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The effect of the photodecomposition of chlorpromazine on lecithin monolayers

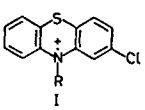
MAHA NEJMEH AND N. PILPEL*

Department of Pharmacy, Chelsea College, University of London, Manresa Road, London, SW3 6LX, U.K.

Chlorpromazine hydrochloride (cpz) is decomposed when irradiated with ultraviolet light, forming H^+, hydrochloric acid, chlorpromazine sulphoxide and 2-hydroxypromazine. The order of the reaction (as measured by the expansion that is produced in a lecithin/cpz monolayer) varies between zero and about $\frac{1}{4}$. The results have been used to explain some of the adverse side effects that are observed when patients are treated with cpz.

Patients who are being treated with chlorpromazine hydrochloride (cpz) for nausea, or more usually for psychotic disorders, frequently suffer adverse side effects. Low dosage of the drug may cause mild photosensitivity with the development, after an incubation period, of a form of dermatitis on parts of the skin that are exposed to sunlight (Zelickson & Zeller, 1964). Higher dosage and more prolonged treatment can produce severe dermatitis which is frequently accompanied by darkening of the skin due to the deposition of melanin in the lower layers of the dermis. Such patients may also suffer damage to the retina, ocular opacity and loss of vision (Satanove, 1965; Mathalon, 1965; Zelickson & Zeller, 1964).

Various authors (Epstein, Brunsting & others, 1957; Blois, 1965; Mitchell Sams, 1966) have ascribed these adverse effects to toxic products which appear to be produced when cpz HCl is irradiated with light and which can apparently react with constituents of the body such as tissue, skin and cell membranes. Zelickson & Zeller (1964) associated the effects with wavelengths above 320 nm, Satanove (1965) with wavelengths below 320 nm, Blois (1965) claimed that cpz formed free radicals (I) in white light which reacted with cellular constituents. Allison, Magnus & Young (1966) showed that one of the effects of such reactions was to increase the permeability of lysozomal and cell membranes to potassium, enzymes and cytoplasm.



I

A convenient *in vitro* method for studying the reaction between cpz and a body tissue under the influence of light is to spread a model cell membrane, e.g. lecithin as a monolayer on the surface of an aqueous solution of the drug and irradiate the system with light of appropriate wavelength (Davies & Rideal, 1963; Pilpel & Hunter, 1970). This technique was used by Felmeister & Schaubman (1968, 1969) who calculated so-called phototoxic indices for five different phenothiazine drugs. Although they assumed that the observed expansion of the monolayer was due to a polymerization process, they were unable to identify the products of the reaction.

In the present work measurements have been made of the changes that occurred in the force area π-A curves of lecithin spread on various aqueous substrates of cpz HCl when they were irradiated with light of two different wavelengths for various periods of time. The concentrations of cpz HCl in the substrates were selected to be similar to those that can be deduced to be present in the epidermal tissue of patients being treated with about 1000 mg of this drug per day (see Friedel, 1976).

The π-A data were corrected for the changes that occurred in the pH of the systems during irradiation and were then employed to establish the overall kinetics of the photochemical reactions between the lecithin and cpz HCl.

Finally the substrates were chemically analysed in order to identify the products of the reaction and possibly gain a better understanding of the cause of the adverse side reactions observed in patients being treated with this drug.

MATERIALS AND METHODS

Materials

The lecithin (L-α-dipalmitoyl phosphatidylcholine DpL) was 99·8% pure from Sigma laboratories, London. Chlorpromazine hydrochloride was puriss grade from May & Baker. Reference standards of

* Correspondence.

chlorpromazine sulphoxide and 2-hydroxypromazine were also obtained from May & Baker. The water used was triple distilled, surface tension 71·5 m Nm^{-1} at 21°, pH 5·8, conductivity 1·5 × 10^{-6} $ohms^{-1}$ cm^{-1} at at 21°. All other reagents were Analar grade.

Apparatus and procedures

The experimental work consisted of spreading monomolecular films of lecithin on dilute aqueous solutions of cpz HCl in a standard Langmuir trough (Davies & Rideal, 1963; Adamson, 1967) and measuring their force area π-A characteristics before and after irradiation with ultraviolet light.

Substrates which had been irradiated for 4 h were then analysed by physicochemical methods to establish the nature of the products of irradiation. (Attempts to analyse the films themselves proved unsuccessful).

