

Original Research Article

Effect of Carboplatin and Methotrexate on Lipid Levels in the Plasma

Membrane of MCF-7 Cells and their Association with Cell Motility

Abstract:

We examine in this study the effect of two antitumor drugs Metothrexate (MTX) and Carboplatin (CPT) on the phospholipid content of plasma membrane of breast cancer cell MCF7. MTX is a folate analog that inhibit dihydrofolate reductase. CPT is a platinum-containing antineoplastic drug that is an analog of cisplatin. It binds with DNA to form intrastrand crosslinks and adducts that cause changes in the conformation of the DNA and affect DNA replication. IC50 for both of these drugs were determined. Proteins concentration and extracted plasma membrane lipids were quantified from control and treated cells. Effect of the two drugs on cell motility was determined. MTX induced 50% cell death at a range of concentration from 50 to 100 μ M. No further decrease in viability was seen above this concentration even at 1 mM. Cpt induced 50% cell death at 5 μ M. It also showed a range concentration that gives a 50% cell death after 24 hrs of incubation. The plasma lipid content of MCF7 were extracted using Blye and Dyer, seperated by HPLC using a C18 reverse-phase column, and compared in presence and absence of the two drugs. The ability of both drugs to affect cell movement was studied using a motility assay. Images of cells moving were collected over a period of 2 hours. Our results show that both drugs did not affect the amount of lipids but lead to doubling in the protein concentration in the plasma membrane. After membrane lipids separation, comparison of Carboplatin treated and control cells showed no change in their separation patterns. However, Methotrexate showed a single change in the lipid eluted at 4.36 min. Cell motility decreased 13.3% and 17.4% with Carboplatin and Methotrexate respectively.

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. Methotrexate, 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, Carboplatin) 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-cyclobutanedicarboxylato)platinum(II), commercially available under the name of Paraplatin or Platinol, is a second-generation 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 Carboplatin-based treatments is considered standard treatment for many cancers (6).

The uptake of Carboplatin 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).

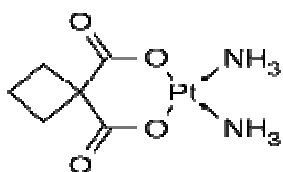


Figure 1. Chemical structure of Carboplatin.

On the other hand, Methotrexate (MTX) (Figure 2), is a folic acid analogue also used in cancer chemotherapy. It is a potent inhibitor of dihydrofolate reductase (DHFR) which is a key enzyme for the biosynthesis of purine and thymidine. 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).

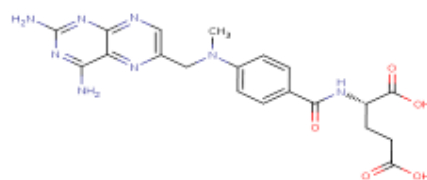


Figure 2. Chemical structure of Methotrexate.

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MTX is transported into cells by reduced folate carrier (RFC) via an 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 cell 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

86 membrane consists mainly of protein and lipids linked together by non-covalent
87 interactions with a mass ratio ranging from 1:4 to 4:1 (10,11).

88 In 1972, Singer and Nicolson described the structural feature of the plasma
89 membrane as fluid mosaic. Individual lipid and protein in membranes have an
90 asymmetric pattern and are free to change constantly (11). Lipids can move either
91 laterally in the plane of the membrane or transversally from one leaflet to the other by
92 flippases, floppases and scramblases (12). Lipids perform multiple important and vital
93 biological roles at cellular and organismal levels. They act as fuel molecules, are
94 crucial structural components of membranes, and considered important signaling
95 molecules (10, 13).

96 Phospholipids are the most abundant lipids in all biological membrane.
97 Phospholipids play essential roles for cell survival. They are involved in the equilibria
98 of the electric charge between the membrane and the surrounding solutions
99 components (14).

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

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

108 Major hallmarks of cancer are the abnormalities in phospholipids metabolism.
109 Larger amounts of phospholipids were shown to be present in various types of tumors
110 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 PC, PI, PE and 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 drug Carboplatin and methothrexate 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.

