THE LIPID CONTENT OF CISPLATIN- AND DOXORUBICIN-RESISTANT MCF-7 HUMAN BREAST CANCER CELLS

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Aim. To perform the comparative study both of qualitative and quantitative content of lipids in parental and drug resistant breast cancer cells. Materials and methods. Parental (MCF-7/S) and resistant to cisplatin (MCF-7/CP) and doxorubicin (MCF-7/Dox) human breast cancer cells were used in the study. Cholesterol, total lipids and phospholipids content were determined by means of thin-layer chromatography. Results. It was found that cholesterol as well as cholesterol ethers content are significantly higher but diacylglycerols, triacylglycerols content are significantly lower in resistant cell strains than in parental (sensitive) cells. Moreover the analysis of individual phospholipids showed the increase of sphingomyelin, phosphatidylserine, cardiolipin, phosphatidic acid and the decrease of phosphatidyethanolamine, phosphatidylcholine in MCF-7/CP and MCF-7/Dox cells. Conclusion. Obtained results allow to suggest that the lipid profile changes can mediate the modulation of membrane fluidity in drug resistant MCF-7 breast cancer cells.

Key Words: MCF-7 cells, total lipids, phospholipids, tumor drug resistance.

Much attention has been recently paid to the lipid-protein interactions as an important component in the biochemical state and the regulation of cellular vital activity [1–4]. These phenomena become of particular importance in the process of transforming normal cells into malignant. The notion of so-called membrane-lipid therapy has even been formulated, which aims to develop drugs that affect the lipid organization and can modulate the localization and activity of membrane proteins [5, 6]. Lipids not only affect the structure of membranes, but also fulfill the relevant regulatory functions in normal and tumor cells. For example, some protein kinase C isoenzymes are activated by phosphatidylserine, phosphatidylethanolamine and diacylglycerol [7–10]. Phosphoinositides, namely phosphatidylinositol, take a direct part in the operation of certain signal pathways in the cell and Ca2+-transport [11]. Negatively charged phosphatidyserine regulates the electrostatic interaction between proteins and membranes [5], and some lipid domains in membranes are peculiar temperature sensors during the reaction of cells to heat shock [12]. According to several studies there is a significant difference in lipid composition for normal and tumor cells of the same histogenesis [13–17]. Thus, the existence of the differences in the level of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylyserine (PS), sphingomyelin (SM) and phosphatidylinositol (PI) has been shown by T.E. Merchant et al. during the comparative study of the lipid content of normal breast tissue cells and tissues of benign as well malignant tumors of human breast by the use of 31P NMR spectroscopy [16]. The same authors found that low-grade differentiated and high-grade differentiated tumors vary in level of PC in colon cancer cells [14]. It was shown that the reduction of cholesterol was observed in Morris 5123 hepatoma cell membranes in comparison with hepatocytes [17].

Besides, the results of our previous studies have shown that changes in lipid composition of tumor cells also occur during the process of drug resistance phenotype development. So in particular, some differences in the phospholipid content of cell membranes of parental and doxorubicin-resistant Guerin carcinoma were found [18, 19]. These data indicate that some minor phospholipids may play an important role in the development of tumor drug resistance. Thus due to the resistance development a malignant cell acquires new properties which are reflected on the both morphological level and change of its molecular and biochemical characteristics [20].

Taking into account above mentioned the study of qualitative and quantitative lipid composition of sensitive and resistant malignant cells is relevant. It can allow to clarify the mechanisms of tumor cells resistance formation and to argue for the expedient using of liposomal forms of anticancer drugs that could probably increase the efficacy of anticancer therapy.

MATERIALS AND METHODS

Cell strains and cell cultivation. The cells of the parental MCF-7/S strain were cultivated in the Dulbecco ISCOVE modified medium (Sigma-Aldrich Chemie GmbH, Germany) supplemented with 10% fetal calf serum (“Sangva”, Ukraine) at 37°C and saturated air with 5% CO2. The cells were subcultured twice a week with a 2–4x10^4 cells/cm² surface plating density. Cell lines resistant to the action of anticancer drugs (MCF-7/CP — resistant to cisplatin and MCF-7/Dox — resistant to doxorubicin) were obtained as presented earlier [24].

