Identification and quantification of ceramide and glucosylceramide in MurineJ774 macrophages cells wild type and Ciprofloxacin resistant cells (CIPR) by liquid chromatography–electrospray ionization tandem mass spectrometry

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Abstract

In previous work, lipid extracted from murine macrophages J774 cells wild type (WT) and from which are resistant to Ciprofloxacin (CIP) antibiotic (surexpressing MRP proteins) were identified and quantified by TLC and phosphorus assay [1] The results showed a decrease of sphingomyelin amount (two fold) in J774 Ciprofloxacin resistant cells as compared to wild type cells.
This suggests a modification of Glucosphingolipids in ciprofloxacin resistant cells. To this aim, we characterized by liquid chromatography coupled to tandem mass spectrometry the ceramide and Glucosylceramide in both types of cells (wild type and CIP resistant cells) as described in Fillet *et al.*, (2002) [2].
The results showed a decrease (15 to 28 %) of different ceramide species (C_{16}-C_{24}) and an increase (25 to 52 %) of the glucosylceramides class (C_{16}-C_{24}) issued from ciprofloxacin resistant cells comparing to the wild type cells.
Taken together our data, we show that the resistance to Ciprofloxacin in J774 cells could be associated with a modification of lipids composition mainly the sphingolipids.

Materials and methods

a) Materials

Cells: we used wild-type (WT) and ciprofloxacin resistant J774 macrophages surexpressing MRP4 protein. The latter were obtained by chronical exposure of WT macrophages to increasing concentrations of CIP (0.1 mM to 0.2 mM) [3].

b) Methods

1-Extraction of lipids

Murine J774 macrophages cells (wild type and surexpressing MRP protein) were rinsed twice with ice-cold PBS, then scraped in PBS and centrifuged at 800 *g*. The resulting pellet was homogenized in distilled water by sonication. An aliquot of the cell homogenate was reserved for determination of protein levels. Ten ng of C_{10}-ceramide (as an internal standard) was added to samples of cell lysates containing 500 µg of protein. Lipids were extracted using Folch's partition with a mixture of chloroform and methanol (2/1, v/v). Samples were then centrifuged at 1500 *g* and the organic phase was evaporated to near dryness under a gentle stream of dry nitrogen. The samples were reconstituted by vortexing with 100 μl of ethanol until they were completely dissolved. To avoid any loss of lipids, the entire procedure was performed in siliconized glassware.

2- Liquid chromatography–electrospray ionization tandem mass spectrometry

We measured ceramide and glucosylceramide intracellular levels as described previously [2] using liquid chromatography-electrospray ionization tandem-mass spectrometry (LC-ESI-MS/MS). Ceramides and glucosylceramides were separated by liquid chromatography on a C18 column and eluted using a solvent gradient made of a mixture of water, acetonitrile and 2-propanol. MS detection was carried out using a Ultima triple quadrupole instrument (Waters, Manchester,UK) configured with a electrospray ionization source used in positive ion mode. Collision-induced fragmentations conducted on ceramides and glucosylceramides produced a well characteristic product ion at m/z 264, making multiple reactions monitoring (MRM) well suited for various ceramides quantitative measurements.

Introduction

Previously, we have described a correlation between overexpression of the multidrug protein Mrp4 and resistance to Ciprofloxacin, a fluoroquinolone antibiotic, in J774 macrophages cells [4,5]. A similar phenomenon was observed with multidrug-resistant in colon cancer cell line, HT29, which displayed over expression of the multidrug resistant protein (Mrp1) [6]. In addition, to changes in the expression level of particular proteins, multidrug-resistant cells exhibit alterations in their sphingolipids composition [7,8]. Consequently, we suggest that overexpression of Mrp4 in J774 Ciprofloxacin resistant cells, could be associated with modification of sphingolipids. Recently, ceramide, glucosylceramides and sphingomyelin were indentified in both J774 wild type and ciprofloxacin resistant cells. Interestingly, a decrease of two fold in sphingomyelin amount has been observed in J774 Ciprofloxacin resistant comparing to the wild type cells [1]. To get more insight the sphingolipids modification between CIP resistant and wild type cells, we quantified using Liquid chromatography–electrospray ionization tandem mass spectrometry, the most predominant sphingolipids: ceramide and glucosylceramides in both types of cells.

