Exp Oncol 2012
34, 2, 97–100

Experimental ONCOLOGY

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

I.N. Todor, N.Yu. Lukyanova, V.F. Chekhun*
R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kiev 03022, Ukraine

***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 phosphatidylethanolamine, 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 Ca^{2+}-transport [11]. Negatively charged phosphatidylserine 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), phosphatidylserine (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 ^{31}P 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% CO_2. The cells were subcultured twice a week with a 2–4x10^4 cells/cm^2 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

Received: May 3, 2012.
*Correspondence: E-mail: todor_igor@yahoo.com
Fax: 38+(044)-258–16–56

Abbreviations used: Ch – cholesterol; ChE – cholesterol ethers; CL – cardiolipin; DG – diacylglycerols; FFA – free fatty acids; LPC – lisophosphatidylcholine; MG – monoacylglycerols; PA – phosphatidic acid; PC – phosphatidylcholine; PE – phosphatidylethanolamine; PG – phosphatidylglycerol; PI – phosphatidylinositol; PL – phospholipids; PS – phosphatidylserine; SM – sphingomielyn; TG – triacylglycerols.

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/ammonia (65:30:10:6, v/v). The second 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% H_2SO_4 in methanol and heated for 5 min at 180 °C. All chromatograms were scanned and scans 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 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 (see 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 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 strain cells (n = 7)

Lipids	MCF-7/S	MCF-7/CP	MCF-7/Dox
Phospholipids (PL)	12.7±1.0	17.7±1.0 ▲	18.7±1.3 ▲
Monoacylglycerols (MG)	6.7±0.1	1.8±0.2 ▼	2.0±0.4 ▼
Cholesterol (Ch)	18.1±1.8	29.0±0.8 ▲	28.2±1.4 ▲
Free fatty acids (FFA)	5.6±0.1	0 ▼	0 ▼
Diacylglycerols (DG)	19.4±0.7	17,6±0.6	15.0±0.5 ▼
Triacylglycerols (TG)	29.6±0.9	8.3±0.7 ▼	18.1±0.4 ▼
Cholesterol ethers (ChE)	7.9±1.2	25.6±0.7 ▲	18.0±1.8 ▲

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. Acylic cardiolipin composition is an important factor that provides the functional reactivation of mitochondrial enzymes, such as cytochrome-c-oxidase. Moreover, acylic 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)

Lipids	MCF-7/S	MCF-7/CP	MCF-7/Dox
Lisophosphatidylcholine (LPC)	5.6±0.4	6.5±0.9	5.9±0.4
Sphingomielyn (SM)	7.6±0.4	12.0±0.5 ▲	9.8±0.2 ▲
Phosphatidylserine (PS)	6.7±0.4	8.8±0.6 ▲	9.4±0.6 ▲
Phosphatidylinositol (PI)	8.4±0.2	7.3±1.0	8.8±0.7
Phosphatidylcholine (PC)	39.4±2.4	30.2±3.0 ▼	30.2±3.1 ▼
Phosphatidylethanolamine (PE)	16.4±0.3	11.8±0.3 ▼	12.8±0.6 ▼
Cardiolipin (CL)	6.7±0.3	9.2±0.4 ▲	8.6±0.2 ▲
Phosphatidic acid (PA)	4.4±0.7	6.8±0.2 ▲	7.4±0.3 ▲
Phosphatidylglycerol (PG)	4.8±0.3	7.4±0.3 ▲	7.1±0.2 ▲

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.

Table 3. The PC/SM ratio in MCF-7 cells (n=7)

MCF-7/S	MCF-7/CP	MCF-7/Dox
5.2±0.3	2.5±0.4 ▼	3.1±0.3 ▼

Note: ▼ – significantly lower (P<0.05) than in MCF-7/S cells.

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 persented 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 phosphatidylserine 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.

