Interaction of a Photosensitizer with Dipalmitoyl Lecithin

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Monomolecular films of lecithin spread on aqueous subphases containing chlorpromazine hydrochloride were studied. The effects of ultraviolet irradiation and pH were determined. The degree of penetration of the chlorpromazine into the lecithin film was found to be dependent on surface pressure, pH, and ultraviolet irradiation. The biological significance of the data is discussed in relationship to a mechanism for photosensitization.

OVER 70 years ago Raab (1) reported the first photosensitized reaction in a biological system, noting that the killing power of acridine on paramecium was markedly increased in the presence of light. Since that time numerous other compounds have been implicated as photosensitizers, including such modern medicinal agents as the sulfonamides, phenothiazines, tetracyclines, and salicylanilides (2). While a number of theories have been advanced to explain this phenomenon (1–3), neither the exact mechanism of photosensitization nor the cellular constituents involved have been clearly established (4). The following fundamental steps, though, are generally assumed to occur: absorption of light by the photosensitizing agent; a photochemical reaction involving the photosensitizing agent, oxygen, and some cellular component; and development of increased cell permeability, probably as a result of the interaction of the photo-produced species with the constituents necessary for maintaining cellular integrity. The resultant clinical developments *i.e.*, erythema, wealing, edema, and in more drastic situations, hemorrhage and irreversible cell damage, are apparently then a consequence of increased cell permeability.

It is reasonable to postulate that this increased cell permeability involves interactions of the photo-produced species with the phospholipids or sterols of the membrane. These components are the principal structural elements which make up the membranes of the epidermal and dermal cells (5), and it would be expected that changes in their structure or orientation might lead to increased cell permeability or lysis.

A number of *in vivo* and *in vitro* models have been employed to study the photosensitization process, including erythrocytes, protozoa, and the skin of test animals (4, 6, 7). While all of these models are useful in assessing the damage produced by the photosensitization process, none of them serve to establish the particular cellular components involved in the reaction. The work of Allison *et al.* (4), however, did indicate that disruption of the membranes of lysosomes and mast cells might be the primary event in photosensitization.

It is the purpose of this work to investigate the usefulness of monomolecular films as models for studying photosensitized reactions with cell membrane constituents. For this initial phase, a spread film of L-α-dipalmitoyl phosphatidylcholine (lecithin) was selected, since it has been reported to be the principal phospholipid of epidermal cells (5). Chlorpromazine hydrochloride (CPZ) was selected as the photosensitizer because of the large number of cases of photosensitization reported implicating this drug (8), and because there is extensive information available concerning the photo-properties of CPZ (8–11).

EXPERIMENTAL

Materials—The L-α-dipalmitoyl phosphatidylcholine (lecithin) was obtained, chromatographically pure, from Mann Chemical Co., New York, N. Y. Chlorpromazine hydrochloride (CPZ), obtained from Smith Kline & French Laboratories, Philadelphia, Pa., was used without further purification. The water used was first deionized with a mixed resin bed, and then double distilled from alkaline permanganate using all glass equipment. All other chemicals were reagent grade.

Equipment and General Methods—Surface tension was measured by means of a Rosano tensiometer (12) using a thin roughened platinum plate. Films were spread on a 900-ml. capacity Langmuir-type trough equipped with a movable Teflon barrier. The trough temperature was maintained constant at 25° ± 0.1 by circulating water from a constant-temperature water bath. The edges of the trough were lightly coated with paraffin to prevent wetting. Ultraviolet irradiation of the films was accomplished by means of a Mineralite model V-41 UV lamp[1] fitted with a filter to screen out radiation below 270 mμ. The lamp was positioned about 50 mm. above the trough. The lecithin was dissolved in hexane–ethanol (95%:5% v/v) and spread on the aqueous subphase using an Agla micrometer syringe.[2] Measurements of pH and absorbance were made with a Beckman Zeromatic pH meter and a Beckman DB spectrophotometer, respectively.

