

Reactive changes of cytophysiological properties, molecular-biological profile and functional metabolic status of cells in vitro with different sensitivity to cytostatic agents under the influence of magnetic fluid

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Abstract: The peculiarities of changes of phenotype properties in MCF-7 human breast cancer cells with different sensitivity to cytostatic agents under the influence of nanoparticles of magnetic fluid were studied. It is defined that the biggest manifestations of magnetic fluid effect both by its essence and direction are observed in cells of resistant MCF-7/Dox line and parental MCF-7/S line in comparison with MCF-7/CP (cisplatin-resistant cell line). In particular, these are more apparent cytotoxic effects and ability to colony-formation, increase of adhesive properties, decrease of invasiveness and proliferative activity, changes of surface charge density and electrokinetic potential as well as lipid composition of cells. The defined differences between MCF-7 cells with different sensitivity to cytostatic agents according to phenotypic manifestations of correlations (magnetic fluid effect – reactive response) are evidence of the individual peculiarities of free radicals formation in cells and development of oxidative stress. The indicated phenomenon of selective reactive changes in cells by effect of magnetic fluid is necessary to take into account in development of systems of directed transport of antitumor drugs.

Keywords: MCF-7 Cells, Nanoparticles of Magnetic Fluid, Drug Resistance, Cytotoxic Effects, Adhesion, Invasion, Proliferative Activity, Free Radical Processes, Oxidative Stress, Lipids

1. Introduction

The modern achievements of medicament therapy of patients with disseminated forms of malignant neoplasms don't exclude the constant search for the new approaches to elaboration of more effective antitumor drugs involving the most progressive and perspective technologies [1]. Such focus of studies is connected, from the one hand, with the fact that most of chemotherapy drugs, which won wide recognition in clinical practice, possess the high toxicity; from the other hand, in a great number of primary oncologic patients is defined the resistance of tumors to the majority of present cytostatic agents. In addition, it is known that resistant to chemotherapy tumor cells are

characterized by high invasive potential, migration ability and as the result – ability to get metastatic phenotype [2].

The modern conceptions of molecular-biological changes in cells define novel strategy of solving the problem of drug resistance overcoming based on application of targeted technologies. In particular, the prospect of elaboration of system of directed transport became possible due to the reception and usage in its composition of metal-based nanomaterials of different origin [3-5]. Such substances, especially metal-based nanoparticles, are of interest, because in the form of nanostructures they get unique properties, which radically distinguish them from their macroscopic analogs and till present time are not enough studied on biological objects. The most perspective resources for construction of antitumor systems of directed

transport are considered to be ferromagnetic liquids, which contain nanoparticles of magnetite – iron oxide from 15 to 75 nm size and substances of pharmacopeial cytostatic agents on the assumption of usage of sources with specially configured outer magnetic field [6-8].

The less studied factor of above-mentioned triad of system of directed transport components are nanoparticles of magnetite (iron oxide). In recent times, it became known that nanoparticles can interact with nucleic acids and proteins in cells, build themselves in membranes, penetrate in cellular organelles and, in that way, change the functions of biostructures [9]. In addition, the accumulation of exogenous pool of iron may influence the molecular epidemiology of ferrous proteins and essentially modify their functional significance [10, 11].

To assess the effectiveness of application of generated ferrous nanomaterial in construction of medical nanocomposites, it is necessary to carry out the series of experimental studies, mainly *in vitro*, for the clarification of changes occurring during the accumulation of this substance in tumor cells.

The aim of our study was to assess the reactive changes, in particular the molecular profile, invasive-metastatic properties and functional-metabolic status of MCF-7 human breast cancer cells with different sensitivity to cytostatic agents caused by effect of magnetic fluid (MF) in different concentrations of nanoparticles.

2. Materials and Methods

The investigation was carried out *in vitro* on cultures of human breast cancer cells of the line MCF-7, sensitive (MCF-7/S) and resistant to cisplatin (MCF-7/CP) and doxorubicin (MCF-7/Dox). The cells of parental line (sensitive to antitumor drugs) were cultivated in modified culture medium Dulbecco ISCOVE (Sigma, Germany) with adding of 10% of fetal bovine serum ('Sangva', Ukraine) at the temperature of 37°C and CO₂ concentration of 5%. The cells have been resowed twice a week with seeding density 2-4x10⁴ cells/cm². From the parental cellular line were obtained resistant to anticancer drugs variants: MCF-7/CP – resistant to cisplatin and MCF-7/Dox – to doxorubicin. The anticancer drugs resistant cells were obtained by growing of cells in culture medium with adding of rising concentrations of cisplatin or doxorubicin. Every two months studied cells were analyzed in order to determine the level of their resistance with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT viability test). On the moment of investigation the level of resistance of MCF-7 cells was 4 for cisplatin and 8 –for doxorubicin.

2.1. MTT Assay

After incubation period 10 µl of MTT were added into every well of 96-well plate. Cells were incubated at 37°C in humid atmosphere for 3 hours and then centrifuged (1500

rpm for 5 min). Violet crystals of formazan were visually detected on the bottom of wells. After removal of the supernatant 50 µl of DMSO were added into wells to dissolve formazan crystals. After 20 min of incubation at room temperature crystals totally dissolved. Optical density in wells was measured by multiwell spectrophotometer (Labsystems Multiskan PLUS) at 540 nm [12].

