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Proton Magnetic Resonance Study of the Interaction of Chlorpromazine Hydrochloride with Lecithin Vesicles

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The interaction of chlorpromazine hydrochloride with lecithin vesicles was investigated through proton magnetic resonance measurements. Sharp $N(CH_3)_2$ signal of the chlorpromazine hydrochloride in D_2O was markedly broadened when the drug was added to a lecithin vesicle-D_2O solution, whereas the broadening was not observed in a dextran-D_2O solution of which viscosity was higher than the vesicle solution. Addition of Eu^{3+} to the vesicle solution induced an upfield shift of outward facing choline methyl signal, but the subsequent addition of the chlorpromazine hydrochloride reversed the induced shift. The vesicles prepared in the presence of Mn^{2+} did not show the PMR signal of choline methyl due to the presence of Mn^{2+} both inside and outside of the vesicles, but when the drug was added to the solution, choline methyl signal appeared again. The results confirmed that the chlorpromazine interacted with polar part of the lecithin vesicles displacing the trivalent ion Eu^{3+} and the divalent ion Mn^{2+} from the vesicle surfaces.

Keywords——chlorpromazine hydrochloride; lecithin vesicles; drug interaction with lipids; shift reagent; PMR study of lecithin vesicles

Lecithin vesicles prepared by ultrasonication of lecithin–water dispersions have been adopted as biological membrane models in various studies. Since proton magnetic resonance (PMR) spectrum of the lecithin vesicles in D_2O gives relatively well resolved signals[2], that is, a strong sharp signal of the choline methyl protons, an intense but rather broad peak of the chain methylenes, and a weak peak due to the terminal methyls (Fig. 1a), the analysis of PMR spectra of mixed-solution of drug and lecithin vesicles could provide some basic informations as to the interaction of the drug with the biological membrane.[3]

In this paper, we report a PMR spectroscopic investigation about the interaction of chlorpromazine with lecithin vesicles. Balzer, *et al.*[4] reported that chlorpromazine inhibited the calcium transport of the sarcoplasmic vesicles isolated from rabbit skeletal muscle. Further they found that chlorpromazine was bound to the lipids of the membranes of the sarcoplasmic reticulm by measuring the radioactivity of the supernatant of the centrifuged lipid suspensions containing labeled chlorpromazine^{35}S.[5] Fujii, *et al.*[6] reported that chlorpromazine induced shape transformation of human erythrocyte membrane. Leterrir, *et al.*[7] measured the signal decay of electron spin resonance (ESR) of spin labeled lecithin multibilayers in the presence of chlorpromazine under ultraviolet irradiation and concluded that the chlorpromazine located in the polar part of the bilayer.

It is known that the vesicles prepared from egg yolk lecithin are impermeable to ions.[8] This property has been applied in PMR spectroscopy to distinguish between internal and

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external surfaces of vesicles using paramagnetic ions such as Eu^{3+} and Mn^{2+}.[9] An addition of Eu^{3+} to the vesicle solution induces an upfield shift of the signal arising from outward facing choline methyl so that the choline methyl signal splits into two peaks signifying outward facing (external) and inward facing (internal). Mn^{2+} is used because of its ability of signal broadening. On reference to the ESR results of Leterrir, *et al.*[7] mentioned above, it would be expected that the PMR experiments employing the paramagnetic ions[10] would directly and precisely elucidate the interaction of chlorpromazine with lecithin vesicles.

Experimental

Lecithin——Egg yolk lecithin purchased from E. Merk was purified by column chromatography on alumina and silic acid, and the purity was monitored by thin-layer chromatography until it gave a singl spot. The purified lecithin was stored in the form of 5% (w/v) chloroform solution.

Preparation of Vesicle Solution——4.0 ml of the lecithin-chloroform solution was placed in a test tube and dried up by passing a stream of N_2 gas and the residue was stored under high vacuum for several hours. Subsequently 4.0 ml of D_2O was added to the dried lecithin so that the concentration of the lecithin vesicle solution would be made up to 5% (w/v). Then the lecithin-D_2O mixture was shaken until it became white milky dispersion. Lecithin vesicles were formed when the dispersion was ultrasonicated by Kaijo Denki Sonicater Model T-A-4201 (Tokyo). During the sonication the test tube was placed in an ice-water bath. 2 min of the sonication was repeated with 1 min of interval. The net sonication time was 10—15 min.