Studies of Nonirradiated Films—The surface pressure (surface tension of the subphase minus the surface tension of the subphase in the presence of the film) of the lecithin film was determined at different areas per molecule on a subphase containing CPZ ($1 \times 10^{-4} M$) at pH values of 3.0 and 6.0. The subphase was adjusted to pH 3.0 and 6.0 with 0.001 *M* HCl and sodium acetate–acetic acid buffer (0.1 *M*), respectively. The ionic strength of the HCl solution was adjusted to 0.1 with sodium chloride. Surface pressure was also determined in the absence of CPZ for comparison.

Studies of Irradiated Films—Films were spread under the same conditions as used for the nonirradiated studies. Irradiation was initiated with the films at zero surface pressure and continued for 2 min. The surface pressure–surface area (π-A) curves were determined either immediately or 5 min. after irradiation.

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[1] Ultra-violet Products, Inc., San Gabriel, Calif.
[2] Burroughs Wellcome Corp., Tuckahoe, N. Y.

Pure lecithin films, *i.e.*, in the absence of CPZ, were irradiated for 30 min. to determine their ultraviolet stability.

To determine the effect of initial pressure, films were irradiated for extended periods of time at two different initial film pressures.

RESULTS AND DISCUSSION

Irradiation of pure lecithin film at pH 3.0 and 6.0 for 30 min. showed no change in surface pressure, demonstrating the stability of these films to the ultraviolet radiation. Irradiation of the pH 3.0 subphase containing 1×10^{-4} *M* CPZ in the absence of the film also produced no change in surface pressure, though some decomposition did occur as evidenced by formation of color and changes in absorbance spectrum. However, at pH 6.0 an increase in surface pressure was observed even in the absence of the lecithin film. This increase in surface pressure apparently was due to the formation of an insoluble film, whose π-*A* characteristics were markedly dependent on rate of compression.

The π-*A* curves for the lecithin on a 1×10^{-4} *M* CPZ subphase at pH 3.0 and 6.0 were the same (Fig. 1). A π-*A* curve for pure lecithin (*i.e.*, in the absence of CPZ) is included for comparison. It is clear that film pressure develops at much larger lecithin areas when CPZ is included in the subphase. This apparently is the result of penetration of the CPZ molecule into the lecithin film leading to the formation of a mixed film. On continued compression of the nonirradiated film it can be seen that the π-*A* curves for both the lecithin and lecithin–CPZ monolayers become identical, due to the ejection of CPZ from the film at about 31 dynes/cm.

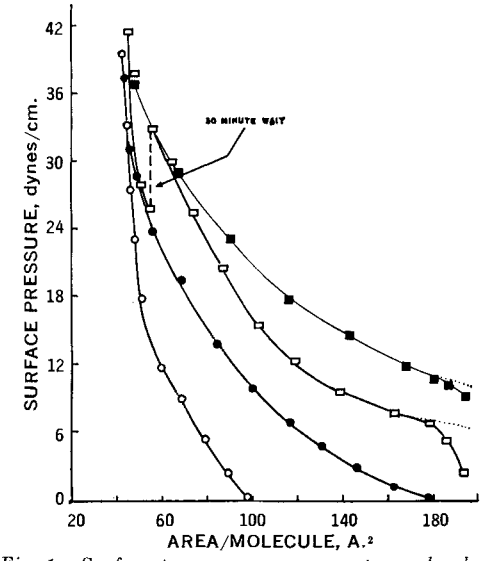

Fig. 1—Surface pressure versus *area per molecule for L-α-dipalmitoyl phosphatidylcholine at pH 3.0 and 6.0, 25°, and ionic strength 0.1. Key:* ○, *zero chlorpromazine HCl at pH 3.0 and 6.0;* ●, 1×10^{-4} M *chlorpromazine HCl at pH 3.0 and 6.0;* □, 1×10^{-4} M *chlorpromazine HCl at pH 3.0 irradiated for 2 min. at zero initial pressure;* ■, 1×10^{-4} M *chlorpromazine HCl at pH 6.0 irradiated for 2 min. at zero initial pressure; dotted lines indicate the curve that results if the readings are initiated 5 min. after the termination of the irradiation rather than immediately after.*

pressure. Similar results have been reported by Zografi *et al.* (13).