In research we studied whether sensitivity of cells and cytotoxic effect were in relation to MF influence, peculiarities of colony-formation of cells with different sensitivity to cytostatic agents, molecular profile according to the expression of adhesion proteins, proliferative potential, invasive activity of cells as well as cytophysiological peculiarities of cells – electrokinetic potential and total surface charge density of cell in connection with data on lipid content, because status of these components of cells functioning provision, certainly, impact the reception and accumulation of investigated factors.

2.2. Assessment of Cells Sensitivity to the MF

For assessment of sensitivity of MCF-7S cell line and its sublines resistant to anticancer drugs – MCF-7/Dox, MCF-7/CP in studied MF with size of ferromagnetic nanoparticles 5-10 nm in 24-well plate was put the suspension of cells in concentration 3-4x10⁴ cells/well (in 1,5 ml of cultivation medium) and cultivated in a moist atmosphere containing 5% CO₂ at temperature 37°C during 24 hours, after that was added the MF in two concentrations (50 and 100 µg/ml) and incubated during 48 hours in standard conditions. The cells were calculated with the help of hemocytometer after staining with trypan blue, the number of cells was defined according to the formula:

$$A/80 \times 2 = X \cdot 10^6 \text{ cells/ 1 ml of medium} \quad (1)$$

where A – cell number calculated in hemocytometer (in 5 squares), x2 – dissolving with trypan blue (1:1).

The cytotoxic effect of MF was assessed with the help of light-optical investigation of cytological specimens of MCF-7 cells after staining according to the Giemsa method at magnification of microscope x40, x100 (oil immersion). Simultaneously it was made the visualization of MF nanoparticles with the help of our modification of Lillie method [13].

2.3. Colony-Formation Analysis

For determination of cells colony-forming ability were used 0,5% of agarized medium layer, on which was stratified 0,33% agar cell suspension after MF influence and incubated in a moist atmosphere at temperature 37°C and 5% CO₂ during two weeks. The generated colonies were stained and evaluated with the help of MTT assay. The results were assessed according to the number of stained colonies, which consist of 40 and more cells.

2.4. Immunocytochemical Assay

The cells for the immunocytochemical analysis were grown on cover glasses, incubated in fixative solution (methanol + acetone: 1:1) during 2 hours at $t = -20^{\circ}\text{C}$, than – with 1% solution of bovine serum albumin (BSA) for 20 minutes. The monoclonal antibodies for defying of proteins were against CD44 (Diagnostic BioSystems), E-cadherin (ThermoScientific), CD325 (N-Cadherin), β -catenin (Diagnostic BioSystems), Ki-67 (MIB, DakoCytomation, Denmark) were applied in standard conditions of cultivation with compliance of conventional algorithm of methodical approaches [14].

The estimation of results was made with the help of optical microscope (x100, oil immersion) with usage of classical method of H-Score:

$$S = 1 \times N_{1+} + 2 \times N_{2+} + 3 \times N_{3+} \quad (2)$$

where S – «H-Score» index, N_{1+} , N_{2+} and N_{3+} - number of cells with low, medium or high expression of the marker [15].

2.5. Cell Invasive Activity Assay

The invasive cells activity after effect of MF was investigated with the help of standard invasion test according to manufacturer's instruction (BD Biociences). It was used the synergetic membrane for invasion (BD BioCoat™ Matrigel™ Invasion Chamber, Bedford, MA) with 8 micron-sized holes. In the top of well was applied the cell suspension with concentration of 5×10^4 cells/well in nutrient medium without adding of bovine serum, in bottom was put the standard growth medium with 10% fetal bovine serum, after that cells were incubated at standard conditions at the temperature of 37°C in a moist atmosphere with CO_2 concentration of 5% during 48 hours. After that membranes were fixed with the help of methanol and dyed in crystal violet. The cells, which remained in top of wells were washed off and analyzed the invaded cell number by optical microscope (x100, oil immersion).

2.6. Zeta Potential and Total Cell Surface Charge Density Evaluation

The calculation of ζ -potential was based on M.Smoluchowski equation. In equation it was substituted the value of linear speed of cells motility in electric field. This velocity was measured with the help of device, in which cells, slurred in special K/Na-phosphate buffer, moved in the middle of square quartz tube. Due to this were eliminated the extraneous mechanical effects on the character of cells movement and appeared the opportunity of using of microvolumes of studied material.

The spacing between platinum electrodes of device was in 5 cm, electric E field tension gradient run up to 20 V/cm, because the voltage in 100 V was applied to electrodes. The M.Smoluchowski equation adapted to biological objects and, automatically, to physiological values of physicochemical parameters, looks like:

$$\zeta = 14U \quad (3)$$

where U – so called electrophoretic mobility expressed in off-system units in the ratio of linear speed of cells motility V in electric field, defined experimentally ($\mu\text{m/s}$), to the field tension gradient (V/cm).

