Chlorpromazine-induced corneal endothelial phototoxicity

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Chlorpromazine, which has been used extensively for the treatment of psychiatric disorders, is known to accumulate in the posterior corneal stroma, lens, and uveal tract. Because it is a phototoxic compound, the potential exists for it to cause cellular damage after light exposure. Specular microscopic perfusion of corneal endothelial cells in darkness with 0.5 mM chlorpromazine HCl resulted in a swelling rate of 18 ± 2 μm/hr, whereas corneas exposed to long-wavelength ultraviolet light for 3 min in the presence of 0.5 mM chlorpromazine swelled at 37 ± 9 μm/hr (p < 0.01). Preirradiation of 0.5 mM chlorpromazine solution with ultraviolet light for 30 min and subsequent corneal perfusion with the solution resulted in a corneal swelling rate of 45 ± 19 μm/hr. Cornea endothelial cells perfused with 0.5 mM chlorpromazine that was preirradiated with ultraviolet light showed marked swelling on scanning electron microscopic examination, whereas those perfused with nonirradiated chlorpromazine were flat and showed a normal mosaic pattern. Combining either 300 U/ml catalase or 290 U/ml superoxide dismutase with chlorpromazine did not alter photoinduction of corneal swelling. The data suggest that corneal endothelial chlorpromazine phototoxicity is secondary to cytotoxic products resulting from the photodynamically induced decomposition of chlorpromazine and is not caused by hydrogen peroxide or superoxide anion generated during the phototoxic reaction.

Key words: cornea, endothelium, photodynamics, phototoxicity, chlorpromazine

Chlorpromazine, a phenothiazine derivative, has been used extensively for the treatment of psychiatric disorders. Numerous reports have listed the ocular complications of systemic administration of chlorpromazine, which include fine deposits in the anterior lens, deep corneal stroma, and corneal endothelium.1-9 These chronic changes appear to be related to total drug dosage, compared with the epithelial keratopathy that has been associated with short-term high dosages.9 It is known that the phenothiazines concentrate in the uveal tissues of the eye, but measurable levels have also been found in the cornea and lens.10 Chlorpromazine is potentially a phototoxic substance,11, 12 since numerous in vitro experiments have demonstrated phototoxicity to bacteria13 as well as photohemolysis14-18 induced by the compound. In patients receiving chlorpromazine, phototoxic as well as photoallergic reactions have been noted in the exposed areas of the skin.5, 6, 19-21 It is thought that the phototoxic reaction results from the action of sunlight on phenothiazines

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in the skin, resulting in an inflammatory response and increased melanin production.\textsuperscript{19,20} Since the cornea is constantly exposed to light and allows the penetration of both long-wavelength ultraviolet as well as visible light, it is of potential concern that patients receiving chlorpromazine for the treatment of psychiatric disorders may be at risk for corneal endothelial cell damage induced by phototoxic reactions of chlorpromazine or its by-products deposited deep in the stroma or in the endothelial cells.

Previous work in our laboratory has demonstrated that corneal endothelial cells are susceptible to photodynamic change, that this reaction is oxygen dependent, and that there is an alteration of corneal endothelial cell bicarbonate flux after photodynamic alteration.\textsuperscript{22,23} It was the purpose of this investigation to determine whether corneal endothelial cells were susceptible to chlorpromazine-induced photodynamic alteration.

**Materials and methods**

**Group 1.** Adult albino rabbits weighing 3 kg were killed with an overdose of intravenous sodium pentobarbital. The eyes were enucleated together with the conjunctival sac and eyelids, and the corneas were mounted in a specular microscope.\textsuperscript{24,27} The specular microscope allows constant perfusion of the corneal endothelial surface at 37°C and +15 mm Hg pressure and permits observation of the endothelial cells as well as sequential measurement of corneal thickness. Silicone oil was placed on the epithelial surface to prevent evaporation. Corneal swelling rates were determined by linear regression analysis, and a comparison of the swelling rates of experimental and control corneas was made by analysis of covariance.\textsuperscript{28,29}

Chlorpromazine was dissolved in Krebs-Ringer bicarbonate with added 0.3 mM reduced glutathione and 0.5 mM adenosine.\textsuperscript{25,27,30,31} Chlorpromazine in a concentration of either 0.1 or 0.5 mM was placed in a beaker 8 cm from the light source and was irradiated for 30 min with long-wavelength ultraviolet light. The ultraviolet light source was a 4 W Mineralight lamp, multiband UV 254/366 nm (Model UVSL-25; Ultra-violet Products, Inc.). The output as rated by the manufacturer is 280 $\mu$W/cm$^2$ at 366 nm at 6 inches.

