Effect of Carboplatin and Methotrexate on Lipid Levels in the Plasma Membrane of MCF-7 Cells and their Association with Cell Motility

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Authors’ contributions

This work was carried out in collaboration between all authors. Author JB designed the study, wrote the protocol and supervised the work. Authors JB and MD supervised the two BAU masters students, authors RD and HS carried out all laboratories work, managed literature search and performed the statistical analysis. Authors JB and JU managed the analyses of the study. Motility assays were done by author MES laboratory at LAU. Cell Culture, membrane isolation and HPLC studies were done by the author at AUB in JU laboratory. Author JB wrote the first draft of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aim: We examine in this study the effect of two antitumor drugs metothrexate (MTX) and carboplatin (CPT) on the phospholipid content of plasma membrane of MCF-7 breast cancer cells. MTX is a folate analog that inhibit dihydrofolate reductase. CPT is a platinum-containing
antineoplastic drug that inter-chelates DNA and inhibits replication. **Methods:** MCF-7 breast cancer cells were treated with different concentrations of CPT or MTX. Plasma membranes were purified and their protein contents were measured with and without drug treatment. We extracted the lipids from plasma membranes of drug-treated and control MCF-7 cells, quantitated, and separated by HPLC using a C18 reverse-phase column. The ability of both drugs to affect cell movement was studied using a motility assay.

**Results:** MTX induced 50% cell death at concentrations ranging from 50 to 100 μM. No further decrease in viability was seen above this concentration even at 1 mM. CPT induced 50% cell death at 5 μM. It also showed a range concentration that gives a 50% cell death after 24 hrs of incubation. Protein levels in plasma membranes of treated cells doubled compared to control. Lipid levels in plasma membranes decrease insignificantly after drug treatment. No changes in separation pattern of lipid extracted from membranes were seen after CPT treatment compared to control; however, MTX treatment showed to a single change in elution pattern in peak at 4.36 min. Both drugs had little effect on cell motility causing a decrease of 13.3% and 17.4% with CPT and MTX respectively compared to control.

**Conclusions:** Our results show that both drugs did not affect the amount of lipids but lead to doubling in the protein concentration in the plasma membranes of MCF-7 breast cancer cells. These drugs did not lead to a significant decrease in cell motility compared to control.

**Keywords:** Antitumor drugs; plasma membrane; lipids; cell motility.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CPT</td>
<td>Carboplatin,</td>
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<tr>
<td>MTX</td>
<td>Methotrexate,</td>
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<tr>
<td>RP-HPLC</td>
<td>Reverse Phase High Pressure Liquid Chromatography.</td>
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1. **INTRODUCTION**

Cancer is the most common malignant disease and a leading cause of death in the world. In the United States and a number of European countries, cancer is the second leading killer after heart disease and strokes [1]. In recent years, the occurrence of cancer has increased rapidly and cancer research became one of the most intense areas of research.

Breast cancer is the leading type of cancer death in women worldwide with more than one million new cases occurring annually [2]. Breast cancer is treated by surgery and/or combined with other treatments such as radiation therapy, hormone therapy and chemotherapy [3]. Different classes of chemotherapeutic drugs are used to treat breast cancer. The most common ones are taxanes, anthracyclines, antimetabolites and alkylating.

Antimetabolites drugs (i.e. MTX, Fluorouracil) interfere with the normal metabolic processes within cells. They interfere with one or more enzymes that are necessary for DNA synthesis [4]. While alkylating drugs (i.e. cisplatin, CPT) bind covalently to nucleophilic moieties in the cellular DNA forming DNA cross-links that cause changes in DNA conformation, resulting in disruption of DNA function and cell death [5].

Carboplatin, cis-diamine (1,1-cyclobutane dicarboxylato)platinum(II) (CPT), commercially available under the name of Paraplatin or Platinol, is a platinum analog of cisplatin. It was introduced into clinical trials in 1981 to overcome the toxic side effects of cisplatin such as nausea, vomiting, neurotoxicity, nephrotoxicity, and ototoxicity. Currently, the use of CPT based treatments is considered standard treatment for many cancers [6].