Thin-layer chromatography method. The qualitative and quantitative composition of total lipids
and phospholipids were determined by thin-layer chromatography method using plates "Sorbfil" PTSH-AF-A ("Imid Ltd", Krasnodar, Russia). To realize this purpose the lipids from the MCF-7/S, MCF-7/CP and MCF-7/Dox cells were extracted using a chloroform/methanol solvent mixture (1:1, v/v). The thin-layer chromatography of total lipids was carried out only in one direction in the hexane/diethyl ether/glacial acetic acid solvent system (85:15:1, v/v) [21]. The thin-layer chromatography of phospholipids was carried out in two mutually perpendicular directions [22]. The first solvent system was chloroform/methanol/benzene/acetone/glacial acetic acid/water (70:30:10:5:4:1, v/v). After the evaporation of the solvent system the plates were treated with 10% H2SO4 in methanol and heated for 5 min at 180°C. All chromatograms were scanned and scanned were imaged with the program Picture J. Lipid content was expressed in percents. Seven experiments with each cell line were performed in this study. The statistical analysis of the results was carried out using Student’s t test. Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

Results presented in Table 1 show marked differences in the total lipid composition between sensitive and resistant MCF-7 cell strains. It has to be marked that used method didn’t allow to determine free fatty acids in resistant cells. It was shown (Table 1) the significant increase of cholesterol in the MCF-7 cells resistant to cisplatin and doxorubicin by 60 and 55%, respectively (P<0.05). At the same time in the MCF-7/CP and MCF-7/Dox cells the increase of cholesterol esters content was recorded. Formation of drug resistance phenotype in human breast cancer cells is also accompanied with significant quantitative changes of mono-, di- and triacylglycerols. For example, in the cells of both resistant strains a much smaller number of monoacylglycerols was observed in comparison with the cells of sensitive strain (P<0.05) was observed. In the MCF-7/CP cells the amount of monoacylglycerols was reduced by 3.7 fold and in the MCF-7/Dox cells by 3.4 fold. The reduction of diacylglycerols and triacylglycerols amount in the cells of both resistant strains was also recorded.

Table 1. The content of total lipids (%) in sensitive and resistant MCF-7 strains (n = 7)

<table>
<thead>
<tr>
<th>Lipids</th>
<th>MCF-7/S</th>
<th>MCF-7/CP</th>
<th>MCF-7/Dox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids (PL)</td>
<td>12.7±1.0</td>
<td>17.7±1.0</td>
<td>▲ 18.7±1.3</td>
</tr>
<tr>
<td>Monoacylglycerols (MG)</td>
<td>6.7±0.1</td>
<td>1.8±0.2</td>
<td>▼ 2.0±0.4</td>
</tr>
<tr>
<td>Cholesterol (Ch)</td>
<td>18.1±1.8</td>
<td>29.0±0.8</td>
<td>▼ 28.2±1.4</td>
</tr>
<tr>
<td>Free fatty acids (FFA)</td>
<td>5.6±0.1</td>
<td>0</td>
<td>▼ 0</td>
</tr>
<tr>
<td>Diacylglycerols (DG)</td>
<td>19.4±0.7</td>
<td>17.6±0.6</td>
<td>15.0±0.5</td>
</tr>
<tr>
<td>Triacylglycerols (TG)</td>
<td>29.6±0.9</td>
<td>8.3±0.7</td>
<td>▼ 18.1±0.4</td>
</tr>
<tr>
<td>Cholesterol ethers (ChE)</td>
<td>7.9±1.2</td>
<td>26.5±0.7</td>
<td>▼ 18.0±1.8</td>
</tr>
</tbody>
</table>

Note: ▲ — significantly higher (P<0.05) than in MCF-7/S cells; ▼ — significantly lower (P<0.05) than in MCF-7/S cells. We also found that the total content of phospholipids is higher in resistant cells than in sensitive ones. The individual phospholipid analysis of MCF-7/S, MCF-7/CP MCF-7/Dox cells has shown that the resistant cells compared with sensitive cells contain more SM and phosphatidylglycerol (Table 2) and, conversely, less PE. Also, the increase of cardiolipin (diphosphatidylglycerol) level in resistant cells (especially in MCF-7/CP) was noteworthy. It is known that cardiolipin is localized both on the inner and outer mitochondrial membrane sheets and in contact places of eukaryotic cells. This substance is necessary for the catalytic activity of several enzymes involved in energy metabolism (provides the coupling of oxidative phosphorylation in mitochondria [23]). The interaction of cardiolipin with mitochondrial proteins is specific. Acyl cardiolipin composition is an important factor that provides the functional reactivation of mitochondrial enzymes, such as cytochrome-c-oxidase. Moreover, acyl cardiolipin composition plays a key role in the initiation of apoptosis (cit. [23]).

