Probing fluoroquinolone-biomembrane interactions on the nanoscale

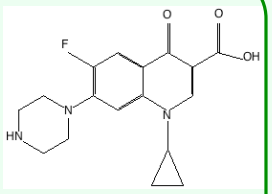
Nathalie Fa[1] , Ingrid Burton[2], Magali Deleu[3], Robert Brasseur[4], Yves Dufrêne[2], Marie-Paule Mingeot-Leclercq[1].

[1]Unité de pharmacologie cellulaire et moléculaire, Université Catholique de Louvain, Brussels (Belgium), [2]Unité de chimie des interfaces, Université Catholique de Louvain, Louvain-la-Neuve (Belgium) [3] Unité de Chimie Biologique Industrielle, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux (Belgium), [4] Centre de Biophysique Moléculaire Numérique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux (Belgium).

ABSTRACT: Fluoroquinolones antibiotics show an intracellular activity that is thought to depend on their interactions with membranes and/or recognition by membranous proteins. Therefore, probing this interaction at the molecular level represents an important challenge in membrane biophysics and pharmaceutical research. We investigated the effects of a fluoroquinolone, ciprofloxacin (CPX), on phospholipid monolayers and bilayers (dioleoyl-phosphatidylcholine (DOPC) : dipalmitoyl -phosphatidylcholine (DPPC), 1:1), using atomic force microscopy (AFM) and complementary biophysical methods.

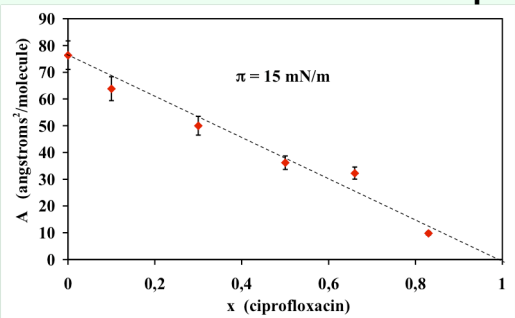
AFM images in air of lipid monolayers showed that ciprofloxacin reduces the size and modifies the DPPC domains, from round to indented shapes. Surface pressure – area isotherms at the air-water interface revealed that the drug does not significantly influence the lateral organization of monolayers and that a very little quantity of CPX is integrated to the lipid monolayer.

Time-lapse AFM images of bilayers in aqueous solution revealed a progressive reduction of the size of the DPPC gel domains. Dialysis experiments on multilamellar vesicles confirmed the ability of fluoroquinolones to bind to lipids, this binding depending on the nature of the lipid and on the pH.This work shows that AFM combined with biophysical approaches represent a powerful tool in pharmacological research to investigate drug-membrane interactions at the molecular level.

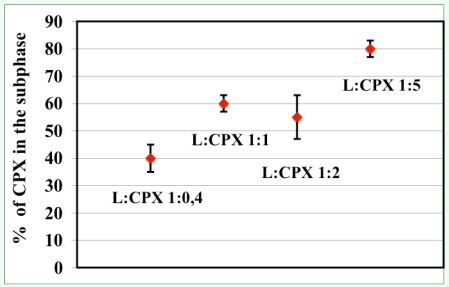


Chemical structure of the CPX

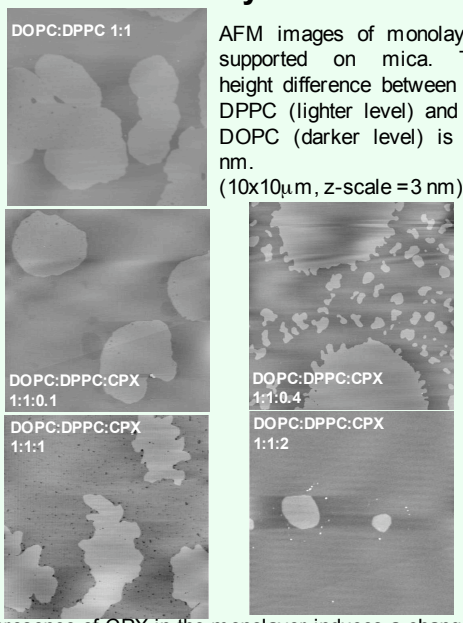
DOPC:DPPC:ciprofloxacin monolayers



Average area per molecule of mixed DOPC:DPPC:CPX monolayers vs. molar fraction of CPX for a surface pressure at the air-water interface of 15 mN/m. The dotted line corresponds to the ideal curve.



