The Photodynamic Effect of Chlorpromazine, Promazine, and Hematoporphyrin on Lens Protein

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Calf lens protein was irradiated with near ultraviolet (UV) light in the presence of the drugs chlorpromazine, promazine, and hematoporphyrin. It then was analyzed by SDS-polyacrylamide gel electrophoresis and amino acid analysis. Marked increases in protein photopolymerization (other than S-S bond formation) and histidine destruction were noted in the presence of these drugs. In all cases, these effects were reduced in the presence of a singlet oxygen quencher, thus suggesting that these photodynamic effects are due, at least in part, to a Type II mechanism. Invest Ophthalmol Vis Sci 25:746-750, 1984

Certain drugs having either a tricyclic, heterocyclic ring system, such as Chlorpromazine (CPZ) or Promazine (PZ)1 (Fig. 1) or a porphyrin ring system such as Hematoporphyrin (HP)2 (Fig. 2) are known to be photosensitizers. Therefore, in addition to their drug action, they can have deleterious side-effects when patients using them are exposed to sunlight. These effects may be exaggerated erythemal responses such as edema and necrosis of the skin.3-5 In addition to phototoxic effects to the skin, some drugs are known to cause phototoxic side effects to the cornea, lens, and retina.6-8

Phenothiazines, such as chlorpromazine, accelerate the formation of cataracts in patients following prolonged use in high-dose therapy.9-13 Although several mechanisms have been proposed for this side-effect,14-20 it is conceivable that the cataracts are due to some photosensitized reaction.

Hematoporphyrin is used in the photoradiation therapy of certain tumors,21 including the treatment of intraocular tumors.22 Although there is, as yet, no direct evidence that HP accelerates cataractogenesis, it is a very efficient photosensitizer2 and is known to produce phototoxic reactions in the skin23 and in retinal tissue.22

The human lens is an organ that is composed of more than 30% protein. The packing24 of these proteins is such that light is transmitted through the lens without distortion. There is no significant absorption of visible light in the lens; however, with the introduction of photosensitizers into this system, long ultraviolet (UV) and visible light is absorbed and could lead to the photosensitized oxidation of certain amino acid residues, such as, histidine, methionine, tyrosine, and tryptophan.25 These changes could lead to light-scattering areas, which are evidenced as an opacification.

Our studies are directed toward: (1) determining whether these drugs can photooxidize lens protein; (2) understanding the fundamental molecular mechanism by which photosensitizing drugs could induce cataractogenesis; and (3) to investigate methods by which this toxic side-effect could be prevented or retarded.

Materials and Methods

Materials

Chlorpromazine, hematoporphyrin, sodium azide, and penicillamine were purchased from Sigma Chemical Co (St. Louis, MO). Promazine was a gift from Dr. Irene Kochevar, Department of Dermatology, Harvard Medical School (Cambridge, MA). Phosphate buffer was prepared from reagent grade sodium salts in glass distilled water. The materials used for sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis were from Bio Rad Laboratories (Rockville Center, NY).

Protein Isolation for Photolytic Experiments

The soluble protein from the cortex of calf lenses is used in these model protein studies. The cortex was isolated using a #7 cork borer, homogenized in 0.1 M phosphate buffer pH 7.4 in a 10:1 proportion to lens wet weight. The homogenate was centrifuged on a Sorvall Superspeed Centrifuge at 12 K for 20 min. The
soluble portion was dialyzed exhaustively against the same buffer at 4°C. Aliquots of this were diluted to the desired concentrations with either buffer (phosphate 0.1 M, pH 7.4) or buffer mixed with other compounds whose properties were being investigated.

Photolysis

Samples were photolyzed using a Hanovia 450-watt mercury lamp equipped with a Kimax filter (1% transmission at 285 nm). A “merry-go-round” irradiation apparatus was used that insured that all parallel samples received equal radiation. The radiant energy incident on the samples being photolyzed was measured with a Yellow Springs Radiometer (Yellow Springs, OH) and was found to be 350 W/m².

Electrophoresis

Aliquots of photolyzed samples were incubated with 0.125 M Tris. HCl and 40 mM dithiothreitol at 45 °C for 30 min in order to monitor crosslinking other than S-S bond formation. The procedure of Lammeli was used for the sample and electrophoresis buffers. The gel composition was 15% acrylamide. Electrophoresis performed at ambient temperature in an LKB electrophoresis apparatus. The gel were stained with Coomasie blue and scanned at 570 nm using a Gilford 2400-S spectrophotometer (Oberland, OH) with gel scanning accessory.

Amino Acid Analysis

Aliquots of the photolyzed samples were taken and subjected to acid hydrolysis (6 N HCl; 16 hr) followed by amino acid analysis using a Beckmann 121 amino acid analyzer (Summerset, NJ).

