PHOTOCHEMICAL STUDY OF THE INTERACTION OF PHENOTHIAZINE DERIVATIVES WITH SPIN LABELLED LECITHIN MULTIBILAYERS

François Leterrier and Roger Kersanté
Division de Biophysique du Centre de Recherches du Service de Santé des Armées, 1 bis rue du Lieutenant Raoul Batany 92140-Clamart, France.

Received January 15,1975

SUMMARY

Nitroxide free radicals are destroyed by ultraviolet irradiation in the presence of phenothiazine derivatives. This property has been used in order to determine the type of interaction of these drugs with spin-labelled lecithin multibilayers. Kinetic measurements of the spin label signal decay under irradiation have shown that chlorpromazine and perphenazine are preferentially located in the polar part of the bilayer, whereas promethazine and oxidized derivatives of chlorpromazine are found principally in the hydrophobic part.

Numerous works have shown that phenothiazine derivatives possess a high affinity for biological membranes (1,2). They induce modifications of the protein structure, as evidenced by the fluorescence and spin label methods (3,4,5,6). These results however, do not exclude the possibility that they interact with the membrane lipids (7). Such interactions have been demonstrated with alcohols and local anesthesies (8,9). In order to test this hypothesis, we have studied the effects of phenothiazine drugs on fatty acid spin labelled lecithin multibilayers which are a well known model system (10.11).

Ultraviolet irradiation of phenothiazine derivatives is known to yield free radicals (12). These reactive species destroy nitroxide free radicals probably by a reduction reaction. This property has been applied to the determination of the location of some phenothiazine drugs inside of the lecithin layers. The principle of the method is the following : nitroxide derivatives of fatty acids substituted at different positions along the chain are incorporated into lecithin multibilayers. The layers are irradiated with UV light in the presence of phenothiazine compounds and the nitroxide paramagnetic signal amplitude recorded as a function of time. Since the positions of the spin labels in the lipidic structure are well known, the measured kinetics give informations on the locations of the drugs in the multibilayer.

MATERIAL AND METHODS

1) Labelled lecithin multibilayers preparation : Pure egg yolk lecithin (Gift of Pr. Garry-Bobo) was dissolved in methanol (10 mg/ml). The spin

labels were added to this solution in a molar proportion of 1.4 %. Spin label I and III were N oxide oxazolidine derivatives of stearic acid substituted respectively in position 14 and 3 relatively to the terminal CH_3. They were purchased from Synvar. Spin label II was a palmitic acid derivative substituted in position 5 (Gift of Dr. Devaux). A drop of the lecithin solution (7 μ l) was deposited on a microscope cover slip, and was immediatly mixed with a drop (7 μ l) of water or of an aqueous solution of the phenothiazine to be studied. The mixture was allowed to evaporate at room temperature under a stream of nitrogen gas. The proportion of phenothiazine molecules in the lecithin layer was 0.8 % or 4 %.

2) Recording of electron spin resonance spectra : The cover slip was fixed at the extremity of a teflon bar and introduced in the cavity of a Varian E 3, ESR spectrometer. The slip could be oriented either parallel or perpendicular to the axis of the magnetic field.

3) Irradiation of the samples : The lecithin layers were irradiated inside of the cavity with an Osram HBO 150 w high pressure mercury lamp. Since all phenothiazines studied show an absorption maximum around 310-320 nm, light of wavelength lower than 300 nm was absorbed with a filter. A 10 cm focal length lens was used in order to focalisate the light beam on the frontal grid of the ESR cavity. A light flux of 1.5 10^5 erg $cm^{-2}s^{-1}$ was measured with an YSI Kettering model 65 radiometer.

4) Drug studied : The following phenothiazine derivatives have been studied : chlorpromazine (CPZ), perphenazine (PPZ), prometazine (PMZ), chlorpromazine sulfoxide (CPZ-SO) and chlorpromazine N oxide (CPZ-NO). All these compounds were a gift from Rhône-Poulenc and were used without further purification.

RESULTS AND DISCUSSION

1) ESR spectra of the lecithin bilayer before irradiation.

Figures 1a, 2a and 3a show the spectra obtained with each of the three spin labels in the absence of drug. As previously demonstrated (10,11), they indicate that the fatty acid chains are rigid and perpendicular to the plane of the film in its polar part, and that they become fluid and randomly oriented in the hydrophobic part. In presence of PPZ, the spectral modifications are not important (fig. 1b, 2b, 3b). With spin label I, the order parameter(S) (13) increases slightly (without drug $S_o = 0.82$; with 4% PPZ, $S_{PPZ} = 0.86$), but the resolution of the spectrum decreases. These result indicate that the drug inhibits partially the formation of the oriented multibilayers, but that the rigidity is enhanced in the area where they are well built.

With spin label III, the order parameter decreases slightly ($S_o = 0.21$, $S_{PPZ} = 0.19$) and the ratio of the amplitudes of the middle line to the high field line of the spectrum is increased (2.25 to 2.66). This result shows that the fatty acid chains are more disorganized but that the viscosity of the hydrophobic part of the film is decreased. No significative differences are observed with spin label II ($S_o = S_{PPZ} = 0,73$).

