts with another molecule directly to give either hydrogen atom or electron transfer. The resulting radicals react further with oxygen or other molecules including dye-peroxy radicals. In the second class of reaction, called Type II, the sensitizer triplet interacts with oxygen, resulting in an electronically excited singlet state of oxygen (O2) [12].

In this study, illumination of the retinal homogenate only did not induce lipid peroxidation. However, the addition of any of the three photosensitizers tested to the irradiated retinal homogenate induced marked lipid peroxidation that occurred in a dose-dependent manner. Such retinal lipid peroxidation induced by...
visible light and photosensitizers produces a similar effect to lipid peroxidation induced by UV light [2]. α-Tocopherol inhibited lipid peroxidation with photosensitizer but SOD did not. α-Tocopherol is known as a potent singlet oxygen quencher [13] and also \( \text{O}_2^- \) scavenger [14]. These results are in accord with the findings of Kellogg and Fridovich [15], who showed that illumination of linolate in the presence of 10 μM rose bengal yielded lipid peroxides whose formation was not prevented by SOD or catalase, but was inhibited by the singlet oxygen scavenger 2,5-dimethylfuran. Peroxidation via a singlet oxygen mechanism has been proposed as the initial step in peroxidation of unsaturated phospholipids [15]. There may be a possible mechanism involving singlet oxygen or radicals.
LIPID PEROXIDATION BY PHOTOSENSITIZERS

other than superoxide anion in photosensitizer-induced lipid peroxidation. In our previous studies, α-tocopherol inhibited UV light-induced lipid peroxidation in a retinal homogenate, but SOD did not do so [16]. Therefore, there may be a common mechanism in UV-light and photosensitizer-induced lipid peroxidation.

Many studies have demonstrated retinal damage due to illumination [17–19]. It has been suggested that singlet oxygen products in retinas exposed to light could trigger chemical reactions that would be damaging to visual photoreceptors [20]. Recently, several investigators have proposed that lipid peroxidation may play a causal role in retinal light damage [6, 7]. Although lipid peroxidation did not occur significantly upon illumination without photosensitizers, some endogenous photosensitizers present in photoreceptor cells in the retina could, in theory, participate in this type of reaction. Lipid peroxidation may be considered as a possible mechanism of retinal light damage [21].

In clinical ophthalmology, fluorescein angiography, which can detect minor pathological changes in the ocular fundus by fluorescein stain, is a frequently used diagnostic tool. The present study suggests the possibility that this clinical test could produce lipid peroxidation and thus promote pathological changes in the fluorescein-stained areas exposed to many bright flashes during the performance of fluorescein angiography.

REFERENCES


Vol. 12, No. 2, 1992


