Characterization at nanometric scale of the interactions between Fluoroquinolones (ciprofloxacin) and membrane lipids

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Abstract

Fluoroquinolones antibiotics are effective intracellular antibiotics, and their activity depends on achieving appropriated concentrations. It is thought that to reach the intracellular environment, fluoroquinolones interacts with the membranes lipid bilayer and/or recognizes the membranous proteins like efflux pumps [1,2]. Therefore, probing drug/lipid interactions at the molecular level represent an important challenge in membrane biophysics and pharmaceutical research.

In this study, we investigated the ciprofloxacin effects on phospholipid monolayers and bilayers (dioleoylphosphatidylcholine (DOPC); dipalmitoyl-phosphatidylcholine (DPPC), (1:1) using fluorescence spectroscopy and complementary biophysical methods. Firstly, we determined by steady state anisotropy fluorescence the binding parameters of ciprofloxacin with the phospholipids monolayer (DOPC) and (DPPC). Secondly, ciprofloxacin stability within a lipid monolayer was studied by Langmuir-Blodgett method, and, finally, its effect on membrane organization and fluidity was investigated using atomic force microscopy (AFM) and nuclear magnetic resonance ^{31}P NMR.

The binding parameters of ciprofloxacin antibiotic with phospholipid monolayers revealed a stoichiometry of one antibiotic per DOPC or DPPC. The affinity of ciprofloxacin with phospholipid monolayers was in the order of $10^5 M^{-1}$. Langmuir experiment showed that ciprofloxacin was partially found in the subphase, and that the drug affected the monolayer stability at high compression level. Furthermore, on bilayers, time-lapse AFM images in the presence of ciprofloxacin revealed an erosion of DPPC gel domains and reduction about 35 % of the area occupied by these domains. In addition, by ^{31}P NMR, we showed that in fluid phase, ciprofloxacin modified the lineshape of the spectra and increased the chemical shift anisotropy values (Δσ) as compared to those of the control DPPC spectra.

Taken together our data indicate that the interaction of ciprofloxacin with phospholipids involves two steps: i) binding to lipid vesicles, and ii) modifications in the membrane fluidity and organization. Finally, our findings provide valuable information for further investigations on fluoroquinolones-lipids interactions.

Materials and methods

a) Materials

Ciprofloxacin antibiotic is provided by Bayer (Leverkusen, Germany). DOPC and DPPC lipids were purchased from Sigma. Lipids vesicles were prepared as described by (Laurent *et al*., 1982).

b) Methods

Anisotropy titrations were performed by adding increasing concentrations of either DOPC or DPPC vesicles to a fixed amount of ciprofloxacin, 5µM, in 10 mM Tris, pH 7.4.

Langmuir experiments. An automatically controlled Langmuir trough, equipped with a platinum Wilhelmy plate was used to obtain the surface pressure–area isotherms of (DOPC: DPPC [1:1]) and DOPC:DPPC mixture with ciprofloxacin monolayers at the air/water interface. Ciprofloxacin was added at increased concentration. The air/water interface was then compressed with two Delrin barriers. The same technique was used to study the release of ciprofloxacin from the interface into the subphase in 10 mM Tris, pH 7.4. The presence of lipids was detected by phospholipid assay and ciprofloxacin was assayed by fluorimetry (λ_{ex}275 nm; λ_{em}430 nm), as described previously (Michot *et al*, 2004)

AFM Imaging. Topographic images of (DOPC:DPPC) (1:1) molar ratio, vesicles were taken using an optical detection system equipped with a liquid cell (Nanoscope IV, Digital Instruments) in 10 Tris, 100 mM NaCl buffer. AFM measurements were carried in the absence and in the presence of 1mM of ciprofloxacin at room temperature.

^{31}P NMR studies. ^{31}P NMR studied the effect of ciprofloxacin on membrane lipid mobility and organisation. Control samples of liposomes (37.5mM in lipids) were treated by adding concentrated drug solutions to reach a final DPPC: drug ratio of 2:1. Typical Fourier transform parameters were applied. Spectra were recorded upon warming of the sample from 30°C to 50°C.