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126 **Materials and Methods:**

127 **1. Cell strain and culture**

128 MCF-7 (human breast cancer cell line) (cat# HTB-22) used in this study was
129 purchased from the American Type Culture Collection (ATCC), Manassas, VA, US.
130 Cells were grown in RPMI medium supplemented with 10% FBS and 2% PEN-Strep
131 at 37°C in a humidified incubator containing 5% CO₂.

132 **2. *In vitro* cytotoxicity assay (MTT Assay)**

133 Cell Proliferation Kit I (MTT) (cat# 11699709001) purchased from Roche was
134 used to determine cell viability. MCF-7 cells were seeded in a flat bottomed 96-well
135 plate at a density of 5000 cells/well in a final volume of 100µl medium in a

136 humidified incubator with 5% CO₂ for 24 h. The cells were treated with 100 µl of
 137 different concentrations of Methotrexate (0.1µM to 50 µM) or Carboplatin ((1 µM to
 138 1 mM) suspended in media lacking FBS and incubated for 24 h. Media was added to
 139 the control. 10µl of 3-(4-5 dimethyl thiazol-2yl)-2,5 diphenyl tetrazolium bromide
 140 (MTT reagent) were then added and the plate was incubated for 4 h, followed by the
 141 addition of 100µl of solubilizing reagent and overnight incubation. The viability of the
 142 cells was determined by the formation of purple formazan product. The absorbance of
 143 the color obtained was read at a wavelength of 595 nm using an ELISA plate reader.
 144 The percent survival was measured according to the following equation:

$$145 \quad \% \text{ survival} = (\text{abs of treated cells} / \text{abs of control cells}) * 100.$$

146 **3. Membrane Isolation**

147 Plasma membrane isolation was carried out as described by Lin *et al.* (18)
 148 with a minor modification. MCF-7 cells were seeded at a density of 2x10⁶ cells/10ml
 149 and then treated with metothrexate and Carboplatin at their determined IC₅₀
 150 for 24 h. Both control and the treated cells were harvested, washed twice with saline,
 151 and pelleted by centrifugation at 1500 rpm for 5 min. Cell pellets were homogenized
 152 in 500µl HEPES-Mannitol solution for 5 min using a 27 gauge needle. The volume
 153 was made up to 3 ml followed by addition of 1M CaCl₂ to a final concentration of 10
 154 mM. Homogenate was stirred for 10 min to evenly distribute the Ca²⁺ cation and then
 155 centrifuged at 3000xg for 15 min. The obtained supernatant was collected and
 156 ultracentrifuged at 100000xg for 24 h using a Sorvall WX Ultra Centrifuge in AH-627
 157 rotor to pellet the plasma membrane. All steps were performed at 4°C.

158 **4. Enzymatic characterization of isolated plasma membrane from MCF-7**

159 The purity of the extracted plasma membrane was assessed by measuring the
160 activities of a membrane specific enzyme as well as marker enzymes for endoplasmic
161 reticulum and mitochondria. The presence of 5'-nucleotidase activity and absence of
162 glucose-6-phosphatase and succinate dehydrogenase from the plasma membrane
163 preparation confirmed its purity from both ER and mitochondria, respectively. Purity
164 of the plasma membrane was assessed by measuring the activity of 5'-Nucleotidase as
165 described by Aronson and Touster (19). Absence of ER and mitochondrial
166 contaminants was confirmed by assessing the activity of glucose-6-phosphatase, and
167 Succinate Dehydrogenase as described by Aronson and Touster (19) and Bonner (20)
168 respectively.

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170 **5. Protein quantification**

171 Proteins concentrations of the cell homogenates and the different fractions
172 were estimated using Bradford assay. Concentrations were determined from a
173 standard curve obtained using different amount of 0.01% of BSA.