CPT uptake across the plasma membrane occurs via a specific mediator, copper transporter 1 (CTR1), which is the major copper influx transporter expressed on the cell membranes [7].

**Fig. 1. Chemical structure of CPT**

On the other hand, methotrexate (MTX) (Fig. 2), is a folic acid analogue also used in cancer chemotherapy. It is a potent inhibitor of dihydrofolate reductase (DHFR) that is a key enzyme in the synthesis of purines, thymidine,
and certain amino acids. MTX is used as a chemotherapeutic agent for different human malignancies such as acute lymphoblastic leukemia, malignant lymphoma, osteosarcoma, breast cancer and head and neck cancer [8].

Fig. 2. Chemical structure of MTX

MTX is transported into cells by reduced folate carrier (RFC) via active transport. Several ATP-binding cassettes (ABC) transporters especially ABCG2 and ABCC1-5 are responsible for effluxing this drug from the cell. RFC is ubiquitously expressed in normal tissues [9]. The clinical efficacy of MTX is often weakened by the attainment of resistance in cancer cells. Mammalian cancer cells’ resistance to MTX can occur due to various mechanisms including a decrease in MTX uptake by RFC, an increase of MTX efflux due to the overexpression of ATP-binding cassette (ABC) transporters, the overexpression of DHFR protein by amplification of DHFR gene, a decreased affinity of MTX to mutated DHFR or a combination of these mechanisms [9].

The ability of cancer cells to invade and metastasize depends on the composition of the plasma membrane. The plasma membrane is an effective barrier that separates the inside of cells from environment and regulates molecular trafficking. Like other biological membranes, the general structure of the plasma membrane consists mainly of protein and lipids linked together by non-covalent interactions with a mass ratio ranging from 1:4 to 4:1 [10,11].

Lipids can move either laterally in the plane of the membrane or transversally from one leaflet to the other by flippases, floppases and scramblases [12]. Lipids perform multiple important and vital biological roles at cellular and organismal levels. They act as fuel molecules, are crucial structural components of membranes, and considered important signaling molecules [10,13].

Phospholipids are the most abundant lipids in all biological membrane. Phospholipids play essential roles for cell survival. They are involved in the equilibria of the electric charge between the membrane and the surrounding solutions components [14].

Malignant neoplastic cells are distinguished from their normal counterparts by alteration in the cellular behavior and disorganization of the plasma membrane [15]. These perturbations are reflected by modification in the content of membrane phospholipids [15], as well as dysregulation in their metabolism [16].

The importance of lipids in cancer biology has started to reveal in the past three decades of research. Extensive studies have elucidated the abnormalities at the surface of neoplastic cells. Such changes are elucidated by alteration in membrane lipids that are considered major cellular indicators of tumor evolution and progression.

Major hallmarks of cancer are the abnormalities in phospholipids metabolism [17,18]. Larger amounts of phospholipids were shown to be present in various types of tumors compared to normal cells [17]. Furthermore, malignant tumors contain larger amounts of both cholesterol and phospholipids than benign tumors. Both programmed cell death and proliferative growth are affected by plasma membrane phosphatidylcholine and choline metabolites [17]. Szachowicz-Petelska et al. [14] quantitated the phospholipids extracted from healthy and cancerous renal tissue from patients and from plasma membrane of non-metastatic colorectal cancer cells. This study concluded that cancerous cells showed an increase in the amount of Phosphatidylcholine (PC), Phosphatidylinositol (PI), Phosphatidylethanolamine (PE) and Phosphatidylserine (PS) in cell membranes. Hence, alteration of membrane phospholipid in malignant transformation and progression represents a major hallmark of cancer cells. Cancer research has focused on the effect of chemotherapeutic drugs intracellularly with less emphasis on their effect on the cell’s plasma membrane. Our study aims at investigating the effect of the two anti-neoplastic drugs CPT and MTX on the lipid levels in the plasma membrane of breast cancer cells (MCF-7) and to evaluate the effect of these drugs on the invasiveness of MCF-7 cells in an In vitro system using time lapse assay.
2. MATERIALS AND METHODS