At both pH values irradiation results in significantly higher pressure at all areas as compared to the corresponding nonirradiated films. The rapid rise in surface pressure seen in the initial part of the π-*A* curves was observed consistently when the surface tension readings were begun immediately after the end of the irradiation. If, instead, the readings were begun 5 min. after the end of the irradiation, this sharp rise was not observed. The initial part of the curves then started at a higher pressure as indicated by the dotted lines (Fig. 1). The remainder of the curves were the same under both conditions. Thus it appears that changes occur, possibly in the composition of the photospecies, its orientation, or its interaction with the film or other components of the system, for a short period after the irradiation has ceased. However, apparently, after this initial period no further changes occur in the photoproduced species over the time of observation. At very high pressures it appears that the π-*A* curves of the irradiated films approach that of the pure lecithin. This suggests that the irradiated component as well as the nonirradiated CPZ is ejected from the film at very high pressures. Because of the uncertainty of the data at pressures very near the collapse pressure, it was not established whether the curves do eventually coincide and whether all of the irradiated components are ejected.

If the compression of the irradiated film on the pH 3.0 subphase was stopped on reaching an area of about 60Å² (33 dynes/cm. pressure) and maintained at this area for 30 min., the pressure gradually decreased to the value previously recorded for the nonirradiated film. Further compression then showed the same π-A characteristics as did the nonirradiated film (Fig. 1). When the compression was stopped at an area of 100Å.² or greater (*i.e.*, an area at which the pressure is about 17 dynes/cm. or less) and maintained at that area for 30 min., no change in surface pressure with time was observed. Thus it appears that the decrease in surface pressure with time, observed at the smaller area/molecule, was the result of a gradual ejection of the photo-produced species from the film rather than any change in the properties of the species itself.

At pH 6.0 the π-*A* curve after irradiation was shifted considerably to the right of that obtained at pH 3.0 (Fig. 1). However, if the surface pressure at each area developed from the irradiation of CPZ at pH 6.0 in the absence of the lecithin was subtracted from this curve, the resultant curve appeared to qualitatively follow that obtained at pH 3.0. Thus it appears that the differences observed at the two pH values are at least in part due to the increase in surface activity which results from irradiation of CPZ alone at pH 6.0. In addition, at pH 6.0 the change in π that resulted after a 2 min. irradiation decreased with increasing film pressure beyond 4 dynes/cm. The opposite effect was noted above an initial pressure of 8 dynes/cm. when the film was irradiated at pH 3.0 (Fig. 2). The reason for these differences was not determined, but they might be due to a number of factors, including the formation of different photo species at each pH value as well as the influence of pH on the rate of the photo reaction and on the photo species–film interaction.

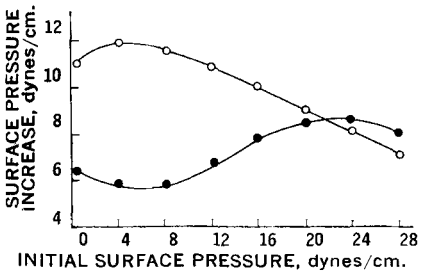

Fig. 2—The increase in surface pressure at various initial surface pressures for L-α-dipalmitoyl phosphatidylcholine at 25°, ionic strength 0.1, and 1 × 10^{-4} M chlorpromazine HCl following ultraviolet irradiation of the film at zero surface pressure for 2 min. Key: ●, pH 3.0; ○, pH 6.0.

