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Phospholipid Spherules as a Model to Assess Photosensitizing Properties of Drugs

ALVIN FELMEISTER and SHRILEKHA V. TOLAT

Abstract □ The effect of a series of UV irradiated and nonirradiated phenothiazine drugs on the chromate leakage from lipid spherules has been determined. All of the drugs studied increased chromate leakage to varying degrees prior to irradiation. However, only chlorpromazine and prochlorperazine exhibited a marked increase in chromate leakage after irradiation. These effects are discussed in terms of the *in vivo* photosensitizing properties of the drugs.

Keyphrases □ Photosensitizing properties—phenothiazines □ Lipid spherules, chromate leakage—photosensitizing properties, phenothiazines □ Phenothiazines—photosensitizing properties □ Spectrophotometry—analysis

Nonphysiologic photosensitized reactions have been studied extensively since Raab first observed the photosensitized activity of acridine toward paramecium almost 70 years ago (1). During the intervening time, much has been learned about such reactions, although their exact mechanism of action has not been completely elucidated. However, it has been reasonably well established that changes in the permeability of the membrane of cells or cell organelles are often induced by light-irradiated photosensitizing agents (2).

It was the purpose of this investigation, therefore, to develop a physical model that might be used to assess photosensitizing agents by measuring their influence on membrane permeability. The phospholipid spherule model developed by Bangham *et al.* (3) was selected for this purpose. These workers have shown that phospholipids, when permitted to swell in an aqueous salt solution, form salt-containing compartments bounded by bimolecular membranes. These structures (spherules) exhibit permeability characteristics that are quite similar to those of biological membranes. Furthermore, these spherules have been shown by numerous researchers to be useful tools for studying membrane–drug interactions, particularly when changes in permeability are involved (4). Therefore, changes in permeability of these spherules induced by light-irradiated drugs should be a measure of the photosensitizing property of these drugs.

Five phenothiazine derivatives were selected for this initial study. Two of these compounds, chlorpromazine and prochlorperazine, have been shown unequivocally to be photosensitizers (5). The other three compounds, promazine, triflupromazine, and fluphenazine, rarely if ever produce photosensitization (5–7).

MATERIALS AND METHODS

The phenothiazine derivatives were used without further purification. These were chlorpromazine hydrochloride and prochlorperazine hydrochloride (Smith Kline & French Laboratories); promazine hydrochloride (Wyeth Laboratories); and triflupromazine hydrochloride and fluphenazine dihydrochloride (The Squibb Institute for Medical Research).

The lipid spherules were prepared by the method of Bangham *et al.* (3) with slight modification. Briefly, egg lecithin and dicetylphosphate (90 and 10 μmoles, respectively) were dissolved in chloroform and placed in a 50-ml. round-bottom flask. The solvent was removed under reduced pressure using a flash evaporator. Six milliliters of a 0.145 *M* potassium chromate solution was then added to the flask, and the lipid material was permitted to swell for 4 hr. at room temperature. At the end of 4 hr., any chromate ion not trapped within the spherules was removed by dialyzing the dispersion against a 0.145 *M* KCl solution for 18–20 hr. One milliliter of the dialyzed suspension of chromate-containing spherules was transferred to each of five cells. By use of a micrometer syringe, 0.05 ml. of a 1×10^{-2} *M* solution in 0.145 *M* KCl of the

drug under investigation was added to each of two of these cells (final drug concentration $\approx 5 \times 10^{-4}$ *M*). The same volume of an UV-irradiated solution of the drug was added to two of the remaining cells. An equal volume of 0.145 *M* KCl was added to the remaining cell, which served as the control. After mixing the material in each cell, 1 ml. of the dispersion was transferred from each cell to a corresponding dialyzing sac. The sacs were sealed and then placed in separate test tubes containing 5 ml. of 0.145 *M* KCl. The test tubes were maintained at 37° in a water bath for 30 min. At the end of this time the sacs were removed and the concentration of chromate ion present in the KCl solution was determined spectrophotometrically (absorbance at 380 mμ). The absorbance, which is proportional to the chromate-ion concentration, was used as a measure of leakage of this ion from the spherule (*i.e.*, lipid membrane permeability).

Known concentrations of chromate ion were determined by this procedure in the presence of the irradiated and nonirradiated phenothiazines (5×10^{-4} *M*) to determine whether either the drug itself or any of its photoproducts interfered with the spectrophotometric analysis. The results obtained were not significantly different from those obtained in the absence of the phenothiazines.