The direction of cell movement under the effect of electric field with certain parameters in the side of cathode or anode gave the opportunity to define the sign of total cell charge; surface density (q) was calculated according to the Quincke-Helmholtz equation:

$$q\delta = \zeta\epsilon_a \quad (4)$$

where δ – thickness of double electric layer, and ϵ_a – absolute inductive capacity [16].

2.7. Micromethod of Thin-Layer Chromatography

Qualitative and quantitative composition of total lipids and individual phospholipids were defined by micromethod of thin-layer chromatography with usage of plates "Sorbfil" TLC-A ("Imid Ltd", Krasnodar, Russia). From cells MCF-7/S, MCF-7/CP and MCF-7/Dox were extracted lipids by mixture of chloroform:methanol (1:1, v/v). The thin-layer chromatography of total lipids was performed only in one direction in system of solvents hexane: diethyl ether: ice acetic acid (85:15:1, v/v). The thin-layer chromatography of individual phospholipids was performed in two mutually perpendicular directions. First system of solvents was used in ratio – chloroform: methanol: benzol: ammonia (65:30:10:6, v/v), second – chloroform: methanol: benzol: ice acetic acid: water (70:30:10:5:4:1, v/v). After evaporation of solvents system plates were processed by 10% H_2SO_4 in methanol and heated for 5 min. at temperature 180°C . The obtained chromatograms were scanned, for the processing of images was used the PictureJ program. The content of lipids was expressed in percentage.

2.8. Statistical Processing of the Results

Statistical processing of the obtained results was carried out with the help of mathematical program of medical and biological statistics STATISTICA 6.0 and in the environment of Microsoft Excel. Calculation and comparison of the significance of differences between the average values was carried out with usage of Student's t-criterion; correlation analysis was carried out using the Pearson correlation coefficient. Significant were considered the differences with the probability not less than 95% ($P < 0.05$).

3. Results

The results of our previous studies conducted *in vitro* in cultures of human breast cancer (HBC) cells with different sensitivity to cytostatics: sensitive (MCF-7/S), resistant to cisplatin (MCF-7/CP) and to doxorubicin (MCF-7/Dox)

have shown that nanoparticles of magnetite (Fe_3O_4), which are added to the cultural medium, are utilized by tumor cells, but have differences in the accumulation of iron depending on their sensitivity to cytostatic agents [13].

So, in 24 hours after cultivation with ferromagnetic in tumor cells of all three lines – MCF-7/S, MCF-7/CP,

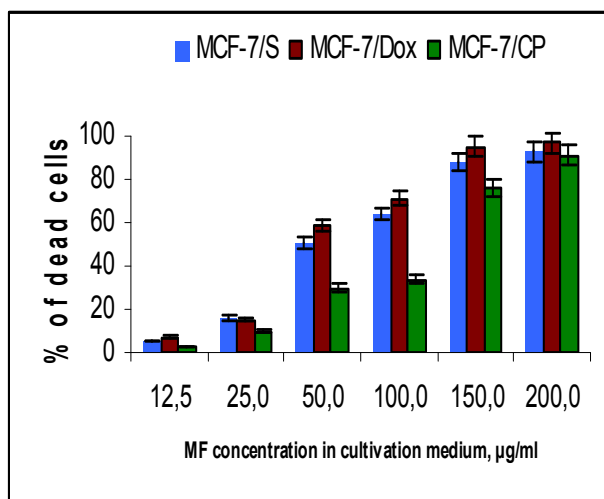


Figure 1. Cytotoxic effect of MF in different concentrations on MCF-7/S, MCF-7/Dox and MCF-7/CP cells. Data is given as $M \pm SD$.

MCF-7/Dox, is observed significant accumulation of nanoparticles of iron. The number of cells with accumulated iron continues to grow up to 48 hours of cultivation; at the same time, the percentage of those, characterized by high content of incorporated ferromagnetic, increases. Dynamics of accumulation of iron in the 72 hour distinguishes itself by stabilization of this process in sensitive (MCF-7/S) and resistant to cisplatin (MCF-7/CP) tumor cells and by the tendency to the further growth in resistant to doxorubicin cells (MCF-7/Dox). It was shown that ferromagnetic nanoparticles are able to arrive in significant amount both to sensitive and resistant to anticancer drugs cells.

The first step on the stage of further studies aimed to determine the peculiarities of functional and metabolic changes in the cells of the line MCF-7 with different sensitivity to cytostatics under the influence of magnetite nanoparticles, was the evaluation of the cytotoxic effect of nanoparticles of magnetic fluid in their use in various concentrations in the same MCF-7 human breast cancer cells.

In previous studies it was shown that the MF in concentrations below 50 µg/ml (12.5 µg/ml and 25 µg/ml) does not provide a significant influence either on parental or on resistant to cisplatin and doxorubicin MCF-7 HBC cells. Instead, the doses of 150 µg/ml and 200 µg/ml caused too strong cytotoxic effect (Fig. 1). Hence, it was used the working concentration of MF of 50 µg/ml and 100 µg/ml. The most significant cytotoxic MF effect comparatively to the control was observed in the MCF-7/Dox cells (up to 70% of the cytotoxicity at MF concentration of 100 µg/ml

and 60% - at concentration of 50 µg/ml). The least impact of MF was detected in the MCF-7/CP cells – up to 30% of antiproliferative/cytotoxic effects in both variants of concentration (Fig. 1). Therefore, the most sensitive to the effect of MF, mainly at the concentration of 100 µg/ml, were MCF-7/Dox cells.