2.1 Cell Strain and Culture

MCF-7 (human breast cancer cell line) (cat# HTB-22) used in this study was purchased from the American Type Culture Collection (ATCC), Manassas, VA, US.

Cells were grown in RPMI medium supplemented with 10% fetal bovine serum (FBS) and 2% PEN-Strep at 37°C in a humidified incubator containing 5% CO2.

2.2 In vitro Cytotoxicity Assay (MTT Assay)

Cell Proliferation Kit I (MTT) (cat# 11699709001) purchased from Roche was used to determine cell viability. MCF-7 (passage number between 15 to 20) cells were seeded in a flat bottomed 96-well plate at a density of 5000 cells/well in a final volume of 100 μl medium in a humidified incubator with 5% CO2 for 24 h. The cells were treated with 100 μl of different concentrations of MTX (0.1 μM to 50 μM) or CPT (1 μM to 1 mM) suspended in complete media or media lacking FBS and incubated for 24 h. Control cells were treated similarly with both types of media lacking the drugs. 10 μl of 3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT reagent) were then added and the plate was incubated for 4 h, followed by the addition of 100μl of solubilizing reagent and overnight incubation. Cell viability was determined by the formation of purple formazan product. Absorbance of the color obtained was read at a wavelength of 595 nm using an ELISA plate reader. The percent survival was measured according to the following equation:

\[
% \text{ survival} = \frac{\text{abs of treated cells}}{\text{abs of control cells}} * 100.
\]

2.3 Membrane Isolation

Plasma membrane isolation was carried out as described by Lin et al. [19] with a minor modification. MCF-7 cells were seeded at a density of 2x10⁵ cells/10ml and then treated with MTX and CPT at their determined IC50 for 24 h. Both control and treated cells were harvested, washed twice with saline, and pelleted by centrifugation at 500 x g for 5 min. Cell pellets were homogenized in 500 μl HEPES-Mannitol solution for 5 min using a 27 gauge needle. The volume was made up to 3 ml followed by addition of 1M CaCl₂ to a final concentration of 10 mM. Homogenate was stirred for 10 min to evenly distribute the Ca²⁺ cation and then centrifuged at 3000xg for 15 min. The obtained supernatant was collected and ultracentrifuged at 100000xg for 24 h using a Sorvall WX Ultra Centrifuge in AH-627 rotor to pellet the plasma membrane. All steps were performed at 4°C.

2.4 Enzymatic Characterization of Isolated Plasma Membrane from MCF-7

The purity of the extracted plasma membrane was assessed by measuring the activities of a membrane specific enzyme as well as marker enzymes for Endoplasmic Reticulum (ER) and mitochondria. The presence of 5'-nucleotidase activity and absence of glucose-6-phosphatase and succinate dehydrogenase from the plasma membrane preparation confirmed its purity from both ER and mitochondria, respectively. Purity of the plasma membrane was assessed by measuring the activity of 5'-Nucleotidase as described by Aronson and Touster [20]. Absence of ER and mitochondrial contaminants was confirmed by assessing the activity of glucose-6-phosphatase, and succinate dehydrogenase as described by Aronson and Touster [20] and Bonner [21] respectively.

2.5 Protein Quantification

Proteins concentrations of the cell homogenates and the different fractions were estimated using Bradford assay. Concentrations were determined from a standard curve obtained using different amount of 0.01% of Bovine Serum Albumin (BSA).