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175 **6. Extraction of the plasma membrane phospholipids**

176 The Bligh and Dyer (21) method was used to extract the total lipids from the
177 isolated plasma membrane. Membranes were suspended mixed with
178 chloroform/methanol (1:2), vortexed very well. Double distilled water was added to
179 the mixture with vigorous vortexing. Samples were then centrifuged at 1000 rpm for 5
180 min at room temperature to separate the aqueous and organic layers. The chloroform
181 layer containing the extracted phospholipids was collected for analysis. The extraction
182 procedure was repeated 3 times on the aqueous layer to remove any residual lipids.

7. Total inorganic phosphate determination

The total inorganic phosphate was determined as described by Oliver H. Lowry and Jeanne A. Lopez with slight modifications (22). 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.

8. Separation of lipids by HPLC

The isolated phospholipids were separated by isocratic RP-HPLC. The separation was performed using a HPLC Discovery HS C18 chromatographic column (25 cm, 4.6 mm, 5 µm) using an isocratic elution 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.

9. Motility Analysis by Time Lapse Assay

Cells were plated on a 35mm petri dish with a density of 2x10⁴ cells/well and incubated under optimal conditions in a humidified incubator with 5% CO₂ at 37°C for 24 h. Carboplatin at IC₅₀ (100 µM) or Methotrexate (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 the average of the distance was measured.

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 $\pm$ the standard deviation (SD). Statistical significance was tested using ANOVA test. P-values less than 0.05 were considered significant.

Results:

1. Effect of Carboplatin on MCF-7 viability

The cytotoxicity of Carboplatin and Methotrexate on MCF7 cells was evaluated in an *in vitro* assay using MTT assay. Cells grown in RPMI media without FBS were treated with Carboplatin or Methotrexate at concentration varying from 1 μ M to 1 mM and from 0.1 μ M to 50 μ M respectively for 24 hours. IC₅₀ was reached at a concentration of 100 μ M for Carboplatin and 5 μ M for Methotrexate (Figure 3 and 4). Additional concentration of Carboplatin up to 1 mM did not show any enhanced cytotoxicity. The same was true for Methotrexate.

Methotrexate on the other hand gave a 50 percent decrease in viability at 5 μ M. Lower doses did not show dose dependent decrease. Higher doses up to 50 μ M did not have enhanced killing effect as well both in the presence and absence of FBS.

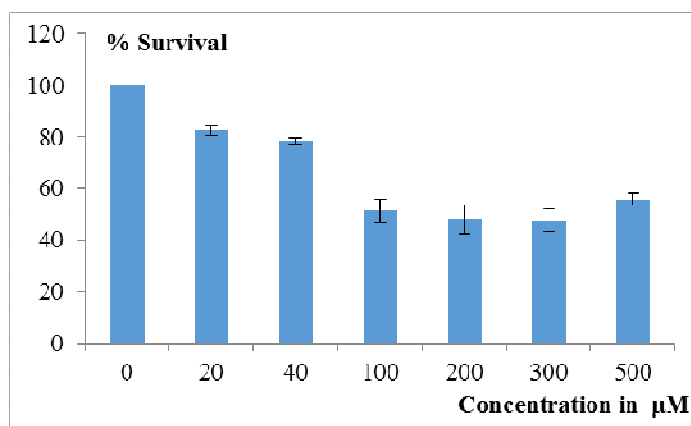


Figure 3. Effect of Carboplatin on MCF-7 viability using MTT assay. Cells were cultured for 24 hours under optimal conditions with Carboplatin at concentrations ranging from 0 to 500 μM . Results represent mean of 3 determination $\pm$ SD.

