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Probing Ciprofloxacin - Lipid Interaction by ATR-FTIR and ^{31}P NMR Spectroscopy

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Abstract

The interactions between the fluoroquinolone ciprofloxacin (CIP) and lipids have been described in the literature. We previously showed that the ability of CIP to induce disorder and modify the orientation of the acyl chains is related to its propensity to be expelled from a monolayer upon compression (*Bensikaddour et al., BiophysicJ, 2008 114843)*. Here, we investigated CIP effects on the transition temperature (Tm) of lipids and on the mobility of phosphate head groups using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) and ^{31}P Nuclear Magnetic Resonance (NMR). The selected phospholipids were DPPC and DPPG. ATR-FTIR experiments showed that CIP had no effect on the Tm of DPPC but increased the order of the acyl chains both below and above this temperature. In contrast, with DPPG, CIP induced a marked broadening effect on the transition with a decrease of the acyl chain order below its Tm and an increase above this temperature. Furthermore, ^{31}P NMR data showed that CIP bound to lipid model membranes and decreased the mobility of phospholipid head groups. As compared to the control samples, the chemical shift anisotropy (Δσ) values of DPPC:CIP (1:1, M:M) and DPPG:CIP (1:1, M:M) were respectively 5 and 9 ppm higher. Altogether, these data have demonstrated that the interactions of CIP with lipids depend markedly on the nature of their phosphate head groups and that the major effects of CIP on the bacterial membranes should be related to interaction with anionic lipid compounds.

Materials and methods

a) Materials

Ciprofloxacin (CIP) antibiotic was provided by Bayer A.G. (Leverkusen, Germany) . DPPC and DPPG lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA) Fig.1. Multilamellar Vesicles (MLVs) and Small Unilamellar Vesicles (SUVs) were prepared in Tris buffer 10mM, pH7.4 as described by Van Bambeke *et al*, (1993).

b) Methods

Infrared spectroscopy (ATR-FTIR). IR Spectra were obtained on a Bruker IFS55 FTIR spectrophotometer (Ettlingen, Germany) purged with N_2 as described previously (Fa *et al*., 2006). 15µl of SUV (50mg/ml) containing DPPC or DPPG were dried under a stream of nitrogen on one side of the germanium internal reflection element using an incident angle of 45° at 20°C. The germanium crystal was placed in an ATR holder for liquid sample, containing ciprofloxacin with molar ratio of one, with an in- and out-let (Harrick, Ossining, NY, USA). Temperature was controlled with temperature-regulated water flowing in a cavity of the cell. Peak positions between 3000 and 2800 cm^{-1} were determined ($\nu_{as}CH_2$ and ν_sCH_2). The software used for data processing was written under MatLab 7.0 (Mathworks Inc., Natick, MA, USA).

^{31}P Nuclear Magnetic Resonance (NMR). The effect of ciprofloxacin on the mobility of phosphate head groups of DPPC and DPPG prepared as MLV was investigated by static ^{31}P NMR experiments. The chemical shift anisotropy (Δσ) depends on the motions of the phosphodiester moiety and can be measured on the spectra by taking the difference of chemical shift between the low field shoulder (σ//) and the high field peak ($\sigma_\perp$): $\Delta\sigma = \sigma_{//} - \sigma_\perp$ (Seelig, 1978). Control samples of 450 µl were prepared from MLV suspension (50mg/ml), in 5mm outer diameter tubes, by adding 50µl of D_2O for locking on the deuterium signal. To investigate the effect of the Ciprofloxacin on the mobility of lipids (DPPC and DPPG) head groups, a defined concentration of drug was added to MLV liposomes to reach a molar ratio of one. Broadband proton-decoupled ^{31}P NMR spectra were acquired by 1D NMR methods on a Bruker AVANCE 500 spectrometer at 202.5MHz. Typical Fourier transform parameters were: 45° (6µs) flip angle, 50 kHz spectral width, and 0.6s repetition times. Sixty thousands transients were accumulated. The chemical shifts were referenced to the isotropic chemical shift of H_3PO_4. All spectra were recorded at constant temperature of 45°.

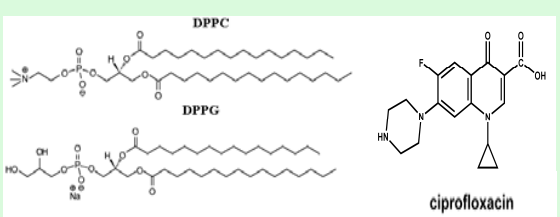

Figure 1 . Chemical structure of lipids in (a) Ciprofloxacin in (b) used in this study

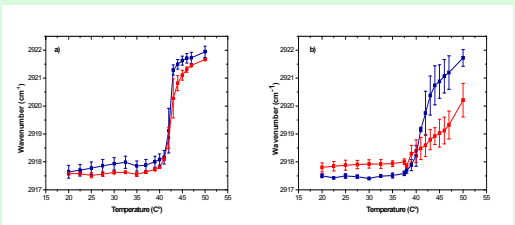

Figure 2. Ciprofloxacin effect on the melting curve of DPPC and DPPG

Evolution of the position of the maximum frequency of the $\nu_{as}(CH_2)$ as a function of temperature for DPPC (a) or DPPG (b) in the absence (bleu square ■) or in the presence of ciprofloxacin (red square ■). Concentration of phospholipids was 50mg/ml. The lipid: ciprofloxacin molar ratio is 1:1

