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Interactions of Fluoroquinolone antibiotics with phospholipids: modification of their physicochemical properties

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Abstract

Probing drug/lipid interactions at the molecular level represents an important challenge in pharmaceutical research and membrane biophysics. Novel insights on these interactions are reported in this study by using steady-state anisotropy fluorescence and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). In this study, two fluoroquinolones have been compared, ciprofloxacin (CIP) and moxifloxacine (MXF). The selected phospholipids were DPPG, DPPC or phospholipids mixtures (DOPC: DPPC), and (DOPC: DPPG). Our results showed that all lipids vesicles tested bound to the CIP antibiotic with a stoichiometry of one (1:1). The binding constants Kapp were in the order of $10^5 M^{-1}$ and the affinity appeared dependent on the negative charge. CIP bound to lipids vesicles with the following preference: DPPG > DOPC: DPPG (1:1) > DOPC: DPPC (1:1). No variation of anisotropy signal was detected in presence of MXF. ATR-FTIR experiments showed that MXF decreased more the all-trans configuration than CIP. However, the surface of the wagging CH_2 of DPPC decreased more in the presence of CIP than MXF. Furthermore, the frequencies of CH_2 stretching vibrations of DPPC as a function of temperature indicated that melting temperature decreases only in the presence of CIP. These data demonstrated that the interactions of CIP and MXF with lipids are different since CIP increases DPPC disorder and decreases the lipid melting temperature, while MXF aligns phospholipids sn_2 chains and diffuse trough the membrane. Finally, this work highlights the biophysical applications of ATR-FTIR and anisotropy fluorescence to investigate lipids-drug interactions.

Materials and methods

a) Materials

Ciprofloxacin (CIP) and Moxifloxacin (MXF) antibiotics were provided by Bayer A.G. (Leverkusen, Germany). DOPC, DPPC and DPPG lipids were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Large Unilamellar Vesicles (LUVs) were prepared as described by Van Bambeke *et al*, (1993).

b) Methods

Steady-State Anisotropy Fluorescence. Anisotropy titrations were performed with an LS55 (Perkin-Elmer Ltd., Beaconsfield, UK) in the T-format at 25 °C, by adding increasing concentrations of liposomes to a fixed amount of antibiotic (5µM) in buffer (10 mM Tris, 100 mM NaCl pH 7.4).

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). The sample of 15µl containing DPPC with different molar ratio of antibiotic (CIP or MXF) was 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 software used for data processing was written under MatLab 7.0 (Mathworks Inc., Natick, MA, USA).

i) Conformation and orientation of DPPC. Non-polarized spectra were recorded in order to analyze the conformation of lipids. However, to get information on the orientation of lipids, dichroic spectra of the complex (DPPC:ATB) obtained by substracting the perpendicular polarized spectra from parallel-polarized ones, were analyzed.

ii) *Melting temperature.* The germanium crystal was placed in an ATR holder for liquid sample with an in- and out-let (Harrick,Ossining, NY, USA) for study drug effect on the melting temperature of DPPC. Temperature was controlled with temperature-regulated water flowing in a cavity of the cell. Peak positions between 3000 and 2800 cm^{-1} were determined and characterize the stretching vibration of the νCH_2 and νCH_3.

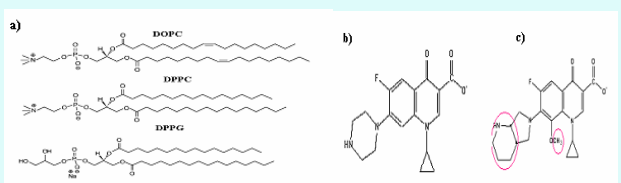

Figure 1 . Chemical structure of lipids in (a) Ciprofloxacin in (b) and Moxifloxacinin (c) used in this study