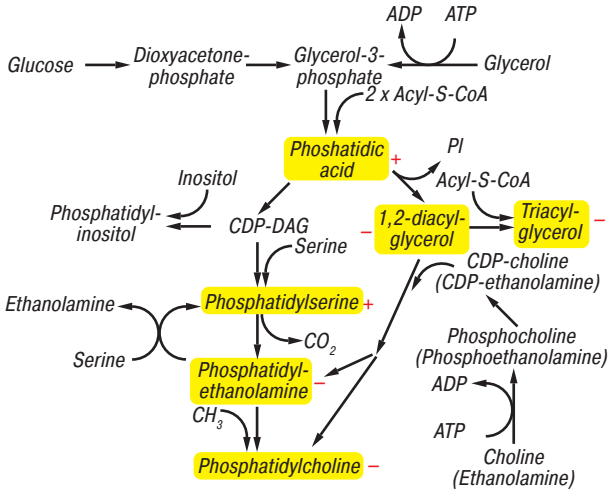

Fig. 1. Scheme of the biosynthesis of certain phospholipids. "+" and "-" means increase or decrease of the corresponding lipids in resistant MCF-7 cells

We suppose that the increase of cholesterol and SM content results in the decrease of membrane fluidity, and the increase of phosphatidylserine 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.

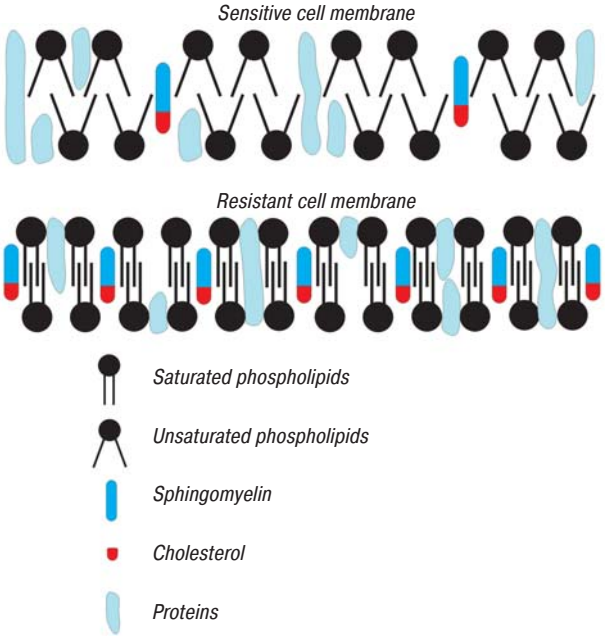

Fig. 2. Structural organization of chemotherapy drugs resistant cell membranes

REFERENCES

1. Lee AG. How lipids effect the activities of integral membrane proteins. Biochim Biophys Acta 2004; **1666**: 62–87.

2. Vögler O. The Gβ/γ dimer drives the interaction of heterotrimeric G proteins with nonlamellar membrane structures. J Biol Chem 2004; **279**: 36540–5.

3. Yeagle PL (ed.) The structure of biological membranes. Second edition. CRC Press, 2005; 590 p.

4. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science 2010; **327**: 46–50.

5. Escriba PV. Membrane-lipid therapy: a new approach in molecular medicine. Trends Mol Med 2006; **1**: 34–43.

6. Chen P, Chien PY, Khan AR, ***et al.*** *In-vitro* and *in-vivo* anticancer activity of a novel gemcitabine-cardiolipin conjugate. Anticancer Drugs 2006; **17**: 53–61.

7. Escriba PV, Sastre M, Garcia-Sevilla JA. Disruption of cellular signaling pathways by daunomycin through destabilization of nonlamellar membrane structures. Proc Natl Acad Sci USA 1995; **92**: 7595–9.

8. Giorgione J, Epand RM, Buda C, ***et al.*** Role of phospholipids containing dosahexaennyl chains in modulating the activity of protein kinase C. Proc Natl Acad Sci USA 1995; **92**: 9767–70.

9. Gomi FM, Alonso A. Structure and functional properties of diacyglycerols in membranes. Prog Lipid Res 1999; **333**: 1–48.

10. Martinez J, Vogler O, Casas J, ***et al.*** Membrane structure modulation, protein kinase Cα activation, and anticancer activity of minerval. Mol Pharmacol 2005; **67**: 531–40.