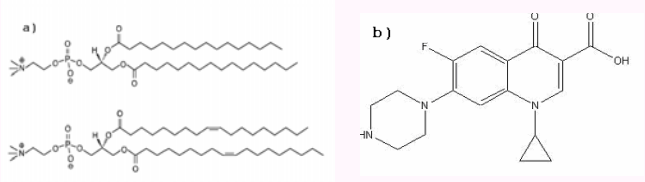

Figure 1 . Chemical structure of the DOPC, DPPC (a) and of ciprofloxacin (b)

2-Ciprofloxacin stability within a lipid monolayer

To gain insight the stability of CIP within a lipid monolayer, we use Langmuir technique. At the first time, we followed the kinetics release of ciprofloxacin from the interface of mixed DOPC:DPPC (1:1) lipids monolayer into the subphase as shown in (Fig. 3a). The amount of fluoroquinolone in subphase increased with its concentration, and reached a plateau within 20 min. Then, we determined the surface pressure versus area isotherms of lipids monolayers without and with increased concentration of ciprofloxacin at the air-water interface (Fig. 3b). Adding of ciprofloxacin to (DOPC:DPPC) monolayers led to an important shift of the curve to the leftt.

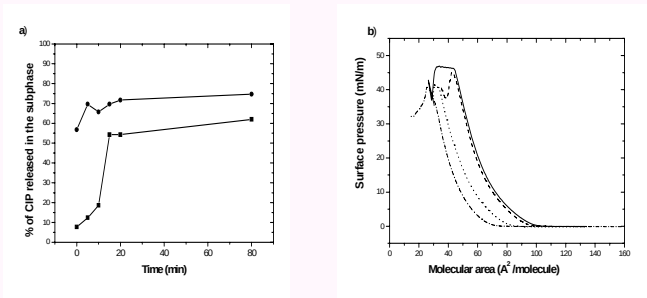

Figure 3. Ciprofloxacin stability within (DOPC:DPPC) monolayer

a) Kinetic of the release of the ciprofloxacin from the DOPC:DPPC (1:1:M) monolayer to the subphase, CIP molar ratio added to monolayer was 0.4 and 1 M , represented in square and circle respectively. (b) Surface pressure–molecular area isotherms of (DOPC:DPPC) alone (continuous line) and in the presence of increased concentration of antibiotic.Ciprofloxacin molar ratio added at 0.4M (discontinuous line), 1 M (dot line), and 2M (dashdot line).

Results

1-Binding parameters of CIP to lipids vesicles

In order to determine binding stoichiometry of CIP/lipids vesicles complex, direct anisotropy titrations were performed by adding increasing DOPC or DPPC (Fig. 1a) vesicles concentrations to a fixed concentration 5 µM of CIP (Fig. 1b). As shown in (Fig. 2), adding of lipids vesicles led to an important increase of anisotropy value. Stoichiometry of binding is deduced from the intersection of the initial slope with the plateau and found to be one for DOPC and DPPC, respectively . The observed binding constant K_{app} was in order of 10^5 M^{-1}. As compared to DOPC, binding the CIP to DPPC, led to a 10 fold K_{app} increase (K_{app}=17.4±0.9 10^5 M^{-1} for DPPC and 1.34±0.4 10^5 M^{-1} for DOPC).

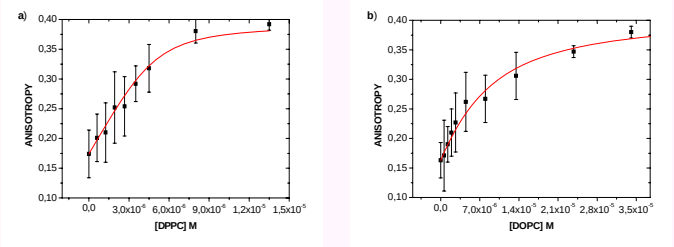

Figure 2 . Binding curve of CIP with DPPC and DOPC vesicles

Binding of CIP to lipids vesicles was followed by steady state anisotropy in the presence of DPPC (a) and DOPC (b) . Antibiotic concentration was 5 uM. Continuous lines corresponds to the fit of the experimental points in order to determine binding constant.