2.6 Extraction of the Plasma Membrane Phospholipids

The Bligh and Dyer [22] method was used to extract the total lipids from the isolated plasma membrane. Membranes were suspended mixed with chloroform/methanol (1:2), vortexed very well. Double distilled water was added to the mixture with vigorous vortexing. Samples were then centrifuged at 200x g for 5 min at room temperature to separate the aqueous and organic layers. The chloroform layer containing the extracted phospholipids was collected for analysis. The extraction procedure was repeated 3 times on the aqueous layer to remove any residual lipids.
2.7 Total Inorganic Phosphate Determination

The total inorganic phosphate was determined as described by Lowry and Lopez with slight modifications [23]. Different amount of inorganic phosphate standard were added to a solution containing 10 N H₂SO₄: 70% HClO₄: H₂O with a ratio of 9:1:40. Samples were vortexed and incubated overnight at 160°C. Tubes were washed with double distilled water followed by addition of 0.9% ammonium molybdate reagent. After vortexing, ascorbic acid solution (9%) was added. The tubes were then incubated at 45°C for 30 min for the blue color to develop. The absorbance of the developed blue color was read at 820 nm.

2.8 Separation of Lipids by HPLC

The isolated phospholipids were separated by isocratic RP-HPLC. The separation was performed using an Agilent 1100 series HPLC with an HS C18 Discovery chromatographic column (25 cm, 4.6 mm, 5 µm) from Sigma. Elution was performed isocratically with a solvent consisting of a mixture of Acetonitrile/Methanol/Water (70:22:8). The flow rate was 0.5 ml/min. The eluted samples were detected at 210 nm. Every run was completed till absorbance returned to baseline.

2.9 Motility Analysis by Time Lapse Assay

Cells were plated on a 35 mm petri dish with a density of 2x10⁵ cells/well and incubated under optimal conditions in a humidified incubator with 5% CO₂ at 37°C for 24 h. CPT at IC₅₀ (100 µM) or MTX (5 µM) were then added for a period of 24 h. For motility analysis, cells were imaged in RPMI media, buffered using HEPES and overlaid with mineral oil on a 37°C stage. Images of moving cells were collected every 60 seconds for duration of 2 hours using a 20X objective lens on Zeiss Observer Z1 microscope. The speed of cell movement was quantified using the ROI tracker plugin in ImageJ software where every cell was tracked over 120 frames. Eight cells were tracked for each time-lapse movie, and average of distances moved were measured.

2.10 Statistical Analysis

All statistical analyses were performed with the standard statistical program SPSS 13.0 PL. The data obtained in this study were expressed as the mean ± Standard Deviation (SD). Statistical significance was tested using ANOVA test. P-values less than 0.05 were considered significant.

3. RESULTS

3.1 Effect of CPT and MTX on MCF-7 Viability

The cytotoxicity of CPT and MTX on MCF-7 cells was evaluated in an In vitro assay using MTT assay. Cells grown in RPMI media with or without FBS were treated with CPT or MTX at concentration varying from 1 µM to 1 mM and from 0.1 µM to 50 µM respectively for 24 hours. No cytotoxic effect was observed with both drugs when mixed in FBS containing media. Cytotoxicity of both drugs was seen only when FBS was eliminated from media. IC₅₀ was reached at a concentration of 100 µM for CPT and 5 µM for MTX (Figs. 3 and 4). Additional concentration of CPT up to 1 mM did not show any enhanced cytotoxicity.

On the other hand, MTX gave a 50 percent decrease in viability at 5 µM Lower doses did not show dose dependent decrease. Higher doses up to 50 µM did not have enhanced killing effect. It did not yield enhanced cell death at concentrations above 50 µM.

3.2 Protein Quantification

Protein concentrations were determined using Bradford Assay. Table 1 summarizes the results. The concentration of proteins in total homogenate did not significantly change upon CPT or MTX treatment. However, the concentration of proteins in plasma membrane increased twice after treatment with both drugs.