The cpz HCl substrates were prepared by dissolving known weights of the material in triple distilled water (using an electromicrobalance for the weighings) and the lecithin was spread from an Agla syringe using 0·5 mg ml^{-1} solutions in 90:10% v/v hexane–ethanol.

The Langmuir trough was of glass with the edges and barriers lightly coated with paraffin wax. It stood in a darkened cabinet (to contain the ultraviolet irradiation) and the source of the light was a Raytech lamp (type S31-430) which was mounted 10 cm above the centre of the trough. The lamp was equipped with two filters, one transmitting between 250 and 320 nm with the peak at 290 nm, the other transmitting at longer wavelengths above 310 nm.

Having spread the monolayer and allowed it to equilibrate for 5 min, it was irradiated in the uncompressed state for the required length of time, and its π-A curve was then plotted using a compression rate of 3·5 × 10^{-2} nm^2 $molecule^{-1}$ min^{-1}, continuing the compression until the film collapsed. A fresh substrate was used for each replicate determination. Temperatures during the measurements were maintained at 20–22°.

Physico-chemical analysis of substrates which had been irradiated for 4 h was achieved by thin-layer chromatography, ultraviolet spectroscopy and mass spectrometry. Ammonium hydroxide was first added to 1 litre of the substrate to make it alkaline; this was then extracted with chloroform. The extracts were concentrated by bubbling nitrogen through them and were then spotted on t.l.c. plates coated with silica gel GF_{254}. After development the spots were eluted with ethanol, centrifuged, and their ultraviolet spectra recorded. The mass spectra of the products were determined with an electron impact mass spectrometer (type VG12F) using the direct probe of the instrument.

Additional experiments were carried out:

(a) to determine the effects of irradiation on the pH of various aqueous solutions of cpz HCl using a w.p.A. pH meter,

(b) to see how the changes in pH resulting from the irradiation of a cpz HCl substrate would effect the π-A characteristics of a lecithin monolayer that had been spread upon it. To this end π-A curves before irradiation were obtained for lecithin spread on cpz HCl substrates whose pH had been adjusted to between pH 5·7 and pH 2 with hydrochloric acid.

All glassware used in the work was carefully cleaned with chromic acid and all the solutions were freshly prepared.

RESULTS

The effects of both short and long wavelength ultraviolet irradiation on the pH of various chlorpromazine hydrochloride solutions in water are shown in Fig. 1. It is seen that the pH decreased with time, the effect being greatest with the short waves.

The effect of the irradiation on the π-A curves of lecithin spread on a representative substrate of cpz HCl is illustrated in Fig. 2 (similar results were produced by irradiating with ultraviolet of longer

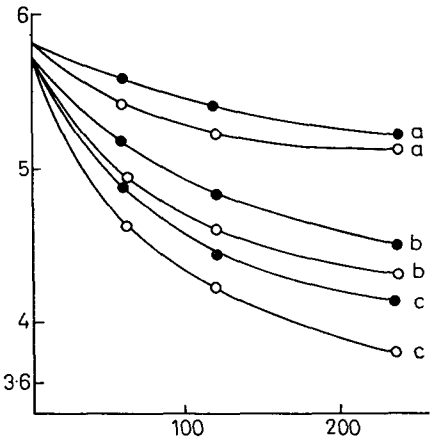


FIG. 1. Effect of ultraviolet irradiation on pH of cpz HCl solutions. ○ Short wave irradiation. ● long wave irradiation. (a) 10^{-5} M cpz HCl. (b) 10^{-4} M cpz HCl. (c) 5 × 10^{-4} M cpz HCl. Ordinate: pH. Abscissa: Irradiation time (min).