Results

1-Binding parameters of Fluoroquinolones to lipids vesicles

To investigate the effect of FQs on the membrane model (liposomes), we evaluated the binding of the antibiotic to the different LUVs liposomes. First, we performed anisotropy titration of CIP antibiotic by liposomes in the absence of salts. Stoichiometry of binding was deduced from the intersection of the initial slope with the plateau (Fig. 2(a) insert). It was found to be one, whatever the nature of lipids. Second, the binding constants, *Kapp,* were determined in the presence of salt, and found to be close to the binding constant value of (10^5 M^{-1}) see table 1. As compared to DPPG liposomes, the decrease in the concentration of negative charge compounds in the liposomes mixture DOPC: DPPG (1:1 M: M), led to a 2.5 fold *Kapp* value decrease. This suggests that CIP may bind to lipids by an electrostatic interaction. This has been confirmed using neutral liposomes (DOPC:DPPC) that significantly altered the K*app* values, too. However the binding parameters of MXF to lipids were not determined since no variation of anisotropy signal was detected even in the presence of DPPG (Figure 2 (b)).

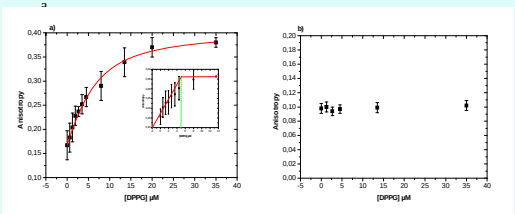

Figure 2. Binding curve of CIP and MXF to DPPG vesicles

Binding of CIP in (a) and MXF in (b) to DPPG vesicles was followed by steady state anisotropy fluoresence. The antibiotic concentration was 5 uM in 10mM Tris, 100 mM NaCl at pH7.4. Continuous lines (a) corresponds to the fit of the experimental point in order to determine binding constant. The inset shows the dependence of anisotropy intensity of ciprofloxacin on the liposomes concentration. The intersection indicates that the saturating liposomes: CIP ratio is 1. No variation of anisotropy signal was observed for Moxifloxacin (b).

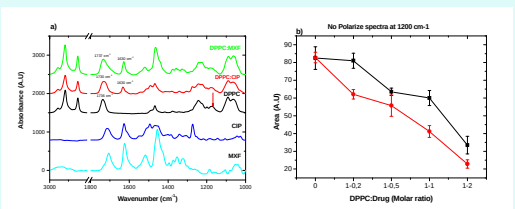

Figure 3. Fluoroquinolones effect on the conformation of DPPC monolayer membrane as revelled by the infrared absorbance spectrum

a) Non polarized spectrum of drug, DPPC and DPPC:Drug with molar ratio of 1:1 were shown. Wavenumber scale was broken from2800-1800cm-1. In (b) Integration peak of non polarized spectrum of DPPC at wagging ν_w CH_2 band (between 1206 and 1193 cm^{-1}) was plotted versus DPPC: Drug molar ratio as indicated; in the presence of CIP square symbol (■) and MXF circle symbol (●).

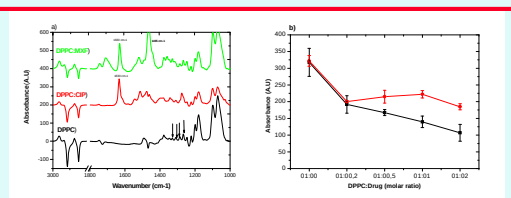

Figure4. Fluoroquinolones effect on the orientation of DPPC monolayer membrane as revelled by dichroic spectrum of ATR-FTIR

The membrane deposed on the germanium plate was prepared from the SUVs liposomes, dried under N_2 flow at 25°C. In(a) Drug molar ratio was 1:1. Dichroic spectrum= $A_{//}$ - $A_{\perp}$, parallel (//) and perpendicular ($\perp$) polarized incident light. Wagging νCH_2 band showed by arrows. In b) Integration peak of dichroic spectrum of DPPC at wagging w CH_2 band (between 1206 and 1193 cm^{-1}) was plotted versus DPPC: Drug molar ratio as indicated; in the presence of CIP square symbol (■) and MXF circle symbol (●).