These observed spectral modifications demonstrate qualitatively

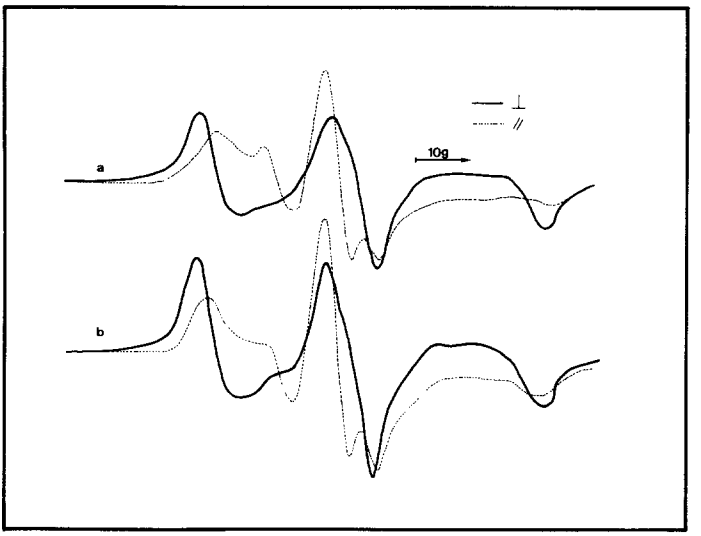

Figure 1 : ESR spectra of lecithin multibilayers spin labelled with I.
a) in absence of drug.
b) in presence of 4 % perphenazine.
Each spectrum was recorded with the plane of the lecithin film either parallel or perpendicular to the axis of the magnetic field.

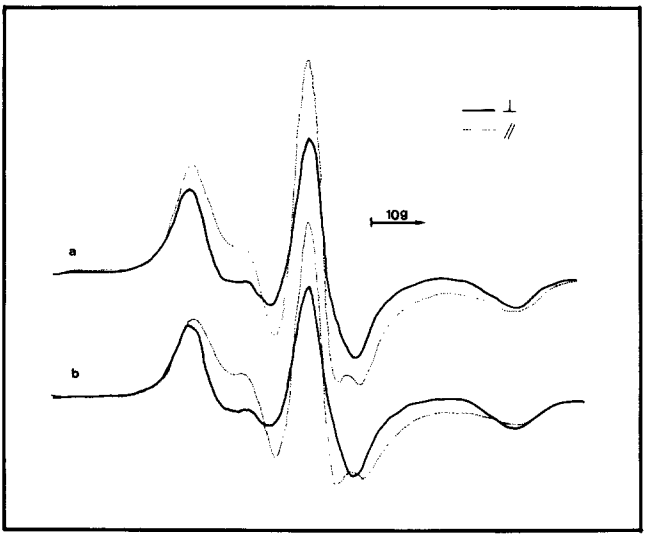

Figure 2 : As in figure 1, with spin label II.

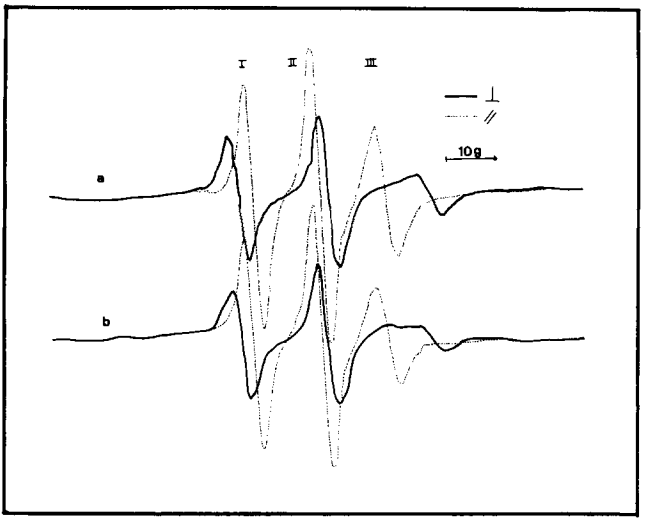

Figure 3 : As in figure 1, with spin label III.

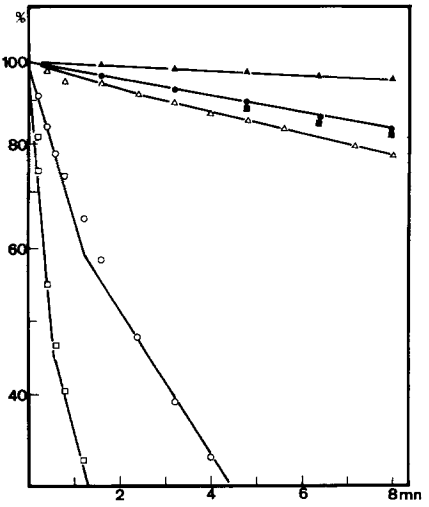

Figure 4 : Kinetics of the spin label amplitude decay as a function of UV irradiation time.
Black symbols : without drug,
White symbols : in presence of 4 % chlorpromazine.
□ spin label I, △ spin label II, ○ spin label III.