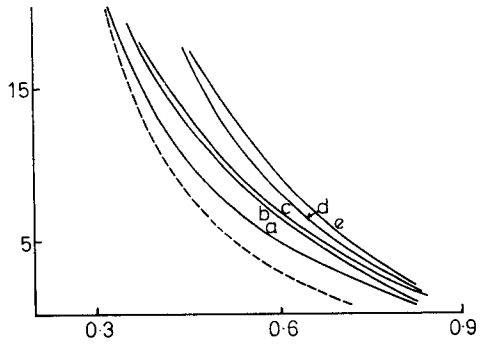


FIG. 2. Effect of short wave irradiation on DpL spread on 5×10^{-4} M cpz HCl. (a) before irradiation and after (b) 45, (c) 90, (d) 180 and (e) 270 min after irradiation. - - - - DpL on water substrate before and after irradiation. Ordinate: Surface pressure π mNm^{-1}. Abscissa: Area per molecule (nm^2).

wavelength). In all systems containing cpz HCl it was found that irradiation caused expansion of the films and a systematic decrease in their collapse pressure. (No effect was produced when the films were spread on pure water.) However, since the irradiation had caused a change to occur in the pH of the substrate, it was necessary to apply a correction to the π-A curves to allow for this pH effect.

This was done by making use of Fig. 3 which shows the π-A curves of lecithin before irradiation on a representative cpz HCl substrate at various pH values between 2 and 6 and Fig. 4 which shows how the area/molecule of lecithin, also before irradiation at an arbitrary surface pressure of 7 mN m^{-1}, varied with pH on three of the substrates.

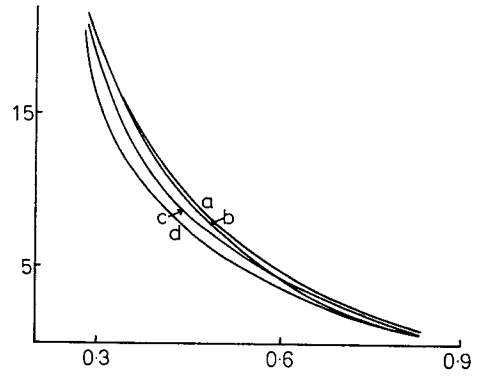


FIG. 3. Effect of pH on π-A curve of DpL spread on 10^{-5} M cpz HCl. pH (a) 4; (b) 4·9; (c) 5·7; (d) 2. Ordinate: Surface pressure (mNm^{-1}). Abscissa: Area per molecule (nm^2).

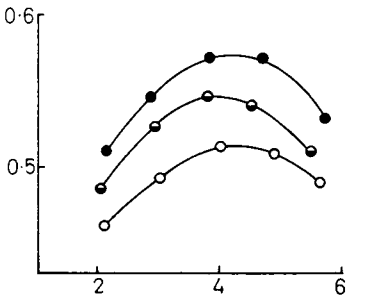


FIG. 4. pH *vs* area per molecule (taken at 7mN m^{-1}) of DpL spread on cpz HCl substrates. cpz HCl (M) ● 5×10^{-4}; ◒ 10^{-4}; ○ 10^{-5}. Ordinate: Area per molecule (nm^2). Abscissa: pH.

Corrected values of the area per molecule of the lecithin on these three substrates at their initial pH (5·5 to 6) at the pressure of 7 mN m^{-1} are plotted against irradiation time in Fig. 5 (short wave irradiation. Similar plots were obtained for long wave irradiation but the expansions produced in the films were then less).

Turning now to the results obtained by analysis of substrates from the lecithin/Cpz HCl systems after various periods of irradiation, Fig. 6 shows that the concentrations of cpz HCl in the substrates (measured by their ultraviolet absorption at 254 nm) decreased with time so that in 4 h between about 15 and 30% of the cpz HCl had been decomposed, the amount being inversely proportional to its initial concentration in solution and depending also on the wavelength of the light employed.

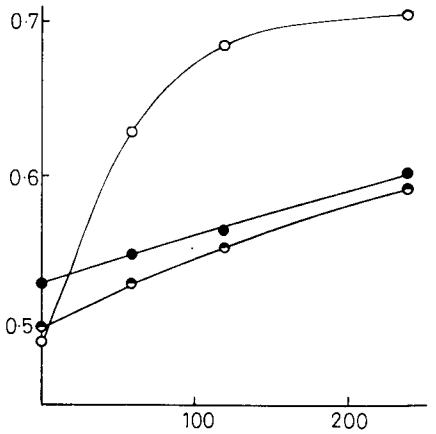


FIG. 5. Effect of short wave irradiation on area of DpL (at 7 mN m^{-1}) spread on cpz HCl substrates after correction to their initial pH. cpz HCl (M) ○ 10^{-5}; ◓ 10^{-4}; ● 5×10^{-4}. Ordinate: Area per DpL molecule (nm^2). Abscissa: Irradiation time (min).

