Effect of The Combination Cytostatics on Cell Line MCF-7

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ABSTRACT

There are many scientific studies dealing with the study of cancer cell lines these days. In oncological research there are important tools determination cell viability. In our study we investigate the effect of three cytostatic drugs on cell line MCF7. We chose these cytostatic drugs: docetaxel (DOC), doxorubicin (DOX) and cyclophosphamide (CPA). We determined the growth curves of cells after 120 hours of their growth for different initial number of cell (2000-10000 cells), and we evaluated the MTT assay for 6 different concentrations of combinations this cytotoxic drugs for 96 hours. MCF-7 cells were exposed to cytostatics for 24 hours. The metabolic activity was evaluated over the 24 hours. Results: According to the statistical analysis, the change in the growth rate was significant ($p < 0.05$, $p < 0.01$) in several cases. This study may be one of the study dealing with the effects of the combination of cytotoxic agents on tumor cell lines MCF-7.

KEYWORDS | CF-7, MTT test, doxorubicin, docetaxel, cyclophosphamide

INTRODUCTION

Chemotherapy is one of the treatment strategies in cancer. Normally, patients who are resistant to chemotherapy show the resistance to multiple antineoplastic agents of different structure and mode of action (1). In vitro studies demonstrated that cancer cells may develop various degrees of resistance to chemotherapeutics through a variety of mechanisms (2). In general, cytostatics selected for combination chemotherapy are active against the tumor when used alone, with different mechanisms of action and minimally overlapping toxicities (3). There are several types of drugs that are used in the treatment of breast cancer. We chose three cytostatic drugs: docetaxel, doxorubicine and cyclophosphamide.

MATERIALS AND METHODS

Cell culture, drugs, viable cell count and MTT test and statistical analysis are the same as in study Trebunova et al, 2012 (4).

RESULTS

In Figure 1 there are the growth curves for different initial number of MCF-7 cells. You can see, that numbers of cells gradually increase. These measurement lasted 120 hours. For further measurements, we chose 4000 cells/ml as initial number.
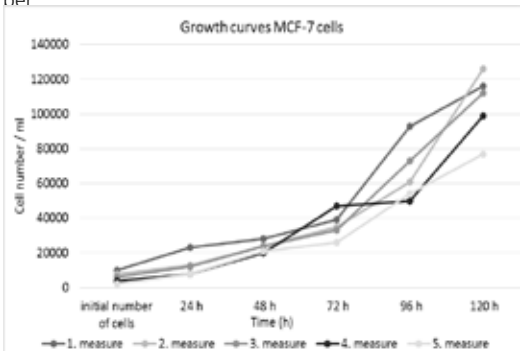
tial number of cells. In 1.measure there is initial number 10000 cells/ml, in 2. 8000 cells/ml, in 3. 6000 cells/ml, in 4. 4000 cells/ml, in 5. 2000 cells/ml.

Cytostatics (six different concentrations, see Table 1) were added approximately 24 hours after seeding. Cells were allowed to grow for 24 hours after the drug treatment. The optical density of each cell was measured spectrophotometrically at 480 nm in an MRX Dynatech ELISA reader (UK).

TABLE -1 SIX DIFFERENT CONCENTRATIONS OF COMBINATION CYTOSTATICS

	I.	II.	III.	IV.	V.	VI.
DOX µg/ml	7.5	7	6.5	6	5.5	5
DOC µg/ml	7.5	7	6.5	6	5.5	5
CPA µg/ml	750	700	650	600	550	500

According to the statistical analysis, the change in the growth rate after 24 hours was significant ($p < 0.05$) for MCF-7 cells treated with combination of cytostatics II in comparison with treated MCF-7 cells with combination of cytostatics III, IV, V and VI. The same results we can see in MCF-7 cells treated with drugs in combination III in comparison with combinations IV, V and VI. In cases, where we used combination of drugs IV and V in comparison with combination V and VI were all results highly significant ($p < 0.01$) (Table 2).

