Differential interaction of novel antitubercular drugs with lipids of varying surface charges: an aspect related to its antimicrobial activity

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Aim

Pulmonary tuberculosis (Tb), an infectious disease caused by Mycobacterium tuberculosis (MTb), is a growing international health concern [1], since it is the cause of approximately two million deaths each year. Consequently, the development of new drugs is required, especially compounds that are able to fight multi drug resistant bacteria (MDR-MTb).

In the current work we study rifabutin (RFB), a drug already used in the therapy of Tb, and its recently synthesized analogue N-acetyl-rifabutin (acetylRFB).

The first part of this work deals with drug-membrane interaction studies using zwitterionic and negatively charged 3D membrane model systems (composed of DMPC and DMPG), that mimic, respectively, the ordinary cell membrane and the mycobacterium membrane (Figure 1).

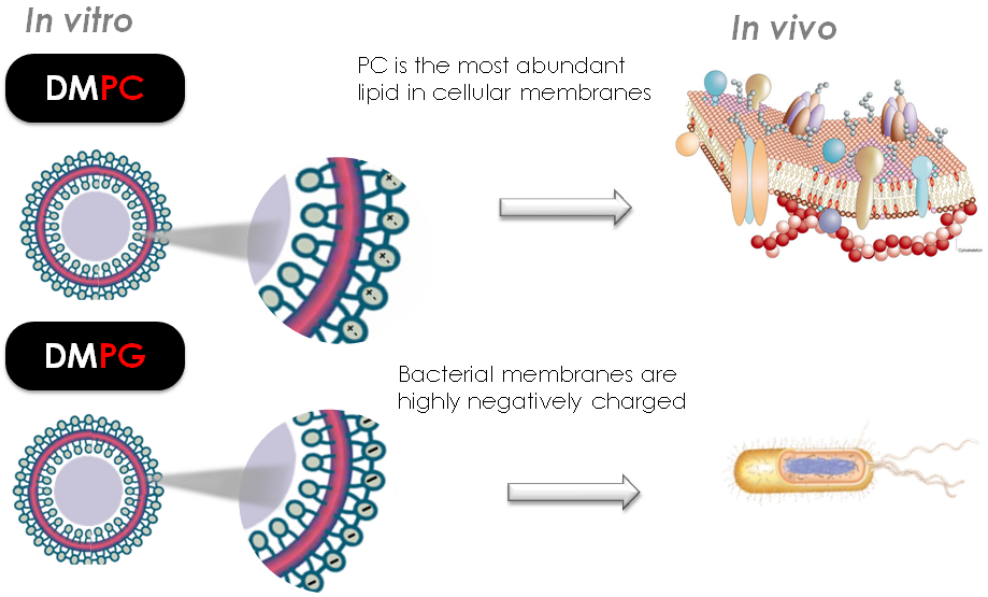

Figure 1: Membrane model systems that mimic the surface charge of biomembranes

Achievements

The results from SAXS and WAXS experiments of DMPC in fully hydrated DMPC at physiological pH are in good agreement with those in the literature. The lamellar lattice constant deduced from the SAXS patterns for DMPC, changes from 6.77 ± 0.05 nm in the $L_{\beta'}$ phase, to 6.25 ± 0.05 nm in the fluid L_{α} phase where the bilayers present highly disordered chains. Deconvolution of the WAXS patterns gives the two parameters of the pseudo-hexagonal lattice of the chain packing, i.e., 4.09 ± 0.05 Å and 4.20 ± 0.05 Å, which are also in good agreement with the literature. Moreover, the diffraction peaks present a high correlation length (ξ) which indicates a good correlation between the bilayers.

The experiments were made considering the hypothesis that the interaction of the drugs with DMPC depended on at least two factors: the drugs' concentration and the initial organization of the lipids. Generally, at the L_α phase, RFB and acetylRFB (Figure 2) increased the long range *d* values, which can be an indicator of enhancement of the water layer. The cooperativity was also affected, being reduced with increasing concentrations of drugs.