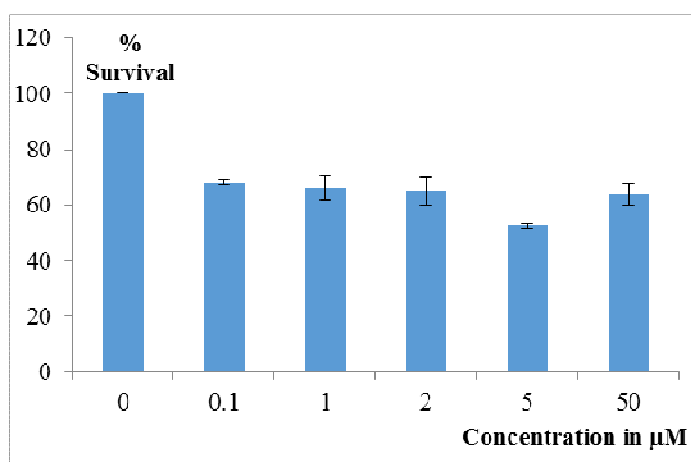


Figure 4. Effect of Methotrexate on MCF-7 viability using MTT assay. Cells were cultured for 24h under optimal conditions with Methotrexate at concentrations ranging from 0 to 50 μM . Results represent mean $\pm$ SD.

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 Carboplatin or Methotrexate treatment. However, the

244 concentration of proteins in plasma membrane increased twice after treatment with
245 both drugs.

246 **Table 1.** Protein concentrations of cell homogenate and isolated plasma membrane from control and
247 Carboplatin or Methotrexate treated cells. Results represent $\pm$ SD of 3 different determinations of three
248 different experiments. P-value in Carboplatin treatment were 0.218 and 0.039 for the cell homogenate
249 and the plasma membrane respectively. For Methotrexate treatment, the p-values were 0.263 and 0.023
250 for the homogenate and the plasma membrane respectively.

Sample	Total protein in control (μg)	Total protein after Carboplatin treatment (μg)	Total protein after Methotrexate treatment (μg)
Total cell homogenate	640.7 ± 8.29	727.2 ± 60.53	591.6 ± 38.56
Plasma membrane	25.12 ± 4.12	51.1 ± 3.5	49.84 ± 5.22

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252 **3. Total inorganic phosphate determination**

253 The amount of inorganic phosphate was determined as described under
254 material and methods. Table 2 summarizes the results obtained. Upon comparing
255 control with Carboplatin treated cells, no significant changes in inorganic phosphate
256 were detected in purified plasma membranes and in total lipids extracted from plasma
257 membranes in Carboplatin treatment. The same is true for Methotrexate treatment
258 except for total cell homogenate.

Sample	Total inorganic phosphate in control cells	Total inorganic phosphate in Carboplatin-treated cells	Total inorganic phosphate in Methotrexate-treated cells
Total cell homogenate	960 ± 6	805.8 ± 65	664.33 ± 82

Plasma membrane	199 ± 29	190 ± 30	116 ± 6
Lipids extracted from plasma membrane	118 ± 12	106 ± 4	105 ± 3

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Table 2. Amount of inorganic phosphate in control and Carboplatin or Methotrexate treated cells obtained from total cell homogenate, plasma membranes, and their extracted lipids. Results represent $\pm$ SD of 3 different determinations of three different experiments. P-value for Carboplatin treatment for the cell homogenate, plasma membrane and the extracted lipids were 0.141, 0.845 and 0.156 respectively. As for Methotrexate treatment, the p values for cell homogenate, plasma membrane and the extracted lipids was 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 Carboplatin and Methotrexate treatment respectively (from 7.96 nmol/ml/ μ g of protein to 2.32 nmol/ml/ μ g in methothrexate treatment and to 3.72 nmol/ml/ μ g in Carboplatin treatment). In the lipids extracted from the plasma membrane, it decreased about 2.4 times (from 4.72 nmol/ml/ μ g to 2.0 nmol/ml/ μ g 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/ μ g of protein versus 1.1 for both treatment).