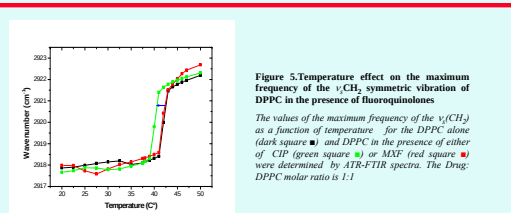

Figure 5.Temperature effect on the maximum frequency of the $\nu_s CH_2$ symmetric vibration of DPPC in the presence of fluoroquinolones

The values of the maximum frequency of the $\nu_s(CH_2)$ as a function of temperature for the DPPC alone (dark square ■) and DPPC in the presence of either of CIP (green square ■) or MXF (red square ■) were determined by ATR-FTIR spectra. The Drug: DPPC molar ratio is 1:1

2-Conformation and orientation of DPPC in the presence of Fluoroquinolones

FTIR analysis provides valuable information regarding the spatial disposition of the drug (CIP, MXF) in relation to the lipid bilayer. First, conformation of the acyl lipid chains was determined using non polarized spectra (Fig. 3a). We provide evidence that MXF decreased more the area of wagging peak at 1200 cm^{-1} than CIP (Fig. 3b). This indicates that the all-trans configuration of the alkyl chain of DPPC decreased more in the presence of MXF.

To get information on molecular orientation of DPPC in the absence or in the presence of the drug, we analyzed dichroic spectra (Fig. 4).

As shown in the figure (Fig.4a), many peaks of drug spectra appeared in the dichroic spectrum of DPPC, notably, at the frequency of 1630 and 1486 cm^{-1} (compare the red and green spectrum with the black one), suggesting a well-organized, well-defined orientation of the drug in the DPPC bilayer. Moreover, the area evolution of the wagging band $\nu_w(CH_2)$ of DPPC as a function of lipid:Drug molar ratio (Fig 4(b)), indicated a decrease of the area at least 60% for CIP and 30% for MXF. This data was in agreement with the angle between the acyl chain of DPPC and a normal at the germanium surface, witch was greater (27°) in the presence of CIP and remained unchanged (20°) in the presence of MXF.

3- Fluoroquinolones effect on the melting temperature of DPPC

To get information on the effect of FQs on the melting temperature of DPPC, 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 or MXF) (Fig.5). The frequencies of the two dominant bands observed at 2850 and 2918 cm^{-1} remained essentially unchanged as the temperature is increased from 20 to 35°C. At temperature between 37 and 42 °C, the two bands centered at 2851 and 2920 cm^{-1}. The melting temperature of DPPC (42C°) was reduced to 40°C in the presence of CIP, while it remained unchanged in the presence of MXF. With further increased in temperature (> 45°C), the peak positions of stretching band remained stable.

Schematic representation of a typical phospholipid with FQs

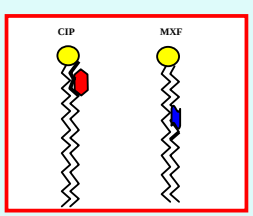

Table 1. Binding parameters of FQ to LUV liposomes obtained from steady state anisotropy experiments

LUV liposomes Composition	CIP $K_{app}(10^5 M^{-1})$	MXF
DPPG	8.6±0.5	No variation of signal
DOPC: DPPG (1:1 M: M)	3.2±0.9	
DOPC: DPPC (1:1 M: M)	1.1±0.2	

Conclusion

This work suggests that, lipids-fluoroquinolones interactions are highly sensitive to the fluoroquinolone investigated. Ciprofloxacin binds to lipids vesicles, probably by electrostatic interactions and remains inserted into the lipids bilayer. As consequence it creates disorder into the DPPC organization and decreases its melting temperature. An increase in the lipophilicity of the drug (MXF vs CIP) enhances the diffusion of moxifloxacin through the membranes, explaining probably why no change in anisotropy and a more important decreased of all trans conformation of lipid chains were observed with this antibiotic.This work highlights the biophysical applications of ATR-FTIR and anisotropy fluorescence to investigate lipids-drug interactions.

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3. Bensikaddour H, Fa N *et al.*, submitted.

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