Comparative cytomorphological investigation of MF effect at concentration of 50 µg/mL and 100 µg/ml on the type of human breast cancer cells growth with different sensitivity to cytostatic agents (MCF-7/S, MCF-7/CP, MCF-7/Dox) have shown that the biggest manifestations of the cytotoxic MF effect, mainly in the concentration of 100 µg/ml, is defined in the MCF-7/Dox cells (Fig. 1). On the one hand, this is a change of cytoarchitectonics of significant part of them with getting the gigantic proportions with the rising of nuclei and nucleoli sizes, especially in the cells with a significant accumulation of MF, what results in their becoming look like the “therapeutic giants”, which have been exposed to cytostatic agents; on the other hand, it is viable cells ability to form the symplasts, what is the evidence of strengthening of their adhesive properties.

The same tendency was observed in the studies of the colony-forming ability of cells in semi-fluid agarized medium after the impact on them of MF in a concentration of 50 and 100 µg/mL. The smallest number of colonies was manifested in the MCF-7/Dox and MCF-7/S cells compared to the MCF-7/CP cells (Fig. 2). At the same time, the MF effect in the studied concentrations was not reflected on colony-forming of the cells resistant to cisplatin: percentage of colonies (absolute index) without the influence – 13.2%, at MF concentration 50 µg/ml – $14.1 \pm 2.1\%$, 100 µg/ml – $13.4 \pm 1.2\%$.

Taking in consideration that only the transformed cells posses the ability to colony-formation in semi-fluid agarized medium *in vitro*, and the number of colonies correlates with their malignance and are the sign of tumorigenicity of cells *in vitro*, data obtained by us, allow us to state that the most apparent inhibitive effect on cytological manifestations of malignant properties of MCF-7 cells is observed in MCF-7/Dox cells after the MF effect in both investigated concentrations. Cause in determination of MCF/CP cells colony-formation after influence of MF the number of colonies didn't change even with concentration of 100 µg/mL and was slightly higher than in control (MCF-7/S), we estimate this fact as absence of reactive response of MCF/CP cells to the effect of MF not only by indicators of its vitality investigation, but also tumorigenicity.

The next series of *in vitro* experiments aimed to define molecular profile of cells of studied lines according to indexes of adhesion and proliferation for clarification of es

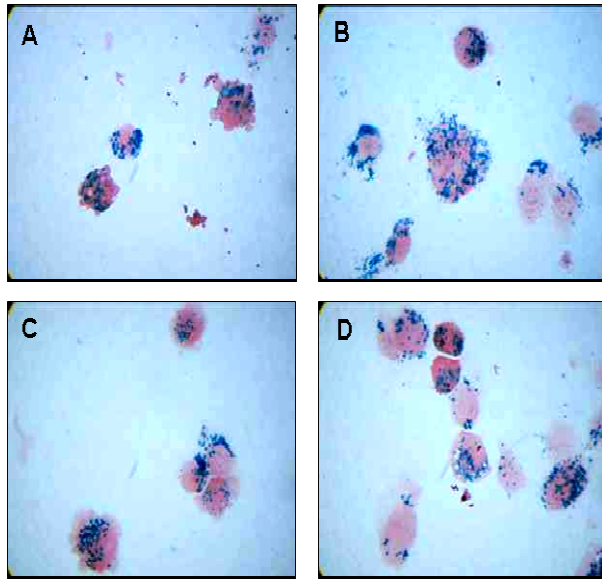


Figure 2. The aftereffects of MF impact on cytoarchitectonics of MCF-7 cells with different sensitivity to cytostatic agents. a) Changes of configuration and effect of blebbing of cytoplasmic membrane in MCF-7/Dox cells, b) MCF-7/Dox cells with big accumulation of MF – 'therapeutic giant', c) less changed cytoarchitectonics of MCF-7/CP cells under the effect of MF, d) formation of symplast by MCF-7/S cells. Staining by modified Lillie method. Magnification. X100, immersion.

Table 1. Immunophenotypic profile of adhesion proteins in MCF-7/S cells after the effect of magnetic fluid

Marker	Level of expression of investigated marker (points)		
	MCF-7/S	MCF-7/S+MF (50 µg/mL)	MCF-7/S+MF (100 µg/mL)
E-cadherin	268±5.7	283±4.6 *	294 ± 4.2*
N-cadherin	59±2.3	11 ±2.5 *	7 ±1.9*
CD44	72 ±3.1	72 ±2.8	70 ±2.6
B-catenin	104 ±2.8	99 ±4.2	102 ±2.4
Ki-67	180 ±4.5	162±3.7 *	157±2.8*

Note: * - $p < 0.05$ comparatively to MCF-7/S cells without effect of agents

Table 2. Immunophenotypic profile of adhesion proteins in MCF-7/CP cells after the effect of magnetic fluid