3.3 Total Inorganic Phosphate Determination

The amount of inorganic phosphate was determined as described under materials and methods. Table 2 summarizes the results obtained. Upon comparing control with CPT treated cells, no significant changes in inorganic phosphate were detected in purified plasma membranes and in total lipids extracted from plasma membranes in CPT treatment. The same is true for MTX treatment except for total cell homogenate.
**Fig. 3. Effect of CPT on MCF-7 viability using MTT assay**

Cells were cultured with CPT at concentrations ranging from 0 to 500 μM in the absence of FBS for 24 hours. Results represent mean of 3 determinations ± SD.

**Fig. 4. Effect of MTX on MCF-7 viability using MTT assay**

Cells were cultured with MTX at concentrations ranging from 0 to 50 μM for 24 h in the absence of FBS. Results represent mean of 3 determinations ± SD.

**Table 1. Protein concentrations of cell homogenate and isolated plasma membrane from control and CPT or MTX treated cells**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total protein in control (μg)</th>
<th>Total protein after CPT treatment (μg)</th>
<th>Total protein after MTX treatment (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell homogenate</td>
<td>640.7±8</td>
<td>727.2±61</td>
<td>591.6±39</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>25.12±4</td>
<td>51.1±4</td>
<td>49.84±5</td>
</tr>
</tbody>
</table>

Results represent ± SD of 3 different determinations of three different experiments. P-value in CPT treatment were 0.218 and 0.039 for the cell homogenate and the plasma membrane respectively. For MTX treatment, the p-values were 0.263 and 0.023 for the homogenate and the plasma membrane respectively.
Table 2. Amount of inorganic phosphate in control and CPT or MTX treated cells obtained from total cell homogenate, plasma membranes, and their extracted lipids

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total inorganic phosphate in control cells</th>
<th>Total inorganic phosphate in CPT treated cells</th>
<th>Total inorganic phosphate in MTX treated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell homogenate</td>
<td>960±6</td>
<td>805.8±65</td>
<td>664.33±82</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>199±29</td>
<td>190±30</td>
<td>116±6</td>
</tr>
<tr>
<td>Lipids extracted from plasma membrane</td>
<td>118±12</td>
<td>106±4</td>
<td>105±3</td>
</tr>
</tbody>
</table>

Results represent ± SD of 3 different determinations of three different experiments. P-values for CPT treatment for the cell homogenate, plasma membrane and the extracted lipids were 0.141, 0.845 and 0.156 respectively. As for MTX treatment, the p-values for cell homogenate, plasma membrane and the extracted lipids were 0.029, 0.1, and 0.386 respectively.

However, when comparing the amount of lipids per microgram of proteins, we noticed that there is a decrease in the total amounts of lipids (as measured by free inorganic phosphate). Total inorganic phosphate (Pi) in plasma membrane decreased 2 to 3 times in CPT and MTX treatment respectively (from 7.96 nmol/ml/µg of protein to 2.32 nmol/ml/µg in MTX treatment and to 3.72 nmol/ml/µg in CPT treatment). In the lipids extracted from the plasma membrane, it decreased about 2.4 times (from 4.72 nmol/ml/µg to 2.0 nmol/ml/µg in both treatment). No significant changes were seen in the concentrations of inorganic phosphate per microgram of protein in total homogenate versus drug treatment (1.5 nmol/ml/µg of protein versus 1.1 for both treatment).

3.4 Separation of Lipids by HPLC

Figs. 5, 6, and 7 show representative chromatograms of extracted lipids from plasma membranes of untreated, CPT, and MTX treated cells respectively. No changes in the elution pattern were noticeable between control and CPT treatment while MTX treatment showed an additional peak eluting at 4.36 min.

Measurement of inorganic phosphate in the eluted peaks at 4, 6.7, and 7.2 min showed that the changes in the ratio of inorganic phosphate between MTX treatment and control were 1.3, 1.9, and 1.22 respectively.