Irradiation of Drug Solution—Three milliliters of a 1×10^{-2} *M* solution of the drug was placed in a standard 1-cm. quartz cell. The cell was positioned 4 in. from the center of a "black light" UV lamp, model 16, Eastern Corp., and exposed for 90 min. The wavelengths emitted by this lamp fall between 290 and 400 mμ. The irradiated solution was then transferred, as previously described, to cells containing the spherules.

RESULTS AND DISCUSSION

The effect of both the irradiated and nonirradiated phenothiazine derivatives on the release of chromate ion from the spherules is summarized in Table I.

Preirradiation—All the nonirradiated drugs tested induced considerably more leakage than that observed with the control. This probably is a measure of the ability of these compounds to interact at a lipid–water interface. Similar effects have been observed with these drugs on other model systems such as erythrocytes, platelets (8), and monomolecular films (9). In fact, phenothiazine–membrane interaction has been proposed as one mechanism of the pharmacologic activity of these drugs (10). The greater leakage induced by chlorpromazine and prochlorperazine apparently is a measure of a greater drug–spherule interaction.

Postirradiation—Of the five drugs studied, only chlorpromazine and prochlorperazine showed any significant change in chromate leakage after irradiation. With both of these phenothiazines, the increase in leakage is considerable. The difference between these two compounds, however, is not significant, indicating that on an equal concentration basis, both of these drugs, when irradiated, induce about the same degree of leakage.

The large increase in leakage induced by the UV irradiation of chlorpromazine and prochlorperazine suggests that such irradiation results in the formation of species that are considerably more membrane active than the parent compounds.

It has been reported that chlorpromazine photopolymerizes *via* a free radical formed by the elimination of the chlorine from the 2-position (11). It is reasonable to postulate that prochlorperazine, which also has a chlorine at the 2-position, will polymerize *via* a similar mechanism. The fact that only these chlorine-containing compounds resulted in increased chromate leakage, coupled with the likelihood that only these compounds will polymerize on exposure to UV radiation, suggests that a photopolymer may be the membrane-active species.

Huang *et al.* (11) postulated that the formation of a photopolymer of chlorpromazine could be responsible for some photosensitized reactions observed in individuals maintained on large doses of this drug.

However, the possibility cannot be eliminated that the *N*-oxide and hydroxy derivatives of chlorpromazine, which were also identified by Huang and Sands (12) as photoproducts, may be responsible to some degree for the observed effects. These products, resulting from the UV irradiation of chlorpromazine, have been shown to be more surface active than chlorpromazine itself (13) and thus more likely to penetrate and disrupt phospholipid membranes.

Table I—Release of Chromate from Phospholipid Spherules Induced by UV-Irradiated and Nonirradiated Phenothiazines

Drug Added	—% of Chromate Released[a]— Nonirradiated Mean[b] ± *SEM*	Irradiated Mean[b] ± *SEM*	Increase due to Irradiation, %[a]
Chlorpromazine	370 ± 17	2790 ± 570	86
Prochlorperazine	428 ± 25	2400 ± 220	82
Promazine	180 ± 30	170 ± 22	—
Triflupromazine	220 ± 50	230 ± 55	—
Fluphenazine	220 ± 40	190 ± 40	—

[a] As compared to that released by the control. [b] Calculated from three to five independently obtained values.

CONCLUSION

Weissman *et al.* (14) have pointed out that artificial lipid spherules appear to resemble natural structures (lysosomes, mitochondria, and erythrocytes) in their release of ions after exposure to lytic agents such as lysolecithin, streptolysin S, a nonionic surfactant (Triton X-100), and steroids. Apparently, these effects, in both natural and artificial systems, are the results of changes in permeability induced by the interaction and subsequent rearrangement of the lipid layers by the lytic agents.

It would appear then that the five phenothiazines are lytic at the concentration used in this study (5×10^{-4} *M*), since they all produced a significant increase in chromate leakage. Such a lytic effect was observed by Ahtee and Paasonen (8) with a series of phenothiazine drugs using erythrocytes, although these authors noted that at lower drug concentration, erythrocyte membrane stabilization occurred rather than lysis. Furthermore, irradiated chlorpromazine and prochlorperazine appear to be much more potent lytic agents than any of the nonirradiated compounds, suggesting the formation of new, more membrane-active species by the irradiation.