Marker	Level of expression of investigated marker (points)		
	MCF-7/CP	MCF-7/CP+MF (50 µg/mL)	MCF-7/CP+MF (100 µg/mL)
E-cadherin	57 ±2.7	61 ±2.1	63 ±2.0
N-cadherin	179 ±3.5	169 ±2.8*	169 ±2.6*
CD44	176 ±5.2	171 ±4.1	173±2.9
B-catenin	125 ±4.1	120 ±2.3	127±2.4
Ki-67	140 ±3.8	136±2.9	139±3.2

Note: * - $p < 0.05$ comparatively to MCF-7/CP cells without effect of agents

Table 3. Immunophenotypic profile of adhesion proteins in MCF-7/Dox cells after effect of magnetic fluid

Marker	Level of expression of investigated marker (points)		
	MCF-7/Dox	MCF-7/Dox +MF (100 µg/mL)	MCF-7/Dox +MF (100 µg/mL)
E-cadherin	28 ±2.0	59±4.6*	71 ± 2.2*
N-cadherin	238 ±5.2	171 ±2.5*	153±1.7*
CD44	234 ±4.6	160 ±2.8*	152 ±2.6*
B-catenin	160 ±4.4	147±4.2*	149 ±2.6*
Ki-67	120 ±2.7	84±2.1 *	89±2.3*

Note: * - $p < 0.05$ comparatively to MCF-7/Dox cells without effect of agents

sence of mentioned differences in investigation of colony-forming and cytotoxic effect. It is determined that under the influence of MF in cells of all lines, mostly in MCF-7S and MCF-7/Dox, occur the changes of proteins expression of intercellular adhesion and proliferation: increase of expression of E-cadherin, decrease of expression of N-cadherin, CD44 and Ki-67 (Table 1, 3). The most apparent changes of expression of these molecular markers were observed in MCF-7/Dox cells: double increase of E-cadherin-positive cells as the result of MF effect in concentration 50 µg/mL and 3 times as much as at concentration 100 µg/mL concerning control; decrease on 30% of N-cadherin, CD44- and Ki-67-positive cells after the influence of MF in both concentrations. The obtained facts correspond with essential cytotoxic effects defined in cells of this line. In MCF-7/CP cells the proliferative activity and expression of molecules of intercellular adhesion were almost not changed (Table 2). In comparison to obtained data were defined the strong correlative connections between expression of markers of cellular adhesion and proliferation in all investigated lines. The matrix of correlative connections is showed in table 4. The discovered by us fact of reducing of the number of CD44-positive cells only in the MCF-7/Dox cells deserves attention, what may be the evidence of a significant selective modeling MF effect on the phenotype of these cell lines.

The discovered by us fact of reducing of the number of CD44-positive cells only in the MCF-7/Dox cells deserves attention, what may be the evidence of a significant selective modeling MF effect on the phenotype of the cells of these resistant lines.

Table 4. Correlation coefficient values between expression of adhesion and proliferation markers in MCF-7/S, MCF-7/CP and MCF-7/Dox cells

Markers	Cell line	E-cadherin	N- cadherin	CD44	β -catenin	Ki-67
E- cadherin	MCF-7/S	1	-0.35*	-0.31*	0.06	-0.44*
	MCF-7/CP	1	-0.30*	-0.29*	0.04	-0.45*
	MCF-7/Dox	1	-0.41*	-0.35*	-0.03	-0.36*
N- cadherin	MCF-7/S	-0.35*	1	0.41*	-0.11	0.32*
	MCF-7/CP	-0.30*	1	0.38*	-0.08	0.27*
	MCF-7/Dox	-0.41*	1	0.40*	-0.13	0.34*
CD44	MCF-7/S	-0.31*	0.41*	1	0.07	0.37*
	MCF-7/CP	-0.29*	0.38*	1	0.06	0.44*
	MCF-7/Dox	-0.35*	0.40*	1	0.10	0.35*
β -catenin	MCF-7/S	0.06	-0.11	0.07	1	-0.02
	MCF-7/CP	0.04	-0.08	0.06	1	-0.05
	MCF-7/Dox	-0.03	-0.13	0.10	1	0.04
Ki-67	MCF-7/S	-0.44*	0.32*	0.37*	-0.02	1
	MCF-7/CP	-0.45*	0.27*	0.44*	-0.05	1
	MCF-7/Dox	-0.36*	0.34*	0.35*	0.04	1

Note: * - $p < 0.05$

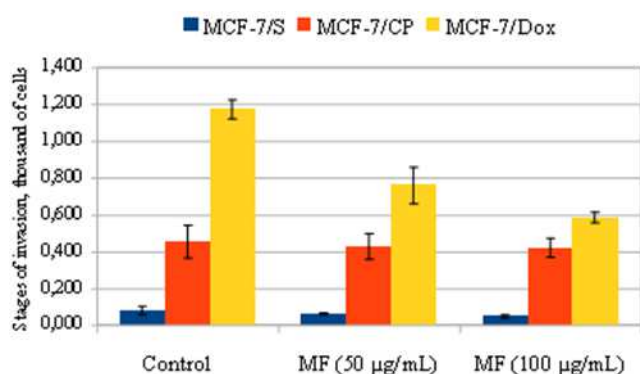


Figure 3. The evaluation of invasive properties of MCF-7 cells with different sensitivity to cytostatic agents after the effect of magnetic fluid in concentration 50 and 100 µg/mL.