Control and experimental corneas were first perfused for 1 hr with Krebs-Ringer bicarbonate solution with added glutathione and adenosine, after which time experimental corneas were perfused in the dark with the preirradiated chlor-
Fig. 2. Scanning electron micrograph of cornea endothelial cells after 3 hr perfusion with 0.5 mM chlorpromazine solution that had been preirradiated for 30 min with long-wavelength ultraviolet light. Endothelial cells appear swollen with marked enlargement of the intercellular spaces and loss of mosaic pattern. (×615).

promazine solution and paired controls were perfused in the dark with either a similar concentration of nonirradiated chlorpromazine or with preirradiated Krebs-Ringer bicarbonate to which 0.5 mM sucrose had been added to maintain osmotic equivalency. Corneal thickness was measured every 30 min over the subsequent 3 hr, after which time corneas were removed, placed in glutaraldehyde with phosphate buffer, and submitted for scanning electron microscopic examination.

Group II. Experimental and control corneas were perfused in the dark for a 1 hr stabilization period with Krebs-Ringer bicarbonate with added glutathione and adenosine to which had been added 0.5 mM chlorpromazine. After 1 hr of perfusion, experimental corneas were exposed to the long-wavelength ultraviolet light source at a distance of 6 inches from the cornea for a total of 3 min. Perfusion was then continued for an additional 2 hr period in the dark with chlorpromazine for an additional 2 hr but were not exposed to ultraviolet light at any time. In a second set of controls, Ringer-perfused corneas were exposed to ultraviolet light for 3 min to determine whether ultraviolet light was producing adverse endothelial effects in this experimental model.

Group III. Corneas were perfused with Krebs-Ringer bicarbonate with added glutathione and adenosine to which had been added 0.5 mM chlorpromazine. Experimental solutions also contained either 5400 U/ml (200 µg/ml catalase) or 280 U/ml (100 µg/ml) superoxide dismutase. After 1 hr of perfusion, experimental and control corneas were exposed to the long-wavelength ultraviolet light source at a distance of 6 inches from the cornea for a total of 3 min. Perfusion was then continued for an additional 1 hr period in the dark with the same solutions.

Results

Group I. Corneas perfused with a chlorpromazine solution that had been exposed to
long-wavelength ultraviolet light for 30 min prior to the perfusion swelled at rates more rapid than corneas perfused with nonirradiated chlorpromazine (Fig. 1). Corneas perfused for 3 hr with nonirradiated 0.1 mM chlorpromazine swelled at 5 ± 1 μm/hr, whereas corneas perfused with preirradiated 0.1 mM chlorpromazine swelled 11 ± 3 μm/hr (p < 0.001). Corneas perfused with nonirradiated 0.5 mM chlorpromazine swelled at 11 ± 1 μm/hr, whereas those perfused with preirradiated 0.5 mM chlorpromazine swelled at 45 ± 19 μm/hr (p < 0.001). Corneas perfused with nonirradiated 0.5 mM sucrose swelled at 10 ± 4 μm/hr, whereas corneas perfused with nonirradiated 0.5 mM sucrose swelled at 6 ± 2 μm/hr (p > 0.05). Scanning electron microscopic examination of corneas perfused with preirradiated sucrose showed a normal endothelial mosaic. Since it was noted that the pH rose from 7.3 to about 7.6 after irradiation of the chlorpromazine solution, a subset of experiments was run with the 0.5 mM preirradiated chlorpromazine in which the pH was readjusted to 7.3; these corneas swelled at rates similar to those without the adjustment of pH.

Scanning electron microscopic examination demonstrated marked swelling of corneal endothelial cells and an increase in the intercellular space after perfusion with preirradiated 0.5 mM chlorpromazine (Fig. 2). In contrast, endothelial cells perfused with nonirradiated 0.5 mM chlorpromazine were flat and showed preservation of the endothelial mosaic (Fig. 3).

