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DIFFERENTIAL SENSITIVITY EVALUATION OF MCF-7 AND MDA-MB-231 HUMAN BREAST CANCER CELLS EXPOSED TO TAMOXIFEN ALONE AND IN COMBINATION WITH ESTRADIOL

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ABSTRACT

The comparative cytotoxic effects of Tamoxifen (TAM) alone or with Estradiol (ES) were studied in estrogen receptor alpha positive (ERα+) MCF-7 and estrogen receptor alpha negative (ERα-) MDA-MB-231 human breast cancer cell lines *in vitro* using cell proliferation, viability, and clonability assays. The cells were incubated with 5 or 7.5µg/mL TAM with or without 0.5µg/mL ES and treated for 24, 48, 72, and 96 hours for cell multiplication and viability. For cloning, the cells were treated similarly, but studied at 96 and 120 hours. In general, MCF-7 cells compared to MDA-MB-231 cells were more sensitive to TAM treatment as evident from cell replication, viability, and clonability. As expected, 7.5µg/mL was more toxic in both cell lines, but more noticeable in ERα+ MCF-7 cells 48 hours post treatment. Estradiol treatment, in general improved the cell multiplication and viability in both cell lines. Although TAM treatments suppressed cloning ability significantly in both cell lines, the effect was less pronounced in MDA-MB-231. The ES and TAM combination treatment further inhibited colony formation. In conclusion, the two cell lines demonstrated differential sensitivity to TAM with or without ES as revealed from cell proliferation and clonability studies.

KEYWORDS: Tamoxifen, Estradiol, human breast cancer cells, MCF-7 ERα +, MDA-MB-231 ERα-, differential sensitivity

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INTRODUCTION

According to the American Cancer Society, breast cancer currently affects more than 200,000 women in the United States and is the most common cause of cancer related death among women[1]. Estrogens regulate growth of human mammary cells by binding to Estrogen Receptor-alpha (ERα) and inducing conformational changes in the receptor's structure, which recruits transcription factors necessary for the expression of several genes required for cell growth[2,3]. Over-stimulation of human breast tissue with estrogens can lead to the expression of oncogenes associated with breast cancer[4]. Selective Estrogen Receptor Modulators (SERMs) such as Tamoxifen (TAM) and Raloxifene bind to ERα and induce changes in the receptor's structure that prevent the recruitment of transcription factors involved in the expression of oncogenes, leading to an accumulation of cells in the G0/G1 phases of cell division and ensue rapid cell death[5,3]. A variety of other SERMs and estrogen receptor blockers are available for the treatment of breast cancer but the most common SERM used is Tamoxifen. Tamoxifen is a non-steroidal anti-estrogen that is used in the treatment of hormone responsive human breast cancer (HBC)[6]. Since its introduction in 1977, TAM is the most widely used chemotherapeutic drug for the treatment of human breast cancer. Although TAM has a strong record of preventing and treating breast cancer in high-risk patients it is not without risk. Its use has resulted in increased incidences of endometrial and uterine cancer in high-risk patients[6]. Thus the Federal Drug Administration (FDA) in USA recommended in 1998 that TAM be the preferred therapy for reducing the frequency breast cancer in patients classified as high risk[7].

Prior research into the effects Tamoxifen on cell proliferation and growth kinetics of ERα positive and ERα negative human mammary carcinoma cell lines found that Tamoxifen exposure resulted in a dose dependent decrease in cell multiplication in human ERα positive breast cancer cells, but not in ERα negative HBC cell lines[8-11]. Prior studies evaluated the effects of TAM and other SERMs on cell growth and apoptosis in ERα+ and ERα- HBC, in addition to human ERα positive (Karpas-620, RPMI-8226) and ERα negative (OPM-2, NCI-H292) myeloma cells[2, 11]. The 4T1 murine breast cancer cell line, an analog to ERα positive HBC cells were used to evaluate the effects of TAM and other SERMs both *in-vitro* and *in-vivo* and found that Tamoxifen alone and in combination with other SERMs resulted in reduced cell proliferation and tumor growth[12, 13]. Additionally, it has been reported that the synthetic estrogen, 17β-Estradiol reversed the cytostatic and cytotoxic effects of Tamoxifen in MCF-7 and T-47D ERα positive cell lines, but not in MDA-MB-231 ERα negative cell lines[14]. The above reports show that the differential cytotoxic and cytostatic effects of TAM alone and in combination with 17 □ -Estradiol on human estrogen receptor positive (ER α+ MCF-7) and estrogen receptor negative (ER α - MDA-MB-231) breast cancer cell lines are inconclusive, and no study on the cloning ability of these two cell lines when exposed to these drugs has been reported. In this investigation the comparative cytotoxic effects of TAM with or without Estradiol were investigated through cell proliferation, viability, and cell morphology using Trypan blue exclusion assay as well as through cell clonability on human ER α + (MCF-7) and ER α - (MDA-MB-231) breast cancer cells.