Table 5. Changes of electrokinetic potential and total surface charge density of MCF-7 cells under the influence of magnetic fluid

Cell lines	χ , mV ($M \pm m$)	q , $\times 10^{-2}$ C/m ² ($M \pm m$)
MCF-7/S:		
Control	15.47 \pm 1.01	-11.09 \pm 0.72
MF 50 µg/mL	13.78 \pm 0.77	-9.88 \pm 0.55
MF 100 µg/mL	11.11 \pm 0.74	-7.97 \pm 0.53
MCF-7/CP:		
Control	8.86 \pm 0.56	-6.35 \pm 0.40
MF 50 µg/mL	9.98 \pm 0.83	-7.16 \pm 0.60
MF 100 µg/mL	9.65 \pm 0.23	-6.92 \pm 0.1
MCF-7/Dox:		
Control	14.27 \pm 0.95	+10.23 \pm 0.68
mf 50 µg/mL	17.11 \pm 0.74	+12.27 \pm 0.53
mf 100 µg/mL	23.40 \pm 0.96	+16.78 \pm 0.69

Note: χ – electrokinetic potential at pH 7.4, q — total surface charge density.

As it is known, that the CD44 is a multifunctional protein that plays a role in adhesion of cells, characterizes their invasive and migration potential, we carried out a

comparative study of invasive properties of the MCF-7 cells after the impact on them of MF. In spite of the fact that the indicators of ‘initial invasion’ of MCF-7/Dox cells were bigger than indexes of lines MCF-7/S and MCF-7/CP, the MF effect was observed only in the MCF-7/Dox cells. It is defined the decrease of invasive properties of the MCF-7/Dox cells, while the applied concentrations of MF didn’t affect the invasion of MCF-7/CP cells (Fig. 3). The above-mentioned fact coincides with the defined before effects and is an evidence of prospect of MF using as effective component of anticancer composite in effect on cells with specific resistance, in this case with acquired insensitivity to anthracyclines. However, the necessity of further studies for the clarification of MF effect target arises, because the defined facts may be connected with changes both in molecules of cells adhesion in acquisition by them of resistant to antitumor drugs phenotype as well as in molecules, which are directly associated with drug sensitivity – P-gp, MDR and others, especially that the data on close connection between P-gp and CD44 exist [17].

In addition, we decided to clarify the question concerning the existence of connection between distinctive features defined in investigations of such important functions of cells as adhesive and invasive properties according to such criteria as total surface charge density and cells electro kinetic potential, in particular to define, if it is observed in these series of experiments more apparent manifestations of changes in MCF-7/Dox cells in comparison to MCF-7/S and MCF-7/CP cells.

The results of comparisons are the evidence of principal difference between reaction of MCF-7/Dox cells and the same MCF-7/S and MCF-7/CP cells on MF effect in investigated concentrations according to its total surface charge index (Table 5). So, the cells of both last lines reacted both on the fact of influence of MF itself and on double increase of its concentration, by significant (MCF-7/S, $p < 0.05$) or insignificant (MCF-7/S, $p > 0.05$) decrease of

negative values of total surface charge approximately from $-10 \times 10^{-2} \text{ C/m}^2$ to $-7 \times 10^{-2} \text{ C/m}^2$ (approximately 30%). At the same time, in MCF-7/Dox cells the effect of magnetic fluid in concentration of 100 $\mu\text{g/mL}$ caused the increase of values of total surface charge to $+16.78 \pm 0.69 \times 10^{-2} \text{ C/m}^2$.

We state that it may be connected with direct pre-dependent expression of positively charged molecules on surface of cells as the result of MF effect, for instance, molecules of P-glycoprotein (Pgp, MDR1).

Table 6. The content of total lipids (%) in sensitive and resistant MCF-7 strain cells before and after incubation (48 h) with MF (n=7).

Lipids	MCF-7/S		MCF-7/CP		MCF-7/Dox	
	Before	After	Before	After	Before	After
Phospholipids (PL)	12.7 \pm 1.0	14.1 \pm 0.7	17.7 \pm 1.0	17.0 \pm 0.6	18.7 \pm 1.3	19.1 \pm 0.5
Monoacylglycerols (MG)	6.7 \pm 0.1	6.9 \pm 0.3	1.8 \pm 0.2	2.0 \pm 0.4	2.0 \pm 0.4	1.3 \pm 0.3
Cholesterol (Ch)	18.1 \pm 1.8	15.3 \pm 0.5 ▼	29.0 \pm 0.8	30.5 \pm 1.0	28.2 \pm 1.4	24.9 \pm 1.1 ▼
Free fatty acids (FFA)	5.6 \pm 0.4	4.7 \pm 0.4	0	0	0	0
Diacylglycerols (DG)	19.4 \pm 0.7	21.5 \pm 0.6 ▲	17.6 \pm 0.6	18.0 \pm 0.7	15.0 \pm 0.5	17.3 \pm 0.2
Triacylglycerols (TG)	29.6 \pm 0.9	30.2 \pm 1.2	8.3 \pm 0.7	9.0 \pm 0.6	18.1 \pm 1.4	21.4 \pm 1.8
Cholesterol ethers (ChE)	7.9 \pm 1.2	7.3 \pm 0.7	25.6 \pm 0.9	23.5 \pm 1.0	18.0 \pm 1.8	16.0 \pm 1.5

Note:

▲ - significantly higher (P<0.05) than in MCF-7 cells before incubation

▼ - significantly lower (P<0.05) than in MCF-7 cells before incubation

Table 7. The content of individual phospholipids (%) in sensitive and resistant MCF-7 cells before and after incubation (48 h) with MF (n=7).

