(*)Corresponding author:
Cellular and Molecular Pharmacology Unit
UCL 7370 avenue E. Mounier 73 B-1200 Brussels, Belgium
Hayet.Bensikaddour@uclouvain.be

Lipid membrane composition of J774 macrophages cells surexpressing MRP protein (resistant to ciprofloxacin) as compared to wild type

Hayet Bensikaddour*[1], Paul. M. Tulkens[1], Françoise Van Bambeke[1] & Marie-Paule Mingeot-Leclercq[1]

[1] Université catholique de Louvain, Faculty of Medicine, Unité de pharmacologie cellulaire et moléculaire, UCL 73.70, avenue E. Mounier 73, B-1200 Bruxelles, Belgium

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Abstract

In view of the critical role of lipids for both drug uptake and activity of MRP proteins [1-2] together with the ability of fluoroquinolones (ciprofloxacin) to interact with lipids [3], we investigated the composition of lipids in Murine J 774 macrophages cells wild type and surexpressing MRP protein (resistant to ciprofloxacin).
Firstly, we identified the lipids composition of J774 macrophages cells wild type (WT) and resistant to ciprofloxacin (CIP). Then, we studied membrane fluidity of liposomes prepared from lipids extracted from the two types of J774 cells (WT and resistant to ciprofloxacin).
Our results showed a decrease of sphingomyelin amount (two fold) and an increase in phosphatidylinositol (1.5 fold) in resistant cells as compared to wild type cells. No significant variation in others phospholipids was observed (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine) and cholesterol content was also similar for the two types of 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.
Studies are currently performed to investigate in details the changes in sphingolipids (sphingomyelin and ceramides). These data might have an important relevance to relate the interaction of ciprofloxacin with lipids and change in the processes involved in cellular accumulation of fluoroquinolones.

Materials and methods

a) Materials

Lipids: Standards lipids (SM, Chol, PC, PE, PI, PS and Cer) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Thin layer chromatography (TLC) and Amplex Red cholesterol Assay Kit were obtained, from VWR international and Invitrogen (Leuven, BE), respectively.
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].

b) Methods

Extraction and separation of lipids by TLC
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 chloroform: methanol (2:1, v/v) and analysed on silica gel concentrating zone plates using chloroform: methanol: acetic acid: water (65:50:4:1, v/v) as mobile phase for phospholipids separation. The ceramides present in the wild type and ciprofloxacin resistant cells were identified by HPTLC silica gel 60 plates using butanol: acetic acid: water (3:1:1, v/v) as mobile phase.
The plates were then exposed to Copper II reagent and comparing with known standards run in parallel.
The phospholipid content in each spot was determined by phosphorus assay and cholesterol concentration was determined using Amplex Red cholesterol Assay Kit.
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.

Table 1. Phospholipids and cholesterol quantification in J 774 macrophages Cells (WT and CIP resistant cells)
Data are mean ± SD (n = 3), (*) Student test (p< 0.05)

Lipid	WT J774 Cells (Percentage %)	CIP Resistant cells (Percentage %)
Sphingomyelin (SM)	14±1	7±1*
Phosphatidylcholine (PC)	14±1	16±2
Phosphatidylinositol (PI)	28±2	38±4*
Phosphatidylserine (PS)	17±1	18±3
Phosphatidylethanolamine (PE)	26±4	23±1
Cholesterol (Chol)	5-7% (35±3 Chol/mg Prot)	5-7% (35±3 µg Chol/mg Prot)

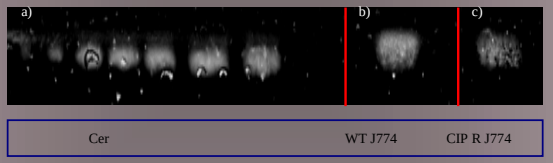

Figure 1. ***Ceramide detection in J 774 macrophages Cells (wild type and CIP Resistant) as revelled by HPTLC***

The bands, in (a), correspond to increasing concentration (from 2 to 15 µg/µl) of ceramide standard as revelled by Copper II reagent. In (b, c) the ceramide bands correspond to WT J774 cells and CIP resistant cells, respectively. Lipids concentration (0.4 mM) were spotted in chloroform: methanol (2:1, v/v)

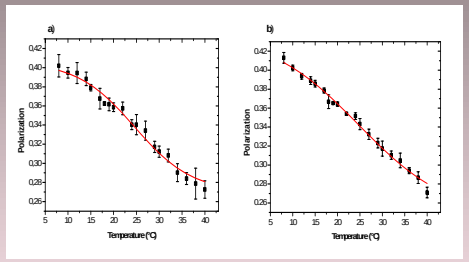

Figure 2. ***Fluorescence polarization thermotropic curves of liposomes prepared from lipids extracted from wild type (a) and ciprofloxacin resistant (b) J774 macrophages. Excitation and emission wavelength were 381 and 426 nm respectively.***

Results

1- Phospholipids and cholesterol contents in murine macrophages cells (Wild type and CIP resistant cells)

Firstly, we checked if the macrophage cells J774 wild type and those surexpressing MRP protein (CIP resistant cells) present a modification in phospholipid composition. Using thin layer chromatography, we observed phospholipids bands corresponding to SM, PC, PE, PI and PS. Then, we quantified by phosphorus assay, the individual phospholipids in both lipid extracts from WT and CIP resistant cells (Table. 1).
The amounts of sphingomyelin decreased (two fold) and phosphatidylinositol increased (1.5 fold) in resistant cells as compared to wild type cells. However, no significant variation in others phospholipids quantity was observed (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine). The content of cholesterol was also similar for the two types of cells.
In addition, we observed a large band in front of the migration of the plate. This band is related to ceramides as revelled by HPTLC using butanol: acetic acid: water (3:1:1, v/v) as mobile phase (Fig.1).

2- Membrane fluidity of liposomes prepared from lipids extracted from wild type and CIP resistant cells

In view of the critical role of lipids for both drug uptake and activity of MRP proteins [1-2] together with the ability of fluoroquinolones (ciprofloxacin) to interact with acidic lipids [7], we tested whether the increase of PI in resistant J774 cells membranes could alter the fluidity of these membranes.
To this end, LUVs liposomes were prepared from lipids extracted from J774 wild type and CIP resistant cells.
The thermotropic fluorescence polarization profiles of the DPH-labelled vesicles are shown in Fig. 2. No change in the thermotropic curves was observed, and the melting temperature *Tm* was 25°C.

Conclusion and summary

In the present study, we identified the lipid composition of J774 membranes cells, wild type and resistant to ciprofloxacin overexpressing MRP protein and determined fluidity property of vesicles prepared from lipids extracted from these two types of cells. Our results show that:
- The fluidity of vesicles constituted from lipid extraction of J774 wild type was similar to that of ciprofloxacin resistant cells.
- The quantity of phosphatidylinositol increased (1.5 fold) and sphingomyelin decreased (two fold) in resistant cells as compared to wild type. Other lipids could be also modified (ceramides and glucosylceramides).
Further experiments are actually under investigation to explore the importance of lipids for cellular accumulation of fluoroquinolones and activity of efflux pumps.

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