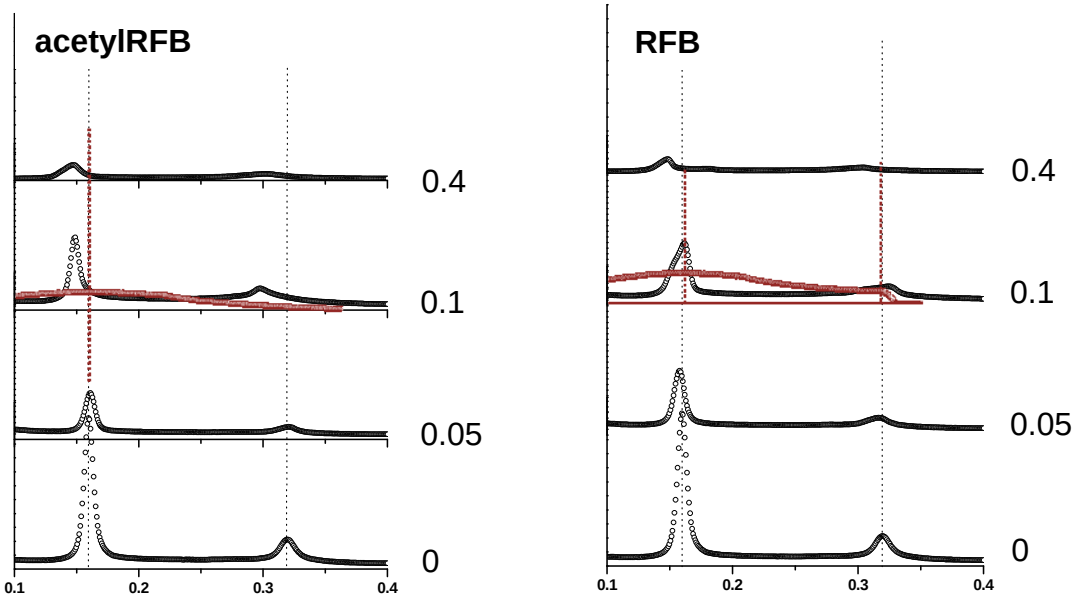

Figure 2: In black: SAXS patterns of DMPC (0); DMPC+acetylRFB and DMPC+RFB (at drug:lipid molar fractions of 0.05; 0.1 and 0.4) in L_α phase (37 ºC). In red: SAXS patterns of DMPG+acetylRFB and DMPCG+RFB (at drug:lipid molar fractions of 0.1) in L_α phase (37 ºC). Solid lines give the best fit of the Lorentzian´s analysis model to the scattered intensities.

The membrane disturbance induced by the drugs was even more pronounced on the negatively charged membranes (Figure 2, red SAXS pattern obtained for a drug:DMPG molar ratio of 0.1) than on the zwitterionic membranes (Figure 2 the black SAXS pattern obtained for a drug:DMPC molar ratio of 0.1). This effect is of great interest with the respect to the effect of drugs perturbing the integrity of bacterial membranes containing large fractions of negatively charged PG like lipids, while mammalian membranes contain large fractions of PCs will be less affected.

At the $L_{\beta'}$ phase and for both the membrane models tested, RFB and acetylRFB influence the chain packing. Only one Bragg peak at 4.05 nm is observed, indicating a change from the orthorhombic unit cell of tilted chains to a hexagonal packing of nontilted chains, which correlates well with the change in the headgroup orientation to a more upright position leading to the decrease of the chain tilt and also correlated with the increase long range distance. The loss tilt angle must be motivated by the penetration of drugs between the membrane phospholipids.

In view of the results obtained from SAXS, WAXS and by ongoing studies of fluorescence quenching, the mechanism by which RFB and acetylRB JC2 permeate through the phospholipids' bilayer must include an electrostatic adsorption at the interface region. This association between drugs and membrane surface is probably the first step that governs the mechanism of interaction of these drugs with bacterial natural membranes.

References

[1] M. Pinheiro, M. Lúcio, J. L. F. C Lima, S. Reis, Nanomedicine **6**, 1413 (2011).