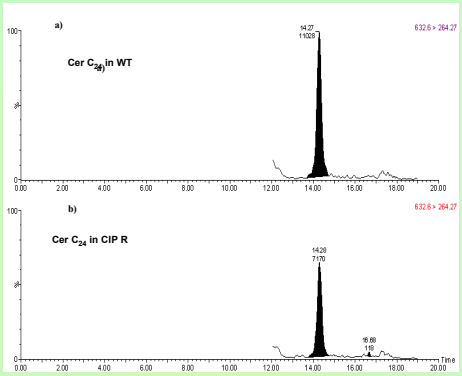

Figure 1. MS spectra of ceramide (C_{24}) in J774 macrophages cells wild type in (a) and CIP resistant cells in (b).

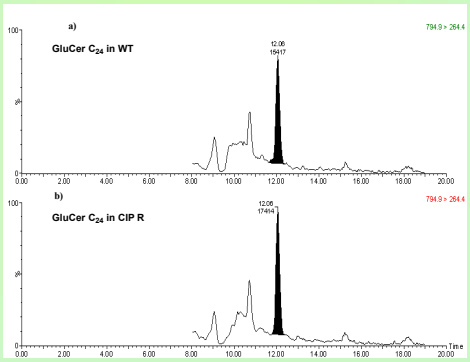

Figure 2. MS spectra of Glucosyl-ceramide (C_{24}) in J774 macrophages cells wild type in (a) and CIP resistant cells in (b).

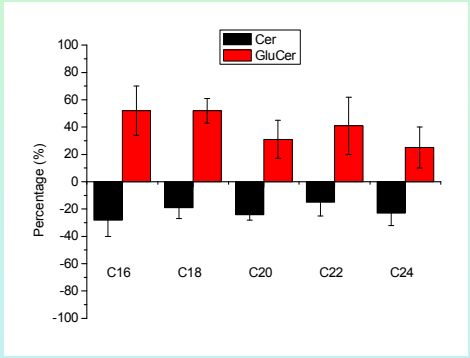

Figure 3. Ceramide and Glucosyl-ceramide levels in J774 ciprofloxacin resistant cells comparing to the wild type cells as revealed by LC-MS

Results

1- Ceramide is reduced in J774 macrophages cells resistant to ciprofloxacin

Quantification of different species of ceramide in J774 wild type and CIP resistant cells extract lipids was conducted using (LC-ESI-MS/MS) as described previously. As can been seen from Figure 1, there is a significant difference between two cell lines with respect to the amount of ceramide (C_{24} is represented).

Similar spectra were obtained with four ceramide species (C_{16} till C_{22}). For comparison, the quantities of ceramide species between wild type and CIP resistant cells were determined using the ratio of the peak areas of natural ceramide to that of the internal standard (Cer C_{10}). It has been noted that the amount ceramide containing in CIP resistant cells was decreased with a percentage of 15 to 28 %, depending to the ceramide species (C_{16}-C_{24}) (Figure 3).

2-Glucosyl-ceramide is enriched in J774 macrophages cells resistant to ciprofloxacin

Since Glucosyl-ceramide (Glu Cer) is produced from ceramide, by glucosylceramides synthase, and the ceramide content decreased in CIP resistant cells overexpressed Mrp4 protein, we further evaluated the Glucosyl-ceramide level in both type cells (wild type and CIP resistant).

As shown in Figure 2, the peak of Glu Cer (C_{24}) of CIP resistant cells is more intense than that of wild type cells. In fact, the resistant cells have approximately 25 % of Glu-Cer C_{24} more than in Wild type. Increased glucosylceramides levels were also observed in four GluCer species (C_{16}, C_{18}, C_{20}, C_{22}) in resistant cells with a percentage of 30 to 50 % (Figure 3).

Conclusion

The present study shows that J774 macrophages cells, resistant to CIP (overexpressed Mrp4) exhibit a deviating sphingolipids composition, mostly, increased levels of glucosylceramides and a decrease of ceramide. A diminution of sphingomyelin was also observed (Bensikaddour *et al.*, 2009).

These results open an outlook to understand the relation that could exist between sphingolipids metabolism and multidrug resistant protein (Mrp4) in J774 macrophages cells.

References

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