3- Ciprofloxacin effect on membrane organization and fluidity

To get more informations on ciprofloxacin membrane interaction, time-lapse AFM topographic images were recorded in solution of (DOPC:DPPC) unilamellar vesicles in the absence and in the presence of 1mM of ciprofloxacin (Fig.4). In the absence of CIP, two discrete height levels reflecting phase separation between solid-like DPPC and liquid-like DOPC were obtained, as those previously published (Berquand *et al*., 2005). The DPPC gel domains were homogenous, with a size ranging from 0.15 to 1.5 µm. When vesicles are incubated with ciprofloxacin, a decrease of the DPPC size domain (35%) was observed, even at short time of incubation (see Fig.4). However, the height differences between gel phase DPPC and fluid phase DOPC remained constant (1.10 ± 0.05 nm) during the experiment. These data support strongly the erosion process by ciprofloxacin. Furthermore, ciprofloxacin effect on membrane mobility was investigated by ^{31}P RMN method.. The experimental chemical shift anisotropy (Δσ) depends on the motion of the phosphodiester moiety, and, measured as a function of temperature on DPPC vesicles, taking the difference of chemical shifts between the low field shoulder($\sigma_{//}$) and the high-field peak($\sigma_{\perp}$). As shown in Fig.5, a sharp decrease of the (Δσ) values was observed around the transition temperature (42°C) (black line). In the presence of cipofloxacin (red line), no significant effect on the phosphate group ordering in the gel phase was observed, whereas in fluid phase, ciprofloxacin modified the lineshape of the spectra and increase the chemical shift anisotropy values compared to those of control DPPC spectra.

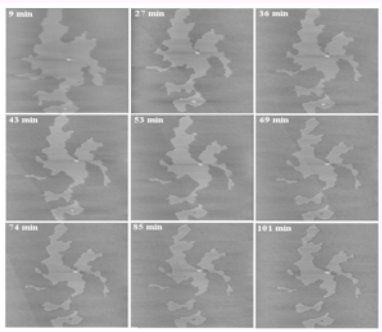

Figure 4. AFM height images of a mixed (DOPC:DPPC) lipids vesicles in the presence of ciprofloxacin

The concentration of CIP was added to lipids vesicles at 1mM. The images was recorded at (20 µm x 20 µm) with z-scale of 5 nm at increasing incubation time as indicated in the figure.

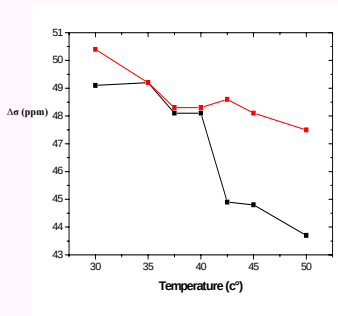

Figure 5. Temperature effect on the chemical shift anisotropy (Δσ) of ^{31}P NMR signals of DPPC liposomes

The values of chemical shift anisotropy as a function of temperature for the DPPC alone (dark square ■) and DPPC with CIP (phospholipid:drug molar ratio of (2:1) (red square ■) were determined by ^{31}P nuclear magnetic resonance (NMR spectra) . (Δσ) values are means of three independent determinations with SD < 1.2 ppm.

Conclusion

This work highlights, lipids- ciprofloxacin interaction, that proceeds via a well-defined two-step pathway:

i) The first step involves binding to lipids vesicles, with a moderate affinity.

ii) The second steps , ciprofloxacin modified the membrane fluidity and its organization.

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