Interaction of the macrolide antibiotic azithromycin with model of membranes

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ABSTRACT:

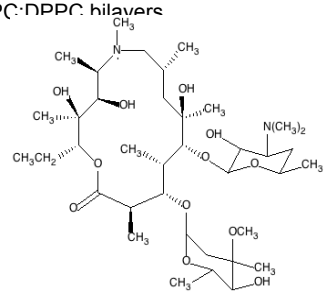
Purpose and methods: The aim of the study was to investigate the influence of azithromycin on the lateral organization of lipids, their phase transition, and the bending modulus of bilayers using real-time atomic force microscopy (AFM) on dioleoylphosphatidylcholine:dipalmitoylphosphatidylcholine (DOPC:DPPC) bilayers, differential scanning calorimetry (DSC) on mulltilamellar vesicles, (MLV) and micropipet experiments on DOPC giant unilamellar vesicles.

Results: **1)** Phase separation was observed between gel domains of DPPC and fluid matrix of DOPC. Time-lapse images collected following addition of azithromycin revealed progressive erosion and disappearance of DPPC gel domains within 60 minutes. **2)** Calorimetric experiments on DPPC and DOPC vesicles showed a suppression of the pretransition and a phase separation respectively. **3)** The micropipet experiments on DOPC giant vesicles revealed a bending modulus divided 2 times. At higher molar ratio of antibiotic (lipid:antibiotic ratio = 50:1), we observed a strong modification in the shape of the vesicles and their potential destruction.

Conclusion: This study could help us to elucidate the physico-chemical membrane properties underlying the effect of drugs on biological processes like inhibition of endocytosis and lysosomal phospholipase activity.

INTRODUCTION:

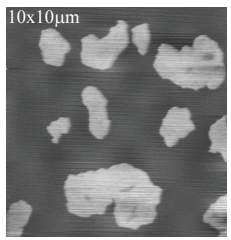
Azithromycin is a dicationic macrolide antibiotic derived from erythromycin A, with a marked amphiphilic character. It was shown to decrease the lysosomal phospholipase activity and to inhibit endocytosis[1]. These effects are related to the ability of azithromycin to interact with lipids and alter the biophysical properties of membranes[2]. In this study, we further investigated the effect of azithromycin on DPPC, DOPC, and DOPC:DPPC bilayers.



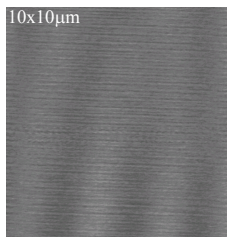
References :

1. J.-P.Montenez, F.Van Bambeke, J.Piret, A.Schanck, R.Brasseur, P. M.Tulkens, M.-P.Mingeot-Leclercq, 1996 Eur.J.Pharmacol **314**:215-227
2. D.Tyteca, A.Schanck, Y.F.Dufrêne, M.Deleu, P.J.Courtoy, P.M.Tulkens, M.-P.Mingeot-Leclercq, 2003 J. Membrane Biol. **192**:203-215
3. W. Helfrich, Z. 1973 Naturforsch. C. C. **28**:693–703

AFM on DOPC:DPPC 1:1 bilayers :

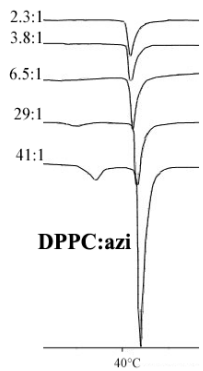


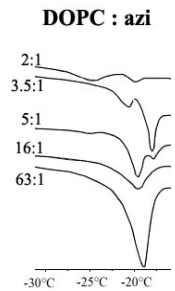
DPPC gel domains (white) in DOPC fluid matrix (dark) ; eight difference:1.10±0.05 nm

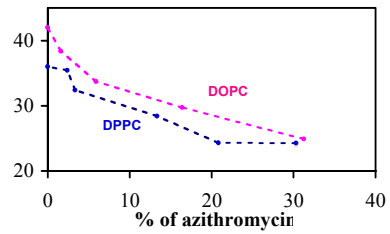


Addition of azithromycin + 60 mn : only one uniform fluid phase visible

DSC on DOPC and DPPC MLVs: :

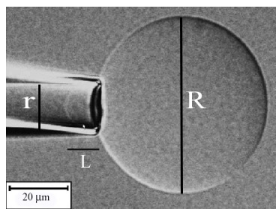


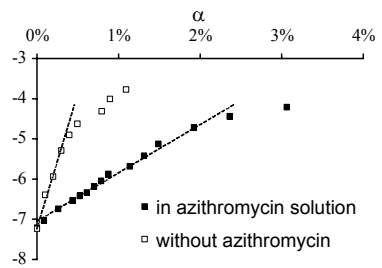




- Suppression of the DPPC pretransition
- Phase separation of vesicles enricched with azithromycin or not
- Strong decrease of the enthalpy change gel -fluid phase for both DOPC and DPPC

Micropipet experiments :





Helfrich's relation[3] where:
σ = tension of the membrane
α = relative increase of the apparent surface

$$\alpha = \frac{A - A_i}{A_i} = \frac{1}{8\,\pi\,k_c} ln\frac{\sigma}{\tilde{\sigma}} + \frac{\sigma}{K_e}$$

k_C = **bending modulus**
= energy necessary to bend the bilayer

$k_C = 22 \pm 2 k_B T$	without azithromycin
$k_C = 9.6 \pm 1.5\ k_B T$	with azithromycin

Actions of the azithromycin on bilayers :

- interaction of azithromycin with polar head groups
- fluidification of DPPC at the DOPC-DPPC interface
- decrease of the enthalpy associated to the gel-fluid phase transition
- enhancement of the fluctuations of the bilayers by mecanical effect of the insertion of azithromycin molecules between the polar head of DOPC molecules.