---

the interaction of phenothiazine derivatives with an organized lipidic structure, but they cannot indicate precisely the part of the bilayer with which the drugs interact preferentially. This information can be obtained with the photochemical measurements.

Drug studied		Control	CPZ		PPZ	PMZ		CPZ-SO		CPZ-NO
Molar fraction (%)		0	0.8	4.0	4.0	0.8	4.0	0.8	4.0	4.0
Spin label	I	23	277	1350	461	133	413	74	185	116
K(min^{-1}x10^{3})	II	5.8	60	63	26	11	32	13	31	13
	III	25	69	757	237	555	1282	208	370	625

TABLE I : Kinetic constants (K : min^{-1} x 10^{3}) of the spin label photochemical reduction in the presence of various phenothiazine derivatives.

2) Effect of UV irradiation.

Without drug, irradiation of the spin labelled lecithin film induces a slow decrease of the esr signal amplitude. In the presence of drug, the decay rate is highly increased (up to 60 times). Figure 4 shows the result obtained in presence of 4 % CPZ : kinetic is first order, at least in the first minutes of irradiation. In table I are given the kinetic constants measured with the different phenothiazine derivatives studied. In all cases, spin label II is the less sensitive. With CPZ or PPZ, spin label I is more rapidly destroyed than spin label III; on the other hand, with PMZ, CPZ-SO or CPZ-NO, spin label III is the most sensitive. At low concentration of drug (0.8 %), the kinetic constants are more difficult to measure, since the decay is first order only during a short time.

Parallel experiments in solution have shown that the three spin labels have the same photosensitivity in the presence of phenothiazine derivatives. The observed kinetic variations are thus no due to a difference in the chemical reactivity of the nitroxide used, but they must be correlated with the relative positions of the phenothiazine nucleus and of the spin label.

The kinetic measurements show that phenothiazine derivatives interact simultaneously with the hydrophobic and with the hydrophilic part of the lipi-

dic film, and that they are practically not present in the intermediate part. This observation is in good agreement with the spectral modification previously discussed.

The preferential location of these drugs varies with their chemical structure. PPZ and CPZ are found predominantly in the hydrophilic part of the bilayer. PMZ and the oxidized derivatives of CPZ, on the other hand, interact more strongly with the hydrophobic part. Furthermore, the differences between the kinetics constants measured with spin labels I and III are more important at low drug concentration : this fact suggests that the multibilayer becomes saturated when the drug concentration increases.

CONCLUSION

The association of the spectroscopic information given by the spin label, with the original photochemical properties of the phenothiazine nucleus has allowed us to precise the location of these drugs in a lipidic model system. This methodology can be applied for the study of natural membranes. We have previously shown (2,3,4) that chlorpromazine and perphenazine induce strong modifications in the proteic structure of isolated membranes (synaptosomes or erythrocytes ghosts), whereas prometazine and oxidized chlorpromazine derivatives show a week effect or even no action. It is interesting to rely these previous results with the fact that chlorpromazine and perphenazine seem to be preferentially located in the part of the bilayer which is itself probably in close interaction with membrane protein.

REFERENCES

1- Seeman, P., Staiman, A., Lee, T. and Chan-Wong, M. (1974). Adv. Biochem. Psychopharmacology, 9, 137-148.

2- Leterrier, F. and Thiery, C. (1974). Adv. Biochem. Psychopharmacology, 9, 77-96.

3- Leterrier, F., Canva, J., Mariaud, J.F. and Calloud, J.M. (1972). C.R. Acad. Sc. Paris, 274. 2094-2096, Série D.

4- Leterrier, F., Rieger, F. and Mariaud, J.F. (1973), J. Pharmacol. Exp. Ther, 186, 609-615.

5- Leterrier, F., Rieger, F. and Mariaud, J.F. (1974). Biochem. Pharm, 23, 103-113.

6- Manian, A.A., Piette, L.H., Holland, D., Groven, T. and Leterrier, F.(1974). Adv. Biochem. Psychopharmacology, 9, 149-161.

7- Zografi, G. and Auslander, D.E. (1965). J. Pharm. Sci., 54, 1313-1318.

8- Hubbel, W.L., Metcalfe., S.M. and Mc. Connell, H.M. (1970). Biochim. Biophys. Acta, 219, 415-427.

9- Butler, K.W., Schneider, H. and Smith, I.C.P. (1973). Arch. Biochem. Biophys. 154, 548-554.

10- Smith, I.C.P. (1971). Chimia, 25, 349-380.

11- Jost. P.. Libertini, L.J., Herbert, V.C. and Griffith, O.H. (1971). J. Mol. Biol. 59, 77-98.

12- Felmeister, A. and Discher, C.A. (1964). J. Pharm. Sci. 53, 756.

13- Hubbell, W.L. and Mc.Connell, H.M. (1971). J. Am. Chem. Soc, 93, 314-326.