Table 2. The content of phospholipids (%) in sensitive and resistant cells of MCF-7 strain (n = 7)

<table>
<thead>
<tr>
<th>Lipids</th>
<th>MCF-7/S</th>
<th>MCF-7/CP</th>
<th>MCF-7/Dox</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysophosphatidylcholine (LPC)</td>
<td>5.8±0.4</td>
<td>6.5±0.9</td>
<td>▲ 5.9±0.4</td>
</tr>
<tr>
<td>Sphingomyelin (SM)</td>
<td>7.6±0.4</td>
<td>12.0±0.5</td>
<td>▼ 9.8±0.2</td>
</tr>
<tr>
<td>Phosphatidylserine (PS)</td>
<td>6.7±0.4</td>
<td>8.8±0.6</td>
<td>▼ 9.4±0.6</td>
</tr>
<tr>
<td>Phosphatidylglycerol (PG)</td>
<td>7.3±1.0</td>
<td>11.8±0.9</td>
<td>▼ 8.8±0.7</td>
</tr>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>39.4±2.4</td>
<td>32.0±3.0</td>
<td>▼ 30.2±3.1</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (PE)</td>
<td>16.4±0.3</td>
<td>11.8±0.3</td>
<td>▼ 12.8±0.6</td>
</tr>
<tr>
<td>Cardiolipin (CL)</td>
<td>6.7±0.3</td>
<td>9.2±0.4</td>
<td>▼ 8.6±0.2</td>
</tr>
<tr>
<td>Phosphatidic acid (PA)</td>
<td>4.4±0.7</td>
<td>6.8±0.2</td>
<td>▼ 7.4±0.3</td>
</tr>
<tr>
<td>Phosphatidylglycerol (PG)</td>
<td>8.4±0.3</td>
<td>7.4±0.3</td>
<td>▼ 7.1±0.2</td>
</tr>
</tbody>
</table>

Note: ▲ — significantly higher (P<0.05) than in MCF-7/S cells; ▼ — significantly lower (P<0.05) than in MCF-7/S cells.

The higher level of phosphatidic acid and phosphatidylserine was defined in the MCF-7/CP and MCF-7/Dox cells comparatively to MCF-7/S cells. Phosphatidic acid is an important point of branching in the biosynthesis of various phospholipids (Fig. 1). Thus, the synthesis of PC, PE and phosphatidylserine is carried out as a result of the hydrolysis of phosphatidic acid and diacylglycerol formation under the influence of phosphatidic acid phosphatase. Another variant of phosphatidic acid metabolism is its conversion into CDP-diacylglycerol which is used in biosynthesis of PI, phosphatidylglycerol and cardiolipin [23].

In accordance with well known data about metabolism of phospholipids we would also like to mention that the differences between sensitive and resistant cells in lisophosphatidylcholine (LPC) and PI content were not observed in our study.

Thus, we found that the formation of resistance to anticancer drugs is accompanied by changes in the composition of lipids in MCF-7 human breast cancer cells. The increase of cholesterol and SM content causes as known the decrease of cell membrane fluidity. This assumption is also confirmed by determination of the PC/SM ratio. PC and SM adapt mainly lamellar configuration of cell membrane and, therefore, significantly influence the membrane stability and fluidity. The content of PC in resistant cells was decreased and SM — increased. Therefore as seen from the data presented in Table 3, the PC/SM ratio in the MCF-7/CP and MCF-7/Dox cells is significantly lower than the same one in sensitive MCF-7/S cells that can indicate the decrease of resistant cells membrane fluidity.

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In general, paying attention to the change of cholesterol and SM amounts in resistant cells, it is worth to say about lipid rafts. Lipid rafts are peculiar areas within the outer sheet of plasma membrane that are enriched with cholesterol and sphingolipids. They are able to selectively incorporate proteins, modify protein-protein and protein-lipid interactions. These membrane microdomains participate in the processes of membranes invagination, signal transduction, endocytosis (cit. [23]).

To explain the obtained results we used the scheme presented in the Fig. 1. It can be seen from the scheme that an increase of phosphatidic acid and phosphatidylserine level is fixed in resistant cells. This situation should result in the increase of PE and respectively to the increase of PC. However, we don’t observe such one. On the contrary, the level of last phospholipids in the resistant cells becomes lower comparatively to basestrain ones. On the one hand, cell malignization is often accompanied by hyperhomocysteinemia, which results in the disruption of methylation processes (i.e., to hypomethylation), that it was shown in our previous works [24, 25]. On the other hand, perhaps, an inhibition of phosphatidylycerine decarboxylation occurs, which leads to the decrease of PE. Along with this, in resistant cells probably also an inhibition of PC synthesis through “diacylglycerol way” carries out.

We suppose that the increase of cholesterol and SM content results in the decrease of membrane fluidity, and the increase of phosphatidylycerine results in the activation of the cytotoxic substances elimination by means of P-glycoprotein, that is in accordance with literature data [25–27]. The obtained data are also confirmed by our previous results and the data of other authors [18, 19, 28]. So, we have proved the role of lipid component in the formation of drug resistance phenotype in malignant cells. We would like to present the hypothetical scheme of membrane in drug resistant cells (Fig. 2). These data may be used for the development of new forms of anticancer drugs which could significantly improve the efficacy of cytostatics through overcome drug resistance.

![Fig. 2. Structural organization of chemotherapy drugs resistant cell membranes](image-url)

REFERENCES