4. Separation of lipids by HPLC

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

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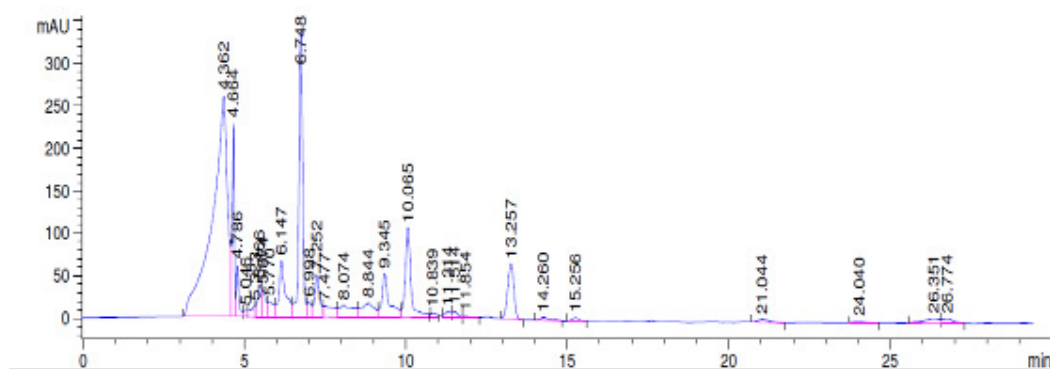


Figure 7. Chromatogram showing the separation pattern of lipids extracted from plasma membrane of Methotrexate-treated cells.

5. Effect of Carboplatin on MCF-7 motility

The motility of MCF-7 breast cancer cells treated with 5 μ M Methotrexate or 100 μ M of Carboplatin resulted in a non-significant decrease by 13.3 % and 17.4% respectively compared to the untreated cells (Figure 8) as observed by time-lapse assay.

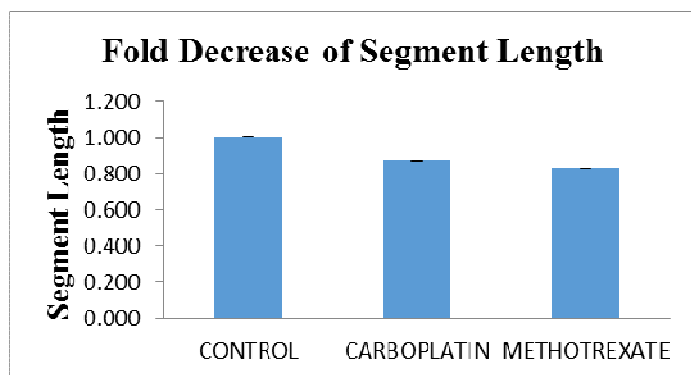


Figure 8. Effect of Carboplatin and Methotrexate on MCF-7 cells' motility. (P-value 0.492 for Carboplatin and 0.341 for Methothrexate).

Discussion:

In this study, we aimed at examining the effect of the anti-cancer drugs Carboplatin and Methotrexate on the lipid levels of the plasma membrane of human

312 breast cancer cell line (MCF-7). In addition, we investigated their effects on the
313 invasive properties of MCF-7.

314 A 50% decrease in MCF-7 viability was obtained at a Carboplatin
315 concentration of 100 μ M. Higher concentrations of Carboplatin up to 1mM were not
316 accompanied by any enhanced cytotoxicity on MCF-7 cells. Carboplatin was shown
317 to be cytotoxic on several cancer cell lines with different reported IC₅₀. For
318 example, IC₅₀ was 289 μ M in human urinary bladder cancer cell line (TCC) (23),
319 0.016 μ M in four leukemic cell lines (CEM, HL60, U937 and K562) (3), and 1.32
320 μ M in human ovarian cancer cell lines (A721, A90, A286, A1, and A121A) (24).
321 Other study performed on epithelial ovarian cancer cell lines (SKOV-3 and OVCAR-
322 3) showed that OVCAR-3 was sensitive to Carboplatin, with an IC₅₀ of 57.3 μ M,
323 and SKOV-3 was resistant to Carboplatin, with an IC₅₀ of 211 μ M. In addition,
324 Carboplatin was shown to inhibit the viability of the ovarian cancer cells BG-1 with
325 an IC₅₀ of 8 μ M (25).