11. Parker PJ. The ubiquitose phosphoinositides. Biochem Sur Trans 2004; **32**: 893–8.

12. Török Z, Tsvetkova NM, Balogh G, ***et al.*** Heat shock protein coinducers with no effect on protein denaturation specifically modulate the membrane lipid phase. Proc Natl Acad Sci USA 2003; **100**: 3131–6.

13. Merchant TE, de Graaf PW, Minsky BD, ***et al.*** Esophageal cancer phospholipid characterization by ^{31}P NMR. NMR Biomed 1993; **6**: 187–93.

14. Merchant TE, Diamantis PM, Lauwers G, ***et al.*** Characterization of malignant colon tumors with ^{31}P nuclear magnetic resonance phospholipid and phosphatic metabolite profiles. Cancer 1995; **76**: 1715–23.

15. Hendrich AB, Michalak K. Lipids as a target for drugs modulating multidrug resistance of cancer cells. Current Drug Targets 2003; **4**: 23–30.

16. Merchant TE, Meneses P, Gierke LW, ***et al.*** ^{31}P magnetic resonance phospholipid profiles of neoplastic human breast tissues. Br J Cancer 1991; **63**: 693–8.

17. Batko J, Plotast-Necas B, Warchol T, ***et al.*** The effect of an experimental neoplastic disease on the flux of sodium and potassium ions across red blood cells and on the lipid composition of their membranes. Acta Biochim Pol 1992; **39**: 317–26.

18. Chekhun VF, Solyanik GI, Kulik GI, ***et al.*** The Seira spectroscopy data of nucleid acids and phospholipids from sensitive- and drug-resistant rat tumors. J Exp Clin Cancer Res 2002; **21**: 599–607.

19. Chekhun VF, Tryndiak VP, Todor IM, ***et al.*** Phospholipids and cholosterol content in tumor cell plasma membranes with different sensitivity to doxorubicin. Ukr Biochem J 2003; **75**: 120–5 (in Ukrainian).

20. Gao C-L, Zhu C, Zhao Y-P, ***et al.*** Mitochondrial disfunction is induced by high levels of glucose and free fatty acids in 3T3-L1 adipocytes. Mol Cell Endocrinol 2010; **320**: 25–33.

21. Kates M. Techniques of lipidology. Isolation, analysis and identification of lipids. M: Mir, 1975; 323 p (in Russian)

22. Vaskovsky VE, Terekhova TA. HPTLC of phospholipid mixtures containing phosphatidylglycerol. J High Resolution Chromatography & CC 1979; **2**: 671–2.

23. Gula NM, Margitich VM. Fatty acids and their derivatives in pathologic states. Kyiv, Naukova Dumka, 2009, 335 p. (in Ukrainian).

24. Chekhun VF, Kulik GI, Yurchenko OV, ***et al.*** Role of DNA hypomethylation in the development of the resistance to doxorubicin in human MCF-7 breast adenocarcinoma cells. Cancer Lett 2006; **231**: 87–93.

25. Chekhun VF, Lukyanova N Yu, Kovalchuk O, ***et al***. Epigenetic profiling of multidrug-resistant human MCF-7 breast adenocarcinoma cells reveals novel hyper- and hypomethylated targets. Mol Cancer Ther 2007; **6**: 1089–98.

26. Mannechez A, Reungpatthanaphong P, de Certaines JD, ***et al.*** Proton NMR visible mobile lipid signals in sensitive and multidrug-resistant K562 cells are modulated by rafts. Cancer Cell International 2005; **5**: 2–12.

27. Martinez MC, Kunzelmann C, Freyssinet J-M. Phosphatidylserine and signal transduction: Who needs whom? Sci STKE 2006; **3**: 318–43.

28. Peetla Ch, Bhave R, Vijayaraghavalu S, ***et al***. Drug resistance in breast cancer cells: biophysical characterization of doxorubicin interactions with membrane lipids. Mol Pharmaceutics 2010; **7**: 2334–48.