Physical chemical properties of Lipids extracted from J774 Macrophages cells wild type and Ciprofloxacin resistant cells (CIP R): Fluidity and membrane potential

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Abstract

The interaction between the Ciprofloxacin (CIP) and the lipids at molecular level has been described in literature. Indeed, CIP bound to lipids and induced disorder and modified the orientation of the acyl chain [1,2]. These data could have a consequence on the lipid composition at cellular level J774 resistant to this antibiotic as shown in (Bensikaddour *et al*., 2009, BS Poster B655 [3]). This modification of lipid composition in resistant cells could have effects on physical chemical properties of membrane.

Here, we investigated the fluidity and membrane potential of lipids extracted from J774 cells wild type and Ciprofloxacin resistant cells by fluorescence measurements. The measurement was done using liposomes constituted from lipids extracted from cells.

Polarization spectroscopy data showed a melting temperature of 25°C in both vesicles (WT and CIP resistant cells) indicating that some other factors might contribute to maintain the fluidity.

Membrane potential studies reveled that lipids extracted from J774 Ciprofloxacin resistant cells bound to the ANS (anilinonaphthalene sulfonates) with a lower affinity as compared to the control. This is in agreement with the presence of more negative charges lipids in J774 CIP resistant cells [3].

This work shows that changes of lipid composition in ciprofloxacin resistant cells could be related to a modification of physical chemical properties of the membrane.

Materials and methods

a) Materials

Cells: we used wild-type (WT) and ciprofloxacin resistant J774 macrophages surexpressing MRP protein. The latter were obtained by chronical exposure of WT macrophages to increasing concentrations of CIP (0.1 mM to 0.2 mM) [4].

Fluorescente probes: diphenylhexatriene (DPH) and 8-anilin-1-naphtalene sulfonic acid (ANS) were obtained from Invitrogen, Molecular probes ® (Leuven, Belgium). The stock solutions of ANS were prepared in methanol.

b) Methods

1-Extraction of lipids

The extraction of lipids from murine J774 macrophages cells wild type and surexpressing MRP protein was performed as described in Bligh and Dyer (1959) and Dennison et al., 2006 [5,6]. Dried samples were dissolved in Tris 10 mM, pH 7.4 to constitue LUVs liposomes.

2-Fluorescence polarization studies

Florescence polarization studies were performed on LUVs liposomes prepared from lipids extracted from J774 cells (WT and CIP resistant cells), in Tris 10 mM pH7.4 buffer at a final concentration of 0.3mM. Incorporation of diphenylhexatriene (DPH) at a molar ration of 1:250 was obtained by preincubation at 37°C during 1 h. The measurement was performed on a LS-55 Perkin Elmer fluorimeter (Perkin-Elmer, Beacons-field, UK). Temperature was raised from 8°C to 40°C with equilibration time of 5 minutes.

3-Membrane potential studies

Liposomes (100 µM) were titrated with a various concentrations of ANS in Tris 10mM, buffer (pH 7.4). The emission coefficients of ANS in the presence of sample, were determined as the line slope of the fluorescence emission intensity at high lipid concentration (1mM) as a function of low concentration of ANS (0.1-1.4 µM) [7]. All measurements were made at 37°C. To obtain the associations constant (Ka), fluorescence titration curves were analyzes using the equation :

$$[ANS]_{bound} = C_{max}\frac{(K_a[ANS]_{free})^b}{1+(K_a[ANS]_{free})^b}$$

Where Cmax is the maximum concentration of ANS bound to lipids, b is the cooperativity parameter. The $[ANS]_{bound}$ and $[ANS]_{free}$ are, respectively, the concentration of bound and free ANS at equilibrium.

Results

1- Thermotropic characterization of liposomes prepared from lipids extracted from J774 macrophages cells WT and CIP resistant

A modification of lipid composition in membranes can modulate its melting temperature. In fact, an increase of the acyl chain order, with a high *trans/gauche* ratio, or increased the rotational freedom with decreasing *trans/gauche* ratio could be observed. To check whether the changes in lipids compositions of J774 macrophages cells resistant to ciprofloxacin could have a modification on the thermotropic behavior of lipids, two LUVs liposomes were prepared from lipid extraction of J774 wild type and CIP resistant cells. The thermotropic fluorescence polarization profiles of the DPH-labelled vesicles are shown in Fig. 1. No change in the thermotropic curves was observed, for both cases, and the melting temperature *T*m was 25°C.