Rate of CPX solubilised in the subphase of a film balance 20 min after deposition of DOPC:DPPC:CPX mixtures (L:CPX) at the air-water interface. The subphase was Tris buffer (10mM, pH 7.4)

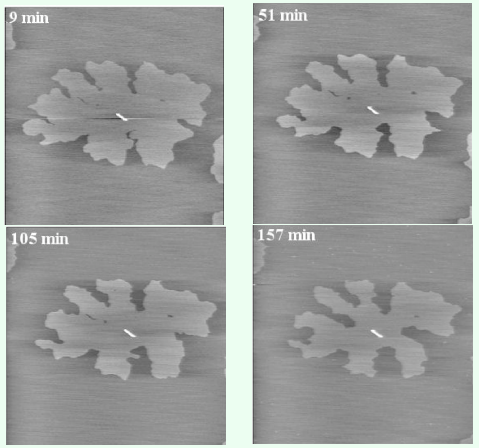


AFM images of monolayers supported on mica. The height difference between the DPPC (lighter level) and the DOPC (darker level) is 0.7 nm. (10x10μm, z-scale =3 nm)

The presence of CPX in the monolayer induces a change of DPPC domain chape and a reduction of their size leading to their disappearance.

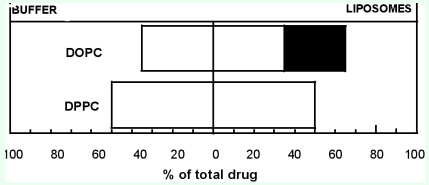
- The interaction between lipids and CPX is very weak ;
- A small amount of CPX is stabilized at the air-water interface with the lipids ;
- The presence of CPX in the monolayer strongly modifies the shape and the size of th DPPC gel phase domains.

DOPC:DPPC:ciprofloxacin bilayers

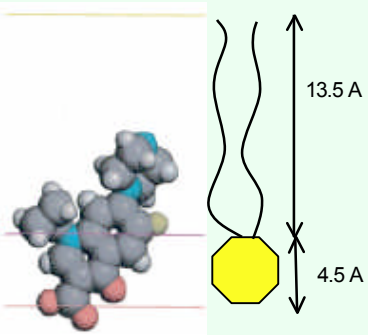


AFM height images (20 μm x 20 μm, z-scale : 5 nm) of a mixed DPPC:DOPC bilayer recorded in Tris/NaCl/CPX solution at increasing incubation time.

- The area of the DPPC gel phase domains decreases linearly with incubaton time in a CPX solution ;
- CPX binds much more to DOPC than to DPPC bilayers ;
- Molecular modeling shows the ability of CPX to penetrate the bilayer.



Equilibrium dialysis of CPX (200 μM) against liposomes (10 mg/ml, either pure DOPC or DPPC) in Acetate/NaCl buffer. Open bars, free drug ; closed bar, bound drug



Position of the CPX molecule in the lipid bilayer determined by a molecular modeling procedure : IMPALA (Lins et al. 2001. Proteins: Structure, Function and Genetics 44, pp. 435-447)

GENERAL CONCLUSION : This work illustrates the complementarity of several methods by analysing the action of CPX (AFM, binding equilibrium, film balance, molecular modeling). We showed that the interaction between CPX and DOPC:DPPC is weak but sufficient to modify the lateral organization of DOPC:DPPC monolayers or bilayers .