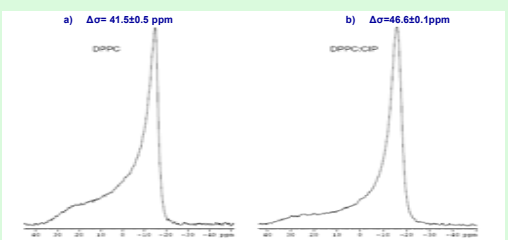

Figure 3. Effect of ciprofloxacin on ^{31}P NMR spectra of large multilamellar vesicles of DPPC

Liposomes (50 mg/ml) were prepared at pH7.4 in 10 mM Tris buffer in the absence (a) and the presence of ciprofloxacin at molar ratio of one (b). The chemical shift (Δσ) values are indicated at the top of spectra.

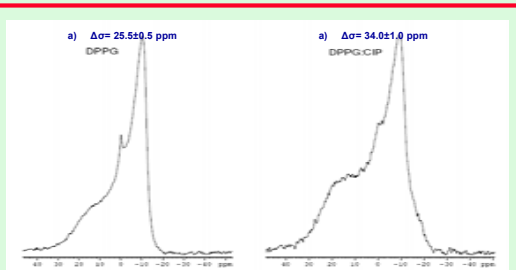

Figure 4. Effect of ciprofloxacin on ^{31}P NMR spectra of large multilamellar vesicles of DPPG

Liposomes (50 mg/ml) were prepared at pH7.4 in 10 mM Tris buffer in the absence (a) and the presence of ciprofloxacin at molar ratio of one (b). The chemical shift (Δσ) values are indicated at the top of spectra.

Results

1- Ciprofloxacine effect on the melting temperature of DPPC and DPPG

To get information on the effect of ciprofloxacin on the melting temperature of DPPC and DPPG, the wavenumber position at asymmetric and symmetric methylene-stretching band $\nu as(CH_2)$, $\nu s(CH_2)$ of DPPC (3100-2800 cm^{-1}) was plotted as a function of the temperature in the absence and the presence of the drugs (CIP) (Fig 2). Pure DPPC and DPPG vesicles (in Tris 10mM, pH 7.4 buffer) exhibit a clear phase transition between a gel phase (L_β) and a liquid crystalline phase (L_α). As expected, the DPPG melting temperature is slightly lower than for DPPC (42°C) and is near (40°C).

The melting curve obtained for DPPC in the presence of ciprofloxacin (Fig. 2a), showed a minor effect. The position of the two dominant bands observed near 2917 and 2849 cm^{-1} (not shown) were slightly below those observed for pure DPPC. The melting temperature remained unchanged (42°C). In contrast, addition of ciprofloxacin to anionic lipid DPPG vesicles induced a significant change in the melting curve (Fig. 2b). A positive shift of $\nu asCH_2$ stretching bands was observed at pre-transition temperatures (between 20-37.5 °C). With further increased in temperature (> 40°C), the peak positions for the C-H stretching band remained significantly below the value found for pure DPPG. These data suggest that ciprofloxacin increased the membrane fluidity, in the gel phase (L_β) and decreased the membrane fluidity in the liquid crystalline phase (L_α).

2- Effect of Ciprofloxacin on the mobility of phosphate heads of DPPC and DPPG

From binding parameters, the binding affinity is strongly dependent on negatively charge phospholipids **(Bensikaddour *et al*., 2007, EBC, Poster 54).** These data led us to suggest that the positive charge microspecies of ciprofloxacin interact preferentially with the negatively charged phospholipid heads group. To investigate this hypothesis we measured by ^{31}P NMR spectroscopy the effective chemical shift anisotropy (Δσ) of the MLV liposomes (DPPC and DPPG) in the presence and the absence of ciprofloxacin at post transition temperature (T=45°C) for both of lipids. As shown in Figure 3 and 4, typical spectra were obtained with multilamellar vesicles for DPPC and DPPG, where signal shapes are characteristic of a bilayer organization with a high-field maximum and a low field shoulder. However, the spectrum of ^{31}P NMR for DPPG (Fig.4) showed an additional peak at 0 ppm. The latter is due to a small population of 100 nm of size as revealed by Nanosizer measurement (data not shown).

The chemical shifts anisotropy (Δσ) values for DPPC and DPPG were 41.5±0.5 and 25.5±0.5 ppm respectively. In the presence of ciprofloxacin, the Δσ value was higher than for control liposome spectra. Indeed, the chemical shift anisotropy (Δσ) values of DPPC: CIP (1:1, M:M) and DPPG:CIP (1:1, M:M) were respectively 5 and 9 ppm higher.

Conclusion

This work supports the view that electrostatic interactions play a major role in the ciprofloxacin –lipid interaction. In fact, the ^{31}P NMR spectra clearly showed evidence of an effect of ciprofloxacin on the negative charged liposomes. In this respect, the Δσ increase for DPPG: CIP (1:1, M: M) vs DPPC: CIP (1:1, M:M) results mainly from the decrease of the fluidity of DPPG at post transition temperature as revealed by ATR-FTIR data.

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References :

1. Bensikaddour H *et al*., (2008): *BiophysicJ* 114843
2. Fa N., *et al.* (2006): *Chem.Phys.Lip.* **144: 108-116.**
3. Van Bambeke F., *et al* (1993): *Eur. J.Pharmacol.* **247**:155-168

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