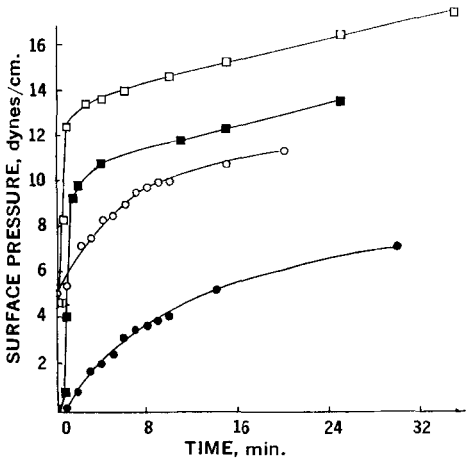

Fig. 3—Surface pressure versus *time of ultraviolet irradiation for L-α-dipalmitoyl phosphatidylcholine at 25°, ionic strength 0.1, and 1 × 10^{-4} M chlorpromazine HCl. At pH 3.0 irradiation started at zero surface pressure (●), and at 5.1 dynes/cm. surface pressure (○); at pH 6.0 irradiation started at zero pressure (■), and at 4.9 dynes/cm. surface pressure (□).*

When irradiation at pH 3.0 was extended beyond 2 min., it was noted that the rate of increase in surface pressure was fairly rapid during the first 7 to 8 min., after which the rate tailed off (Fig. 3). To determine whether this change in rate was a direct result of the increase in pressure that accompanied irradiation, and thus possibly a change in concentration and/or orientation of CPZ in the film, the effect of compression prior to irradiation was studied. The lecithin film, spread on a subphase containing 1 × 10^{-4} *M* CPZ at pH 3.0 was compressed to 5.1 dynes/cm. prior to the start of irradiation. The same rapid increase in pressure was noted, followed by tailing off after about 7–8 min. (Fig. 3). At pH 6.0 the increase in pressure developed much more rapidly and reached a higher pressure before leveling off (Fig 3). However, at both pH values the initial pressure (within the limited range studied) seemed to have no significant influence on either the rate of increase or total increase of surface pressure which resulted from irradiation. Thus it appears that the surface pressure which develops during the course of irradiation does not influence the photo reaction.

To determine whether the irradiated interaction was dependent on the presence of the film, the subphase at pH 3.0 was irradiated for 2 min. prior to spreading of the lecithin. The π-*A* curve of the lecithin film spread on this pre irradiated subphase was not significantly different from that obtained in previous experiments when the film was spread first and then irradiated for 2 min. at zero initial pressure.

CONCLUSION

Seeman *et al.* (14) have shown that CPZ penetrates the membrane of erythrocytes *in vitro*, producing membrane stabilization at concentrations of 10^{-4} *M* or lower, and lysis at concentration above 10^{-4} *M*. These effects have been related to the surface activity and the ability of this drug to penetrate spread monolayers (14). In view of this, and the results of this preliminary study, it appears that in CPZ mediated photosensitized reactions this drug may first penetrate the membranes of the cells of the dermis or basal layers of the epidermis at levels sufficient to stabilize these cell membranes. This is quite reasonable since plasma and tissue levels of CPZ are normally well below 10^{-4} *M*. Subsequent irradiation would tend to increase the pressure within the cell membranes until it reaches a level equivalent to that produced by lytic concentrations of CPZ. The result would be increased cell permeability, with its subsequent clinical effects.

It appears that a monomolecular lecithin film is a useful model to study the interactions of photosensitizing agents at cell membranes. Additional work is currently in progress to determine the effects of other photosensitizing agents on this model as well as the influence of antioxidants and ultraviolet absorbers.

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Keyphrases

Dipalmitoyl lecithin monomolecular films
Chlorpromazine HCl-lecithin film interaction
UV irradiation of film
Surface pressure-effect on film
Photosensitization-relation to chlorpromazine HCl penetration