Lipids	MCF-7/S		MCF-7/CP		MCF-7/Dox	
	Before	After	Before	After	Before	After
Lisophosphatidylcholine (LPC)	5.6 \pm 0.4	5.4 \pm 0.3	6.5 \pm 0.9	6.0 \pm 0.3	5.9 \pm 0.4	5.0 \pm 0.3 ▼
Sphingomyelin (SM)	7.6 \pm 0.4	5.0 \pm 0.2 ▼	12.0 \pm 0.5	11.5 \pm 0.5	9.8 \pm 0.2	6.7 \pm 0.4 ▼
Phosphatidylserine (PS)	6.7 \pm 0.4	6.0 \pm 0.3	8.8 \pm 0.6	8.0 \pm 0.5	9.4 \pm 0.6	7.0 \pm 0.3 ▼
Phosphatidylinositol (PI)	8.4 \pm 0.2	8.1 \pm 0.4	7.3 \pm 1.0	7.2 \pm 0.4	8.8 \pm 0.7	8.2 \pm 0.4
Phosphatidylcholine (PC)	39.4 \pm 2.4	44.4 \pm 2.0	30.2 \pm 3.0	31.2 \pm 2.4	30.2 \pm 3.1	45.0 \pm 3.2 ▲
Phosphatidylethanolamine (PE)	16.4 \pm 0.3	17.3 \pm 1.0	11.8 \pm 0.3	12.0 \pm 0.6	12.8 \pm 0.6	15.1 \pm 0.4 ▲
Cardiolipin (CL)	6.7 \pm 0.3	5.0 \pm 0.3	9.2 \pm 0.4	9.1 \pm 0.5	8.6 \pm 0.2	5.1 \pm 0.3 ▼
Phosphatidic acid (PA)	4.4 \pm 0.7	4.2 \pm 0.2	6.8 \pm 0.2	7.0 \pm 0.3	7.4 \pm 0.3	4.7 \pm 0.4 ▼
Phosphatidylglycerol (PG)	4.8 \pm 0.3	4.6 \pm 0.2	7.4 \pm 0.3	8.0 \pm 0.3	7.1 \pm 0.2	3.2 \pm 0.3 ▼

Note:

▲ - significantly higher (P<0.05) than in MCF-7 cells before incubation

▼ - significantly lower (P<0.05) than in MCF-7 cells before incubation

It was defined that incubation of MCF-7/S and MCF-7/Dox cells with MF nanoparticles during the 48 hours brings to the changes in lipid composition. According to the data, given in tables 6 and 7, the level of cholesterol, sphingomyelin and cardiolipin decreased, and level of diglycerides and phosphatidylcholine, on the contrary, increased.

At the same time, changes in composition of lipids were more manifested in MCF-7/Dox cells, then in MCF-7/S cells. The above-mentioned changes caused the increase of fluidity of membranes and increase of its permeability. However, MF practically didn't influence the lipid composition of MCF-7/CP cells.

So, in *in vitro* experiments we defined the optimal concentration of MF, which besides the cytotoxic effects caused the reactive cytophysiological metabolic changes in MCF-7 human breast cancer cells with the most essential aftereffects in MCF-7/Dox cells. It is important to take in account the mentioned fact of MF effect's selectivity in generation of nanocomposites of directed transport of anticancer drugs. especially with the aim to overcome the

drug resistance.

4. Conclusions

The determination of cytophysiological and molecular-biological profile of MCF-7 cells with different sensitivity to cytostatic agents is evidence of that the most manifested reactive changes according to all investigated indexes are observed in MCF-7/Dox and MCF-7/S cells in comparison to MCF-7/CP with prevalence of changes in resistant to doxorubicin cells. In particular, they are: decrease of invasive properties and metastatic potential, changes of molecular profile of cell-cell adhesion proteins, (increase of E-cadherin and decrease of N-cadherin, CD44 and Ki-67 expression).

At the same time, in resistant to doxorubicin cells we defined more essential changes in total surface charge density and electrokinetic potential as well as in lipid content what caused increase in the fluidity of cytoplasmic membranes, and, generally, resulted in the increase of cells sensitivity to cytostatic drugs (according to

data of our previous studies) due to more active uptake and longer contact.

So, the defined differences in changes of biological properties of MCF-7 tumor cells under the influence of MF with prevalence in resistant to doxorubicin cells must be taken into account in development of nanocomposites. MF with mentioned modifying effects together with effect of anticancer drugs may function itself as a factor which will enhance and amplify the anticancer activity of cytostatics in generated nanosystems.