Long UV Absorbance Spectra of Drugs

The long UV absorbance maxima of promazine is 310 nm; of chlorpromazine is 310 nm; of hematoporphyrin 395; 500–600 nm.

Results

For these photolytic experiments, the soluble protein of the outer cortex of calf lenses was used. The proteins were at a concentration of 2.0 mg/ml. This protein consists of a mixture of alpha, beta, and gamma crystallins and was used as such.

Figure 3 depicts the scan of an SDS gel electrophoresis of this protein mixture taken at 570 nm after staining with Coomasie Blue. The scan consists of a number of peaks in the 20–30 K dalton region. Upon photolysis (5 hr), there is a slight decrease of the proteins.

FIG. 3. Effect of long UV light irradiation upon lens protein. Gel scanning of Coomasie blue-stained electrophoresis gels. Protein (2.0 mg/ml) irradiated for 5 hr is indicated by (-----). The dark control is indicated by (----).
of a broad peak at, or approximately, 45 K dalton. This is probably due to photolytic dimerization of the crystallins.

Upon photolysis in the presence of $10^{-3}$ M PZ (Fig. 4) there is a much greater decrease of the proteins in the 20–30 K region than in the lens protein photolyzed without the photosensitizer. In addition, there is an increased production of higher molecular weight species especially those above 100 K daltons. These results suggest that PZ promotes photopolymerization. The addition of both azide ($10^{-2}$ M) and penicillamine ($10^{-2}$ M) decreased this photopolymerization. But azide, which is a singlet oxygen quencher, is not nearly as efficient as penicillamine, a free radical quencher, in negating photopolymerization. CPZ gave analogous results.

The photolysis of calf lens protein in the presence of HP (Fig. 5) under the same conditions as above resulted in significantly greater destruction of the protein than shown for PZ and CPZ. Again, both azide ($10^{-2}$ M) and penicillamine ($10^{-2}$ M) decreased photopolymerization with penicillamine offering greater protection. In contrast to the phenothiazines, however, the photosensitized oxidation of the lens protein in the presence of HP and penicillamine does not result in the substantial protection of the protein from photodamage.

To determine more specifically the molecular mechanism by which photopolymerization occurs, the changes in histidine composition of lens protein, with and without photosensitizers, were examined kinetically (Fig. 6). As indicated, 20–25% histidine was destroyed in the presence of PZ and CPZ after 3 hr irradiation, whereas there is little or no change in histidine without these sensitizers. In the presence of HP,
there is almost complete destruction of histidine over the same period of time.

Discussion

The photolysis of lens protein in the presence of PZ, CPZ, and HP accelerates the photopolymerization of those proteins, with HP producing the most dramatic effects. The possible photochemical mechanism by which this occurs was investigated. Both the partial quenching in the presence of azide and the destruction of histidine suggests that this photodynamic inactivation of lens protein is due, at least in part, to a Type II (singlet oxygen mediated) mechanism.

Many drugs that have a tricyclic, heterocyclic ring system are known to be photosensitizers and some of these drugs are known to accelerate cataractous formation. The exact relationship between the photochemistry and the phototoxicity is unknown, although several researchers have suggested that both endogenous and exogenous sensitizers may play a role in cataractogenesis.

In the case of CPZ, there is evidence that persistent drug therapy given in high dosages causes cataracts. Several mechanisms have been suggested, namely: (1) the absorption of the drug by the uveal pigment granules; (2) dehydration through the cornea; (3) disruption of the lens membrane; (4) change in electrolyte balance.

There has been suggestive evidence that photosensitization also may be a mechanism. The results from this present study suggests that cataract formation could be caused or accelerated by photosensitized oxidation reactions with lens protein.

Hematoporphyrin currently is being used in the photoradiation therapy of intraocular tumors. This study indicates that it is a very efficient photosensitizer. It already has been demonstrated to cause damage to retinal tissue. Therefore, possible side-effects involving the eye should be taken into account when the drug is administered.

Penicillamine, a free radical scavenger, greatly retards photopolymerization, ie, crosslinking. In the lens there are relatively high concentrations of glutathione. It also is a free radical scavenger and it has been hypothesized that glutathione performs a similar function, that is, protecting the eye against endogenous photosensitized reactions. The long-term use of high levels of these drugs could overload the existing protective system in the lens resulting in cataractogenesis. The addition of exogenous quenchers or scavengers such as penicillamine in any long-term therapy involving a phototoxic drug may serve to retard these phototoxic side-effects.

Key words: cataracts, phototoxic effects, photooxidation, photosensitization, chlorpromazine, promazine, hematoporphyrin

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References