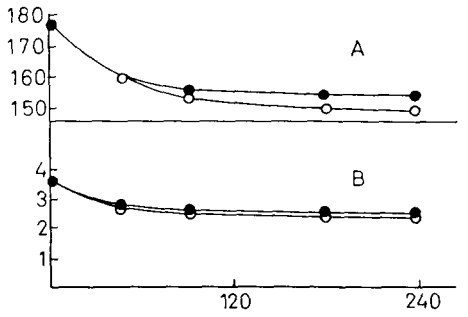


FIG. 6. Effect of ultraviolet irradiation on concentration of cpz HCl solutions.
A 5×10^{-4} M cpz HCl ○ short wave, ● long wave irradiation. B 10^{-5} M cpz HCl ○ short wave; ● long wave irradiation.
Ordinate: cpz HCl concentration (mg litre^{-1}). Abscissa: Irradiation time (min).

It is seen from Fig. 5 that the greatest changes in the area per molecule of the lecithin occurred with the lowest concentration of cpz HCl in the substrate and this could be partly due to the fact, noted above, that the percentage of cpz HCl decomposed was highest at the low concentration. Also at concentrations greater than about 5×10^{-4} M, cpz HCl increasingly adsorbs at the air/water interface, causing a decrease in the surface tension of water. Similar adsorption in the lecithin monolayer might be expected to cause changes in the packing arrangement of the molecules and their ion binding capacity and this could also contribute to the result obtained.

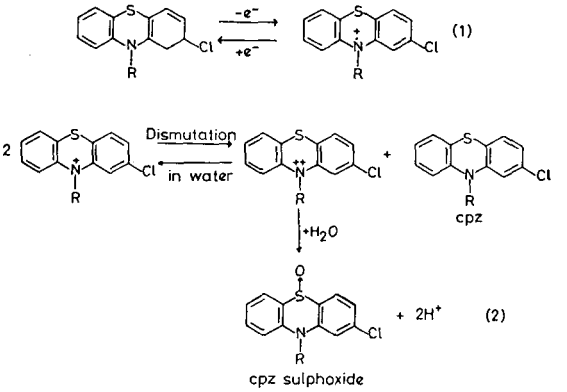
T.l.c. analysis of aliquots of substrates after 4 h irradiation revealed 3 spots when viewed with ultraviolet light at 254 nm. The R_F values of these spots using three different solvent systems are listed in Table 1. The spots were eluted and comparisons made of their R_F values and of their ultraviolet spectra with those of prepared standards. Spot 1 was identified as unchanged cpz HCl. Spot 2 (which was only a minor product) appeared to be chlorpromazine sulphoxide and Spot 3 to be 2 hydroxypromazine. The identity of the latter was confirmed by mass spectrometry.

Table 1. *Chromatographic results.*

Solvent system % by volume	R_F values Spot 1	Spot 2	Spot 3
Benzene–methanol–diethylamine 75 : 15 : 10	0·72	0·6	0·5
Chloroform–acetone–diethylamine 88 : 2 : 10	0·8	0·72	0·2
Benzene–ethanol–ammonia 80 : 20 : 1	0·5	0·31	0·2

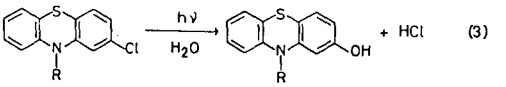
DISCUSSION

We consider first the chemical changes that ultraviolet irradiation had produced in the various systems. The decrease in pH noted in Fig. 1 and the formation of chlorpromazine sulphoxide have been observed by previous investigators (Felmeister & Discher, 1964; Huang & Sands, 1964, 1967). The sulphoxide is probably formed (Felmeister & Discher, 1964) by



Although the amounts detected in the present work were small, the liberation of H^+ could certainly contribute partially to the observed decreases in pH. Chlorpromazine sulphoxide is less surface active than the HCl (Zografi & Auslander, 1964, 1965) and in the amounts formed would not therefore be expected to contribute to the expansion that was observed when the lecithin films on cpz HCl were irradiated (Figs 2, 5).