Thus, the results of this investigation suggest that if either of these latter two drugs accumulate in membranes of cells or cell organelles, even at levels below that required to produce lysis, irradiation could convert them to species with significant lytic activity. Such lytic species in turn could lead to increased cell membrane permeability and subsequent edema and inflammation. In contrast, the lytic potential of the other drugs studied would be expected to remain relatively unchanged after exposure to UV irradiation. Based on this finding, it appears that of these five phenothiazines, only chlorpromazine and prochlorperazine should produce direct photosensitized cutaneous reactions.

The available clinical data generally support this postulation. Both chlorpromazine and prochlorperazine are consistently reported to produce photosensitization (5). In addition, both also are capable of producing this effect in all exposed individuals if a sufficient drug concentration level is achieved. In contrast, based on the available clinical reports, triflupromazine and fluphenazine are essentially nonphotosensitizing (5, 6), and promazine has been reported as a photosensitizer in only one early study (7).

This preliminary study does indicate that lipid spherules are useful models to assess photosensitizing properties of the phenothiazine drugs. Studies with additional known and potential photosensitizers are currently underway to validate the usefulness of this model for other groups of compounds.

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Dermatitic Effect of Nonionic Surfactants IV: Phospholipid Composition of Normal and Surfactant-Treated Rabbit Skin

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Abstract □ Results of the authors' previous experiment indicated that the primary site of action of topically applied surfactants (polysorbate 85 and polyoxyethylene ether 96) is in the epidermal membranes. To elucidate the interaction of surfactants with biological membranes, the composition of epidermal phospholipids and the rate of biosynthesis of major phospholipid components were determined by utilizing thin-layer chromatographic, spectrophotometric, and radiotracer techniques. Results indicated that the major lipid components are cholesterol, lecithin, lysolecithin, phosphatidyl ethanolamine, and sphingomyelin. The treatment with surfactant did not induce any significant change in the phospholipid composition. The biosynthetic and turnover rates of all identified phospholipids, however, were greatly increased (two to four times) in the surfactant-treated skin. Available data suggest that the tested surfactants damaged the epidermal membranes. A role of surfactants in increasing the absorption of medicinal substances was also proposed in view of these results and other reports regarding the effect of surfactants on biological membranes.

Keyphrases □ Phospholipid composition, rabbits—normal, surfactant-treated skin □ Nonionic surfactants—dermatitic effect, rabbits □ TLC—analysis □ Scintillometry—analysis, ^{32}P-incorporation □ Spectrophotometry—analysis

In a multiphase system the molecules of a surface-active agent align and orient themselves at the interface. In a biological system the membranes provide the interface. The concentration of a surfactant in a tissue because of its hydrophilic–lipophilic character is the highest at, or in, the biological membranes; therefore, the site of action of a topically applied surfactant is very likely in the epidermal membranes.

Unfortunately, it is difficult to design experiments to test the action of surfactants on biological membranes. At present, the exact structural configuration of membranes is not defined because of the lack of reliable techniques to study membranes at cellular or molecular level. Recent reviews describe most of the presently accepted theories relating to the structure and function of biological membranes (1–5). Changes in membranes induced by surfactants or any other agents can be studied only by indirect methods. On the basis of the presently accepted concepts of biological membranes, a qualitative and/or quantitative change in lipid composition of a tissue may indicate structural changes and, consequently, functional changes in the membranes. Results of previous investigations (6–8) indicated that the treatment with surfactant preparations induced an increase both in the content and in the biosynthetic rate of epidermal phospholipids, nucleic acids, and acid-soluble material. This increase was explained by the assumption that the surfactants damaged the biological membranes by either rupturing the membranes or replacing certain phospholipid molecules in the continuous phospholipid micelles present in the membranes. Phospholipid molecules present in ruptured membranes and those that are possibly replaced by surfactants were measured along with newly formed molecules during the analysis of skin tissue.

The higher rate of biosynthesis of epidermal phospholipids was explained by the reasoning that it was expected in order to repair the surfactant-damaged membranes or to regenerate membranes. A further step in this project was to find out whether the surface-active agents interact with the membrane as a whole or with only certain components of the membrane. If the surfactants disrupt or damage the membrane as a result of hydrophobic or micellar interactions or by hydrogen bonding and a completely new membrane is regenerated, then the content and the rate of biosynthesis of all phospholipid membrane components will be increased. On the other hand, if the surfactant interacts with certain phospholipid molecules participating in the membrane, *i.e.*, a molecule can replace one phospholipid molecule present in the lipid micelles, one should find that the content and the rate of biosynthesis of those particular phospholipids would be increased more than that of other phospholipid components. The determination of phospholipid composition and the rate of biosynthesis of each phospholipid component in the control and sur-