In CPT treatment, changes in inorganic phosphate was seen mainly at 13.2 min where the ratio between Pi in CPT treatment to control was 0.6.

3.5 Effect of CPT and MTX on MCF-7 Motility

Motility of MCF-7 breast cancer cells treated with 5µM MTX or 100 µM of CPT resulted in a non-significant decrease by 13.3% and 17.4% respectively compared to the untreated cells (Fig. 8) as observed by time-lapse assay.
Fig. 6. Chromatogram showing the separation pattern of lipids extracted from plasma membrane of CPT treated MCF-7 cells

Fig. 7. Chromatogram showing the separation pattern of lipids extracted from plasma membrane of MTX treated cells

Fig. 8. Effect of CPT and MTX on MCF-7 cells’ motility

Results represent mean of 3 determinations ± SD. (P-value 0.492 for CPT and 0.341 for MTX)
4. DISCUSSION

In this study, we aimed at examining the effect of the anti-cancer drugs CPT and MTX on the lipid levels of the plasma membrane of human breast cancer cell line (MCF-7). In addition, we investigated their effects on the invasive properties of these cells.

A 50% decrease in MCF-7 viability was obtained at a CPT concentration of 100 µM. Higher concentrations of the drug up to 1mM were not accompanied by any enhanced cytotoxicity on MCF-7 cells. CPT was shown to be cytotoxic on several cancer cell lines with different reported IC50. For example, IC50 was 289 µM in human urinary bladder cancer cell line (TCC) [24], 0.016 µM in four leukemic cell lines (CEM, HL60, U937 and K562) [3], and 1.32 µM in human ovarian cancer cell lines (A721, A90, A286, AI, and A121A) [25]. Other study performed on epithelial ovarian cancer cell lines (SKOV-3 and OVCAR-3) showed that OVCAR-3 was sensitive to CPT, with an IC50 of 57.3 µM, while SKOV-3 cells were resistant to CPT with an IC50 of 211 µM. In addition, CPT was shown to inhibit the viability of the ovarian cancer cells BG-1 with an IC50 of 8 µM [26]. The IC50 of CPT on MCF-7 cells determined by Kwon et al. was 62.19 µM after 72 hrs incubation [27] while Zhou et al. obtained IC50 of 120 µM with similar incubation time [28].

As for the effect of MTX on the viability of MCF-7, cell viability was almost the same (~50% cell death) at MTX concentrations ranging between 0.5µM and 5µM. However, at 10 times higher drug concentrations, the maximum decrease in viability obtained was only 35%.

Generally, the cytotoxicity of anti-cancer drugs is directly correlated with its uptake and cellular accumulation. The obtained effects of both drugs on the viability of MCF-7 cells in our study might be attributed to several factors. First, the drugs could have been chelated by the proteins found in the FBS (we can exclude this possibility as we omitted the FBS from the media used in our experiments); second, the unavailability of sufficient transporters on the cell surface to allow their entry; third, the likelihood that the parental MCF-7 cells cell passage have developed drug resistance due to the propagation of an inherently resistant subclone. Additionally, the cell passage might play a role in the cytotoxicity assay.

CPT enters the cell through active transport via the major copper influx transporter, the copper transporter 1 (Ctr1), considered as the main path of platinum drugs import into the cell [29]. Ctr1 controls the cytotoxicity of platinum drugs by affecting drug uptake. Absence of Ctr1 expression significantly reduced the cellular uptake of platinum drugs making mammalian cells in vitro and in vivo resistant to these drugs [30]. Quantitation of the cellular CTR1 mRNA and protein in plasma membrane should be performed to confirm this notion.