326 As for the effect of MTX on the viability of MCF-7, cell viability was almost
327 the same (~50% cell death) at Methotrexate concentrations ranging between 0.5 μ M
328 and 5 μ M. However, at 10 times higher drug concentrations, the maximum decrease in
329 viability obtained was only 35%. Methotrexate (MTX) has been actively used in the
330 treatment in several malignancies, including head and neck, breast, and bone tumours,
331 leukemias and lymphomas (8). It is generally established that the viability of cancer
332 cells is inversely proportional to the increase in the concentration of drug used. In this
333 study, cell viability was almost the same (~50% cell death) at Methotrexate
334 concentrations ranging between 0.5 μ M and 5 μ M. However, at 10 times higher drug
335 concentrations, the maximum decrease in viability obtained was only 35%.

336 Generally, the cytotoxicity of anti-cancer drugs is directly correlated with its
337 uptake and cellular accumulation. The obtained effects of both drugs on the viability
338 of MCF-7 cells in our study might be attributed to several factors. First, the drugs
339 could have been chelated by the proteins found in the FBS; second, the unavailability
340 of sufficient transporters on the cell surface to allow their entry; thirdly, the likelihood
341 that the parental MCF-7 cells cell passage have developed drug resistance due to the
342 propagation of an inherently resistant subclone. Additionally, the cell passage might
343 play a role in the cytotoxicity assay.

344 Carboplatin enters the cell through active transport via the major copper influx
345 transporter, the copper transporter 1 (Ctr1), considered as the main path of platinum
346 drugs import into the cell (26). Ctr1 controls the cytotoxicity of platinum drugs by
347 affecting drug uptake. Absence of Ctr1 expression significantly reduced the cellular
348 uptake of platinum drugs making mammalian cells *in vitro* and *in vivo* resistant to
349 these drugs (27). Quantitation of the cellular CTR1 mRNA and protein in plasma
350 membrane should be performed to confirm this notion.

351 On the other hand, Methotrexate primarily enters the cell by an active
352 transport mechanism through the action of the reduced folate carrier (RFC). At a very
353 high concentration ($> 20\mu\text{M}$), the drug may enter through passive diffusion. Some
354 studies have proved that acidic pH may facilitate drug entrance through passive
355 diffusion and may hinder entrance through active transport. Folic acid may also hinder
356 Methotrexate uptake by the cells since it binds to the RFC transporter thus competing
357 with the drug (28). The concentration of folic acid in RPMI medium is $2.26\ \mu\text{M}$
358 almost half the concentration of Methotrexate used in this study. Hence, we speculate
359 that the effect seen may be due to the competition between the two chemicals for RFC
360 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 a potential target for chemotherapeutic drugs. These activities are reflected by alterations in the membrane lipids composition. Todor *et.al.* studied the total lipid content in cisplatin and doxorubicin-sensitive and resistant human breast cancer cells (MCF-7) (29). 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 (29). 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

386 measured by inorganic phosphate content; however, this does not reflect changes at
387 the level of the different types of phospholipids. The changes in Pi in the different
388 collected peaks confirm our suggestion (data not shown for both drugs).

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

396 As for our second aim that targets the effect of Carboplatin and Methotrexate
397 on MCF-7 invasiveness, our results showed that Carboplatin at 100µM caused a
398 13.3% decrease in the motility of MCF-7 compared to the control cells while
399 Methotrexate gave a 17.4 % at 5 µM. However, these decreases were not significant.
400 Invasiveness and metastasis are the main cause of death of breast cancer patients (30).
401 Chemotherapy drugs play a crucial and important role in regulating and controlling
402 cell invasion and metastasis. Menhofer *et. al.* showed that chondramide exerts its
403 effect by disrupting the actin cytoskeleton and hence decrease the ability of cells for
404 contraction (31). Chen *et.al* studied the effect of Carboplatin on the level of actin
405 polymerization and showed that actin binds Carboplatin and leads to decreased
406 expression and disorganization of several cytoskeletal proteins providing a molecular
407 and cellular basis for the known defect in endocytosis in these cisplatin resistant cells
408 (32). Hence, Carboplatin may play a role in inhibiting cellular contractility and thus
409 cellular invasion.

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