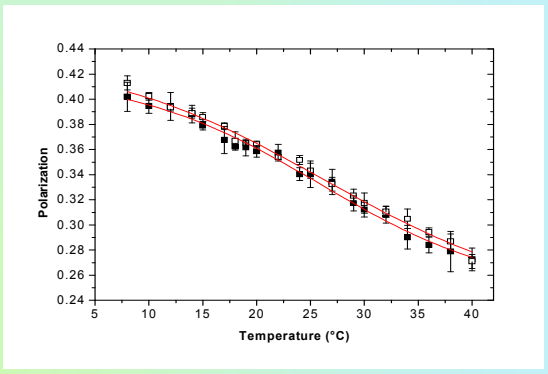

Figure 1. Fluorescence polarization thermotropic curves of liposomes

Fluorescence polarization thermotropic curves of liposome constituted from lipid extraction of J774 cells wild type (close square ■) and ciprofloxacin resistant (open square) . Liposomes (0.3 mM) were prepared at pH7.4 and DPH probe was incorporated with a molar ration of (1:250). Excitation and emission wavelength were 381 and 426 nm respectively.

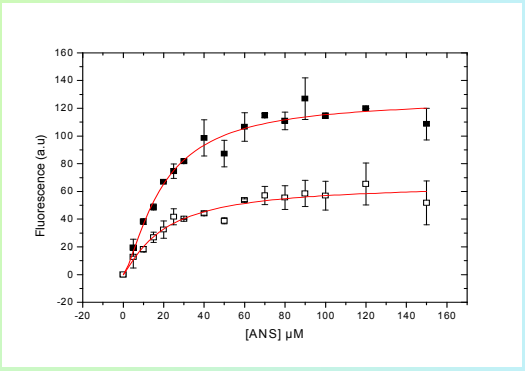

Figure 2. Fluorescence titration of ANS in the different liposomes tested

Variation of fluorescence intensity as a function of ANS concentration for the liposomes mimicking wild type (close square ■) and ciprofloxacin resistant (open square) membranes J774 macrophages cells. Liposomes (100 µM) were prepared at pH7.4 in Tris 10 mM buffer and ANS concentration was added from 5 to 150 µM. Excitation and emission wavelength were 380 and 480 nm respectively

2- Interaction of ANS with lipids extracted from J774 macrophages cells WT and CIP resistant

To obtain information regarding the membrane potential of different vesicles tested, fluorescence titration curves with ANS probe were analyzed (Fig 2). The binding parameters of ANS to vesicles, was calculated by determining the emission coefficient of the probe in different liposomes tested. When ANS bound to the lipids vesicles, its fluorescence intensity increased until to reach a plateau. The maximum of the curve of WT vesicles is clearly above that of CIP resistant liposomes curve, suggesting a higher affinity of ANS to the WT liposomes.

The association constant value (Kas) of ANS bound to WT liposomes is (75± 3) 10^{-3} µM. Whereas, and due to the difficulty to obtain the emission coefficient of ANS in liposomes prepared from lipid extracted from CIP resistant cells, the association constant of ANS bound to theses liposomes was undetermined.

Conclusion

In the present study, we determined, the fluidity and membrane potential of lipids extracted from J774 cells wild type and CIP resistant cells by fluorescence measurements. Our results show that:

- The fluidity of vesicles constituted from lipid extraction of J774 Ciprofloxacin resistant cells was similar to that of wild type cells.
- Membrane potential studies revealed that lipids extracted from J774 Ciprofloxacin resistant cells bound to the ANS (anilinonaphthalene sulfonates) with a lower affinity as compared to the wild type lipids vesicles.

These results suggest repulsion between ANS (negatively charged probe) and lipids extracted from ciprofloxacin resistant cells, which contain probably more negative charge lipids comparing to the wild type cells

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