PMR Measurements——PMR spectra were measured by Varian A-60 spectrometer. Prove temperature was within the range of 35—38° and sufficient time was allowed for the sample to come into thermal equilibrium before the measurements were initiated. The pD value[11] of the vesicle solution was *ca.* 5.4, and after addition of chlorpromazine-HCl it decreased to *ca.* 4.9. All measurements were therefore carried out under these weakly acidic conditions.

Results and Discussion

PMR spectrum of the lecithin vesicles in D_2O is illustrated in Fig. 1a, in which a sharp choline methyl signal and broad methyl and methylene signals of fatty acid chains were observed. As already mentioned, on addition of Eu^{3+} to the vesicle solution the internal and external choline methyl signals were separated as shown in Fig. 1b.

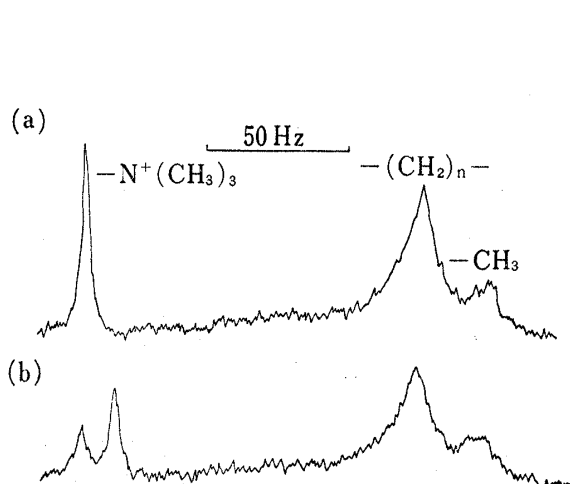

Fig. 1. PMR Spectra of 5% (w/v) Lecithin Vesicles
(a) in D_2O; (b) after addition of Eu^{3+} (9.6 mM) to (a).

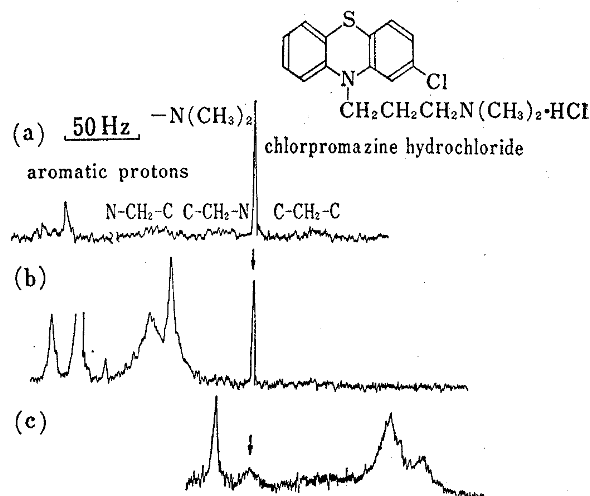

Fig. 2. PMR Spectra of 28 mM Chlorpromazine-HCl
(a) in D_2O; (b) in 5% (w/v) dextran-D_2O solution; (c) in 5% (w/v) lecithin vesicles-D_2O solution.

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On the other hand, PMR spectrum of chlorpromazine-HCl in D_2O did not show highly resolved signals except $N(CH_3)_2$ protons as observed in Fig. 2a. This may be partly because of signal splittings by spin-spin coupling and also partly of poor signal to noise ratio due to the low concentration of this experimental condition (28 mM), while $N(CH_3)_2$ signal was sharp and strong enough to be monitored. When chlorpromazine–HCl was added to the vesicle solution, the signals from the vesicles exhibited no change, but $N(CH_3)_2$ signal of chlorpromazine showed an extreme broadening (Fig. 2c). Since this signal broadening might have been attributed to viscosity of the vesicle solution, a 5% (w/v) dextran–D_2O solution having fairly the same viscosity as the sample was examined to certify the viscosity effect. The relative viscosity of the vesicle solution was 1.2 and that of the dextran solution was 1.6 at 38°. Fig. 2b shows the spectrum of chlorpromazine–HCl dissolved in the dextran solution. No significant broadening of the $N(CH_3)_2$ signal was observed from the dextran solution even though its viscosity was higher than that of the vesicle solution. This result confirmed that the broadening was essentialy caused by the interaction of the chlorpromazine with the lecithin vesicles.