On the other hand, MTX primarily enters the cell by an active transport mechanism through the action of the reduced folate carrier (RFC). At a very high concentration (> 20 µM), the drug may enter through passive diffusion. Some studies proved that acidic pH may facilitate drug entrance through passive diffusion and may hinder entrance through active transport. Folic acid may also hinder MTX uptake by the cells since it binds to the RFC transporter thus competing with the drug [31]. The concentration of folic acid in RPMI medium is 2.26 µM almost half the concentration of MTX used in this study. Hence, we speculate that the effect seen may be due to the competition between the two chemicals for RFC binding thus, leading to uptake defects, target amplifications or alterations in polyglutamation. Furthermore, reduction in the RFC expression on the surface of the cancer cells may contribute to reduced potency.

Cancer cells perform their activities through their plasma membranes which are considered as
potential target for chemotherapeutic drugs. These activities are reflected by alterations in the membrane lipids composition. Todor et al. [32] studied the total lipid content in cisplatin and doxorubicin-sensitive and resistant human breast cancer cells (MCF-7). Marked differences in the total lipid composition between sensitive and resistant MCF-7 cell strains were found. Cholesterol as well as cholesterol esters content were significantly higher but diacylglycerols content were significantly lower in resistant cell strains than in parental cells. In addition, the analysis of individual phospholipids showed increased levels of sphingomyelin, phosphatidylserine, cardiolipin, phosphatidic acid and decreased levels of phosphatidylethanolamine, phosphatidylcholine in cisplatin and doxorubicin resistant MCF-7 cells compared to sensitive MCF-7 cells [32]. The same study found that alterations and changes in the lipid composition of MCF-7 cancer cells were accompanied with the formation and development of resistance to anticancer drugs. A decrease in membrane fluidity is caused by increased levels of cholesterol and sphingomyelin, and cytotoxic substances elimination by means of P-glycoprotein is activated by increased phosphatidylserine content. Additionally, characterization of the plasma membrane lipid content of non-metastatic human colorectal cancer cells was studied [14]. The obtained results showed a modification of the plasma membrane structure and function in neoplastic lesions reflected by changes in the membrane’s phospholipids and free fatty acids content. Cancer transformation was accompanied by an increase in total phospholipids as well as a decrease in free fatty acids levels. Our results did not show an overall change in the plasma lipids content as measured by inorganic phosphate content; however, this does not reflect changes at the level of the different types of phospholipids. The changes in Pi in the different collected peaks confirm our suggestion (data not shown for both drugs).

Cancer research has used proteomics as a tool for the identification of biomarkers and chemotherapeutic targets. Recently, lipidomics have gained a great attention as a way to detect biomarkers and eventually a potential therapeutic target. A change in the lipidomics of cancer cells may affect the way this cancer is approached. Alteration in the levels of different plasma membrane lipids might open new perspectives in identifying efficient drugs that can overcome classical resistance mechanisms exhibited by cancer cells.

As for our second aim that targets the effect of CPT and MTX on MCF-7 invasiveness, our results showed that CPT at 100μM caused a 13.3% decrease in the motility of MCF-7 compared to the control cells while MTX gave a 17.4% at 5 μM. However, these decreases were not significant. Invasiveness and metastasis are the main cause of death of breast cancer patients [33]. Chemotherapy drugs play a crucial and important role in regulating and controlling cell invasion and metastasis. Menhofer et al. [34] showed that chondramide exerts its effect by disrupting the actin cytoskeleton and hence decrease the ability of cells for contraction. Chen et al. [35] studied the effect of CPT on the level of actin polymerization and showed that actin binds CPT and leads to decreased expression and disorganization of several cytoskeletal proteins providing a molecular and cellular basis for the known defect in endocytosis in these cisplatin resistant cells. Hence, CPT may play a role in inhibiting cellular contractility and thus cellular invasion.

5. CONCLUSIONS

Our results show that both CPT and MTX lead to changes in protein level in the plasma membrane of MCF-7 cells. However, they exhibited an insignificant decrease in the amount lipids of these membranes. Furthermore, both drugs did not lead to significant decrease in cell motility compared to control.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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27. Kwon Y, Park JY, Kim WK. In vitro histoculture drug response assay and in


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