TABLE-2 STATISTICAL ANALYSIS OF CYTOSTATICS TREATING MCF-7 CELLS AFTER 24H GROW

	MCF7/WT	I.	II.	III.	IV.	V.	VI.
MCF7/WT		0	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
I.			p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
II.				p<0,05*	p<0,05*	p<0,05*	p<0,05*
III.					p<0,05*	p<0,05*	p<0,05*
IV.						p<0,01*	p<0,01*
V.							p<0,01*

*Represents significant difference in groups of different concentrations of cells.

Figure 1: Growth curves MCF-7 cells with different ini-

According to the statistical analysis, the change in the growth rate after 48 hours was significant (p<0.05) for MCF-7/wt (untretaed) cells in comparison with treated cells with combination of cytostatics I. Statistical significance p<0.05 we can see in cases of treated cell line with combination of drugs III in comparison with combinations IV, V, and VI. In cases, when we used combination IV and V in comparison with combination V and VI were all results highly significant (p<0.01) (Table 3), they are the same as the results after 24 hours.

TABLE-3 STATISTICAL ANALYSIS OF CYTOSTATICS TREATING MCF-7 CELLS AFTER 48H GROW

MCF7/WT	I.	II.	III.	IV.	V.	VI.
MCF7/WT	p<0,05*	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
I.		p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
II.			p<0,05*	0	p<0,01*	p<0,01*
III.				p<0,05*	p<0,05*	p<0,05*
IV.					p<0,01*	p<0,01*
V.						p<0,01*

*Represents significant difference in groups of different concentration of cells.

According to the statistical analysis, the change in the growth rate after 72 hours was highly significant (p<0.01) for MCF-7 cells treated with combination of cytostatics II in comparison with treated cells with combination of cytostatics III and V. For combination III in comparison with combination V were the results significant alike. Statistical significance p<0.05 we can see in cases of treated cell line with combination of drugs II in comparison with combinations IV and VI. In cases, when we used combination IV in comparison with combination V and VI were results significant (p<0.05) (Table 4).

TABLE-4 STATISTICAL ANALYSIS OF CYTOSTATICS TREATING MCF-7 CELLS AFTER 72H GROW

MCF7/WT	I.	II.	III.	IV.	V.	VI.
MCF7/WT	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
I.		p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
II.			p<0,01*	p<0,05*	p<0,01*	p<0,05*
III.				p<0,05*	p<0,01*	p<0,05*
IV.					p<0,05*	p<0,05*
V.						p<0,01*

*Represents significant difference in groups of different concentration of cells.

In Table 5 we can see highly significant results in more cases. For example for MCF-7 cells treated with combination of cytostatics II in comparison with treated cells with combination of cytostatics III, IV, V and VI (p<0.01). For combination III in comparison with combination IV, V and VI and for combination V in comparison combination VI were all the results highly significant alike.

TABLE-5 STATISTICAL ANALYSIS OF CYTOSTATICS TREATING MCF-7 CELLS AFTER 96H GROW

MCF7/WT	I.	II.	III.	IV.	V.	VI.
MCF7/WT	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05	p>0,05
I.		p<0,05*	p<0,05*	p<0,05*	p<0,05*	p<0,01*
II.			p<0,01*	p<0,01*	p<0,01*	p<0,01*
III.				p<0,01*	p<0,01*	p<0,01*
IV.					p<0,01*	p<0,05*
V.						p<0,01*

*Represents significant difference in groups of different concentration of cells.

CONCLUSIONS
Considering the chemotherapeutic activity of cyclophosphamide as a prodrug that is converted in liver to active forms, we attempted to evaluate the possible effect of the addition of the cyclophosphamide to doxorubicin and docetaxel on the metabolic activity of the MCF-7 breast cancer cell line. These results suggest that concentrations of these cytostatic drugs have ambiguous effect on MCF-7 cells in vitro. The possibility of a new combination chemotherapeutic regimen leading to improvements in the treatment of breast cancer requires to re-value the concentration of drugs and the deeper understanding of the molecular mechanism of cell being killed by combination of doxorubicin / docetaxel / cyclophosphamide.

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