Further investigation was carried out with the aid of paramagnetic ions Eu^{3+} and Mn^{2+}. As already seen in Fig. 1b, the PMR spectrum of the lecithin vesicle solution containing Eu^{3+} in bulk aqueous phase showed two separate choline methyl signals. When the chlorpromazine–HCl was added stepwise to this solution, the internal choline methyl signal at the lower field did not show any change, but the external choline methyl signal progressively shifted downfield (methylene signal of the vesicles was taken as an internal reference) toward the position of the internal choline methyl signal. These observations are illustrated in Fig. 3. This behavior indicated that the chlorpromazine diffused to the vesicle surfaces and interacted with polar part of the lecithin displacing the Eu^{3+} ions from the vesicle surfaces.

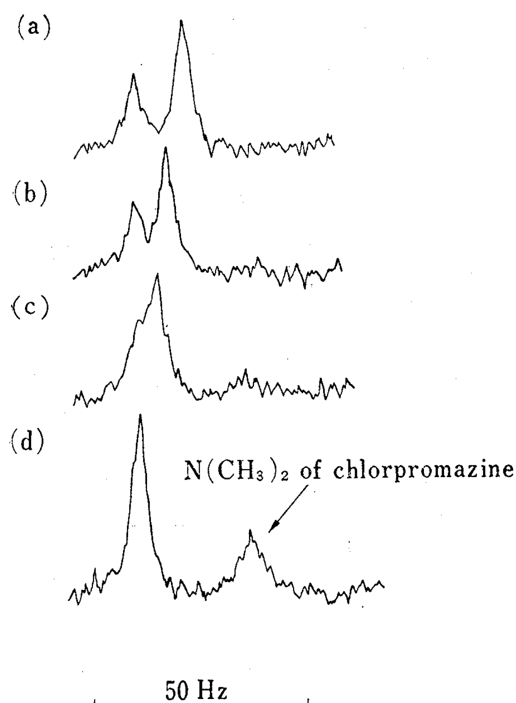

Fig. 3. Effect of Chlorpromazine–HCl on PMR Signal of Choline Methyl of 5% (w/v) Lecithin Vesicle Solution containing 9.6 mM Eu^{3+}

Chlorpromazine–HCl: (a) control; (b) 4.9 mM; (c) 11 mM; (d) 31 mM.

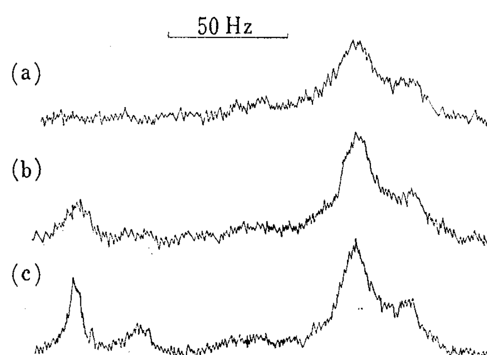

Fig. 4. Effect of Chlorpromazine–HCl on PMR Signal of Choline Methyl of 5% (w/v) Lecithin Vesicles prepared in D_2O containing 10 mM Mn^{2+}

Chlorpromazine–HCl: (a) control; (b) 11 mM; (c) 34 mM.

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Similar result was obtained using Mn^{2+} but with a modified method. When lecithin was sonicated in D_2O containing Mn^{2+}, Mn^{2+} ion was enclosed in cavities of the vesicles and also existed in the bulk aqueous phase, so that both of the inward and outward facing choline methyl signals were broadened to disappear (Fig. 4a) by the paramagnetic effect of the Mn^{2+} ion. But when chlorpromazine–HCl was added to this sample, the choline methyl signal appeared again as seen in Fig. 4b and c. This also showed that the chlorpromazine displaced the Mn^{2+} ion from the vesicle surfaces as in the case of Eu^{3+} ion.

From the PMR observations, we have concluded that the chlorpromazine interacts with lecithin vesicles at the polar part of the vesicle surfaces and displaces the trivalent cation Eu^{3+} and the divalent cation Mn^{2+} from there, that is, chlorpromazine prevents the cations from contacting to the membrane surfaces.

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