Felmeister & Schaubman (1969) suggested that the expansion might be due to the production of dimers or polymers of cpz; but none could be detected in this work. Since in the absence of cpz the lecithin films appeared to be unaffected by ultraviolet light, it is concluded that the expansion was due primarily to the second reaction product, 2-hydroxypromazine, the presence of which in these irradiated systems was also noted by Grant (1974). The 2-hydroxypromazine could be formed as follows:



and the liberated HCl would again contribute to the decreases observed in the pH of the systems.

On this assumption one can now employ the data in Fig. 5 to determine the kinetic order of the overall reaction between cpz HCl and the lecithin monolayer.

This was done at an arbitrary surface pressure of 7 mN m^{-1} applying the differential method for the order of a reaction

$$\frac{dc}{dt} = -K_A C^{n_c}$$

where c is the reactant concentration; K_A is the reaction rate constant; t the irradiation time; and n_c the order with respect to concentration or true order.

Assuming that the change in area of the monolayer is proportional to the change in concentration of the reactant, then the order of the reaction n_c is given by the slope of the plot of $-\log dA/dt$ *vs* $-\log c$; dA/dt being the initial rate of expansion of the films.

The values were found to be about 1/2 under short wave and zero under long wave irradiation (Fig. 7).

If one uses the integration method and solves (by a process of trial and error) the general equation (Laidler, 1950),

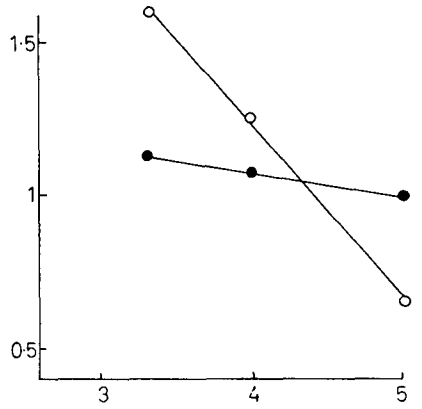


FIG. 7. —Log initial rate of expansion of DpL film (ordinate) on irradiation *vs* —log concentration of cpz HCl (abscissa) ○ short wave irradiation slope n_c = 0·56; ● long wave irradiation slope n_c = 0·079.

$$K = \frac{1}{t\,(n_t - 1)} \left[\frac{1}{(a\text{-}x)^{n_t - 1}} - \frac{1}{a^{n_t - 1}} \right]$$

in which n_t is the order with respect to time; K the reaction rate constant; a the increase of area/molecule (also taken at 7 mN m^{-1}) at the end of the reaction (taken after 4 h); x the increase in area/molecule at irradiation time t; one can obtain the order with respect to time n_t. The values were found to be 0·75 for all the systems. In the above treatment it is appreciated that several simultaneous reactions could be taking place in the monolayer. It would be extremely difficult to analyse each reaction separately. Nevertheless the present simplified treatment gives some indication that inhibition is occurring due to the products of the reaction. This can be deduced from the fact that n_t was found greater than n_c; it seems that the reaction slows down faster than it would do had it followed the true order (Laidler, 1950).

If it is assumed that at similar levels of concentration there is a connection between the photodecomposition of cpz HCl *in vitro* and *in vivo*, then one can use the present results to explain certain observations that have been reported in the literature.

Cpz HCl is seen to be decomposed by wavelengths both above and below 310 nm, the reaction being faster at the shorter wavelengths and more dependent on concentration (kinetic order n_c about $\frac{1}{2}$) than at the longer wavelengths (kinetic order n_c about zero). This agrees with the findings of Zelickson & Zeller (1964), Blois (1965) and Satanove (1965) that cutaneous side effects occur at both wavelength ranges.

There is, however, no general agreement in the literature on the nature of the photodecomposition products formed in the *in vivo* experiments, Epstein & others (1957), Forrest, Forrest & Berger (1958), Blois (1965).

The present finding that hydrogen ions and hydrochloric acid might be produced by reactions (2) and (3) could explain the sensitivity, inflammation or dermatitis that usually precedes the deposition of melanin (pigmentation) in the skin of patients being treated with large doses of this drug.

The other reaction product now detected, namely 2-hydroxypromazine, which appears to have been responsible for the expansion of the lecithin monolayer, might also be expected to produce the increase in the permeability of cell membranes that was reported by Allison & others (1966). Such changes in permeability could occur in the melanocytes of patients taking cpz HCl and accelerate the deposition of melanin in exposed areas of their skin (Van Woert, 1970).

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