References

- [1] A.L. Lainé and C. Passirani, "Novel metal-based anticancer drugs: a new challenge in drug delivery," *Curr. Opin. Pharmacol.*, vol.12, pp.420-426, August 2012.
- [2] S. Alexander, P. Friedl, "Cancer invasion and resistance: interconnected processes of disease progression and therapy failure," *Trends Mol. Med.*, vol. 18, pp. 13-26, January 2012.
- [3] R. Ghosh, L.C. Singh, J.M. Shohet and P.H. Gunaratne. "A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells," *Biomaterials*, vol. 34, pp. 807-816, January 2013.
- [4] X. Chao, Z. Zhang, L. Guo, J. Zhu, M. Peng, A.J. Vermorken, W.J. van de Ven, C. Chen and Y. Cui, "A novel magnetic nanoparticle drug carrier for enhanced cancer chemotherapy," *PLoS One*, vol. 7, e40388, October 2012.
- [5] H. Zhang H, C. Wang, B. Chen and X. Wang, "Daunorubicin-TiO₂ nanocomposites as a "smart" pH-responsive drug delivery system," *Int. J. Nanomedicine*, vol. 7, pp. 235-242, July 2012.
- [6] L. Zhao, M. Huo, J. Liu, Z. Yao, D. Li, Z. Zhao and J. Tang, "In vitro investigation on the magnetic thermochemotherapy mediated by magnetic nanoparticles combined with methotrexate for breast cancer treatment," *J. Nanoscience Nanotechnology*, vol.13, pp. – 741-745, February 2013.
- [7] V.F. Chekhun, O.V. Yurchenko, L.A. Naleskina, D.V. Demash, N.Yu. Lukianova and Yu.V. Lozovska, "In vitro modification of cisplatin cytotoxicity with magnetic fluid," *Exp. Oncol.*, vol.35, pp.15-19, March 2013.
- [8] A.P. Khandhar, R.M. Ferguson, J.A. Simon and K.M. Krishnan, "Enhancing cancer therapeutics using size-optimized magnetic fluid hyperthermia," *J. Appl. Phys.*, vol.111, pp. 7B306-7B3063, April 2012.
- [9] M.P. Alvarez-Berrios, A. Castillo, J. Mendéz, O. Soto, C. Rinaldi and M. Torres-Lugo, "Hyperthermic potentiation of cisplatin by magnetic nanoparticle heaters is correlated with an increase in cell membrane fluidity," *Int. J. Nanomedicine*, vol. 8, pp. 1003-1013, March 2013.
- [10] V.F. Chekhun, A.V. Kirilenko, V.V. Bondar, N.Yu. Lukianova, I.N. Todor, I.P. Kondratenko, I.N. Kudryavaya and A.D. Podoltsev, "Nanocomposites as means of solution of actual problems in oncology," *Materials of 3rd International scientific conference "Nanostructural materials – 2012: Russia – Ukraine - Belorussia"*, p.12, November, 2012. [In Russian].
- [11] V.F. Chekhun, I.N. Todor, N.Yu. Lukianova, O.R. Melnikov, B.Ya. Momot and I.K. Khayetsky, "About mechanisms of antitumor action of ferromagnetic nanoparticles on tumor cells," *Siberian Journal of Oncology*, Suppl.2., pp. 207-208., September 2009 [Materials of Russian scientific-practical conference with international participation, dedicated to 30th anniversary of Institute of Oncology, Siberian Branch of RAMS "Modern Oncology: achievements and perspectives of development," 2009]. [In Russian]
- [12] Niki M. Towards an optimized MTT assay / M.Niki, M. Otto / *J. Immunol. Meth.* – 1990. – v. 130, № 1. – P. 149-151
- [13] L.A. Naleskina, N.Yu. Lukianova, L.M. Kunskeya, D.V. Demash and V.F. Chekhun, "Visualization of the Features of the Distribution and Accumulation of Iron Nanoparticles in Human Breast Cancer Cells Sensitive and Resistant to Antitumor Drugs after Cultivation with Liposomal Ferromagnetic During Various Time Intervals," *Cytology and Genetics*, vol.45, pp. 395-399, December 2011.
- [14] Y.L. Chao, C.R. Shepard and A. Wells, "Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition," *Mol. Cancer*, vol.9, pp. 179-197, October 2010.
- [15] R.A. McClelland, D. Wilson and R. Leake, "A multicentre study into the reliability of steroid receptor immunocytochemical assay quantification," *Eur. J. Cancer* vol. 27, pp. 711-715, July 1991.
- [16] A.A. Sokolovskaya, T.N. Zabolotina and D.Yu. Blokhin, "CD95-deficient Jurkat/A4 cells are resistant to drug-induced apoptosis," *Exp. Oncol.*, vol. 23, pp. 174-180, September 2001.
- [17] G. Bozzuto, P. Ruggieri and A. Molinari, "Molecular aspects of tumor cell migration and invasion," *Ann. Ist. S. Santia*, vol. 46, pp. 66-80, January 2010.