**Group II.** Corneas perfused for 3 hr with 0.5 mM chlorpromazine but exposed to ultraviolet light for 3 min after 1 hr of perfusion swelled at 37 ± 9 μm/hr, whereas those perfused with a similar concentration of chlorpromazine but not exposed to ultraviolet light swelled at 18 ± 2 μm/hr (p < 0.01; Fig. 4). Corneas perfused with Krebs-Ringer bicarbonate and exposed to ultraviolet light for 3 min swelled at 7 ± 3 μm/hr, whereas
corneas perfused with the same solution in the dark for 3 hr swelled at 5 ± 2 μm/hr (p > 0.05).

**Group III.** Corneas perfused with 0.5 mM chlorpromazine and either 5400 U/ml catalase or 290 U/ml superoxide dismutase and exposed to ultraviolet light for 3 min swelled at rates statistically similar to those treated without catalase or superoxide dismutase in the perfusing solution (Group II). This indicates that the toxic reaction is not mediated by hydrogen peroxide or superoxide anion.

**Discussion**

The results of this experiment have demonstrated that corneas perfused with photoactivated chlorpromazine swell at more rapid rates than corneas perfused in the presence of chlorpromazine that has not been photoactivated. It is apparent from the Group I series of experiments that cytotoxic photoproducts are produced during exposure of chlorpromazine to ultraviolet light and that these products persist in the solution in the dark after the ultraviolet light exposure is discontinued. It is known that ultraviolet irradiation of chlorpromazine hydrochloride results in the formation of a semiquinone free radical intermediate and that disproportionation of this free radical yields chlorpromazine-5-oxidehydrochloride, which is the precursor of other degradation products. Some of the degradation products have been identified as H⁺, hydrochloric acid, chlorpromazine sulphoxide, and 2-hydroxypromazine. These and other metabolites may be either more or less toxic than the parent compound. Previous work has demonstrated that chlorpromazine photosensitization is an oxygen-independent process in contrast to photosensitization reactions with rose bengal. Since photodynamic alteration of endothelium by chlorpromazine was not altered by catalase or superoxide dismutase, chlorpromazine phototoxicity is not secondary to hydrogen peroxide or superoxide anion. This is compatible with the results of previous work.

It should be noted that perfusion with nonirradiated chlorpromazine did result in
some corneal swelling, but it was not nearly as rapid as that found with the irradiated compound. Previous work has estimated that irradiated chlorpromazine is about 100 times more effective in producing red blood cell hemolysis than is the nonirradiated compound. It is also important to note that ultraviolet irradiation of 0.5 mM sucrose perfusing solution in group I and irradiation of Krebs-Ringer-perfused controls in group II did not result in significant swelling. This demonstrates that there were no other phototoxic chemicals in the Krebs-Ringer solution and that ultraviolet irradiation of rabbit corneas for 3 min does not cause corneal swelling.

The site of attack of photosensitized chlorpromazine on the endothelial cell membrane has not been identified. However, in the red blood cell it has been shown that lysis results from a detergent mechanism whereby the lytic agent enters the membrane and disrupts the structure, but there is no primary alteration of the membrane components such as fatty acids. It has been postulated that chlorpromazine may first penetrate the membranes of cells and that subsequent irradiation and production of photoproducts results in increased cell permeability.

The 0.5 mM concentration of chlorpromazine found in this experiment to be phototoxic to corneal endothelium is of the same order of magnitude as that found in some other systems. This is a considerably higher concentration than the plasma levels found in patients receiving the compound, which are in the order of 10 to 400 ng/ml (2 × 10^-5 to 10^-3 mM). It would be expected that aqueous humor concentrations would approximately be of the same order of magnitude as serum levels unless the drug is bound to protein. It can be seen from this experiment that the concentrations required to induce a photosensitization reaction are many times higher than that found in the serum and than that which would be expected to be present in the aqueous humor. Potts found 0.27 ng drug/gm wet weight cornea 5.5 days after a single 5 mg/kg dose. However, it is of concern that chlorpromazine and/or its products are deposited deep in the corneal stroma and possibly in the endothelium and that concentrations in these localized regions may be many times higher than that found in the serum or aqueous. Indeed this is the case in the skin, where the compound has been found to accumulate and can result in phototoxic reactions. At this time it is not known whether exposure to long-wave ultraviolet light (such as is found in sunlight) in patients who are receiving chlorpromazine can potentially result in destruction or physiologic alteration of endothelial cells. The results of this experiment demonstrate that endothelium is potentially sensitive to this phototoxic compound and suggest that studies utilizing clinical specular microscopy and pachometry in patients receiving this compound may be of potential interest.

REFERENCES