MATERIALS AND METHODS

Tamoxifen citrate (TAM) and 17β-Estradiol (ES) were purchased from Sigma Chemical Co (St. Louis, MO., USA). TAM and ES were dissolved in 0.2 mL DMSO (Sigma Chemical Co., St. Louis, Mo., USA) in distilled/deionized water to a concentration of 1μg/mL. Dulbecco's Modified Eagle Medium (DMEM),

0.25% Trypsin-EDTA, 0.4% Trypan Blue, Antibiotics (10,000 units penicillin/ 10,000 µg streptomycin per mL), Fetal Bovine Serum (FBS), and 1X Phosphate Buffered Saline (PBS) were purchased from GIBCO (Invitrogen, Carlsbad, CA., USA). T-25(25 cm^2) culture flasks, six well plates, and 35mm petri dishes were purchased from VWR Scientific (Radnor, PA., USA).

(i) ***Cell Culture***

MCF-7(ERα +) and MDA-MB-231(ERα -) HBC cell lines were obtained from Dr. Robert Kurt, Department of Biology, Lafayette College (Easton PA., USA) and were cultured in T-25 culture flasks in DMEM supplemented with 10% FBS and 0.8% penicillin/streptomycin in 37°C humidified incubator with 7.5% CO_2 in air.

(ii) Cell Proliferation and Viability Assay

Exponentially growing cells were harvested from T-25 culture flasks using 1mL 0.25% Trypsin-EDTA diluted with 1mL 1X PBS. Each well of 6 well plates containing 4mL DME-10 was inoculated with 40,000 cells of either MCF-7 or MDA-MB-231 cells and treated with 0, 5, or 7.5µg/mL TAM alone or in combination with 0.5µg/mL ES. Cell multiplication and viability were determined by harvesting cells at 24 hour intervals over the course of 96 hours. Cell numbers were enumerated using the improved neubauer hemacytometer under a compound microscope and viability was assessed using trypan blue exclusion method. The experiments were performed twice in duplicate.

(iii) Cell Cloning

The harvested MCF-7 and MDA-MB-231 cells were treated with 0, 5, or 7.5 µg/mL TAM with and without 0.5µg/mL ES. One hundred treated cells were plated in duplicate in each 35mm petri plates containing 2mL DME-10, and colonies counted at 96 and 120 hours under an inverted microscope. The experiment was repeated twice.

(iv) Data Analysis

Data were analyzed using a two tailed, unequal variance Student's T-Test, One-way ANOVA, and Tukey's Test at the 95% confidence interval. The graph was constructed using Microsoft Excel 2010.

RESULTS

Cell proliferation of MCF-7 was reduced throughout the course of treatment when treated with 5 and 7.5µg per mL Tamoxifen (TAM) compared to untreated controls as can be seen in Figure 1($p < .05$). This difference was also found between two concentrations of drug up to 72 hours. Viability of MCF-7 cells exposed to both concentrations of TAM was significantly reduced ($p < .05$) only after 48 hours compared to controls. Combination treatment with ES lessened TAM's toxic effect at all study periods (Table 1). In general, MDA-MB-231 cells were less sensitive to TAM alone or in combination with ES in terms of cell multiplication and viability (Figure 1 and Table 1). The difference between two cell lines was evident especially at 5µg/mL of drug with ES up to 96 hours. The 7.5µg/mL concentration was found to be equally toxic between each cell line. Like MCF-7 cells, the ES combination treatment demonstrated a noticeable protective effect against TAM especially in viability assay.

Table 1

Viability of MCF-7 and MDA-MB-231 cells represented as a percentage of live cells as determined by 0.4% trypan blue exclusion assay. (+) different from control, (^) different from control+ES, (*) different from 7.5µg/mL TAM, and (++) different from 7.5µg/mL TAM + 0.5µg/mL ES determined by Students T-Test (p < .05). (▼) different from MCF-7 by one-way ANOVA.

	MCF-7				MDA-MB-231			
	Percentage of Live Cells							
Time (hours)	24	48	72	96	24	48	72	96
Control (DMSO)	95	94	97	95	97	97	96	95
Control + 0.5µg ES	94	95	93	94	94	96	95	96
5µg TAM	89	80	68 +	60 + *	91▼	89▼	79 +▼	68 *▼
5µg TAM+ 0.5µg ES	88	84	82	71 ^	91 ++	92	90	78 ^
7.5µg TAM	69	58 +	54 +	25 +	84 +▼	78 +▼	56 +▼	35 +▼
75µg TAM+ 0.5µg ES	73	73 ^	68 ^	42 ^	85▼	81▼	77 ^▼	53 ^▼

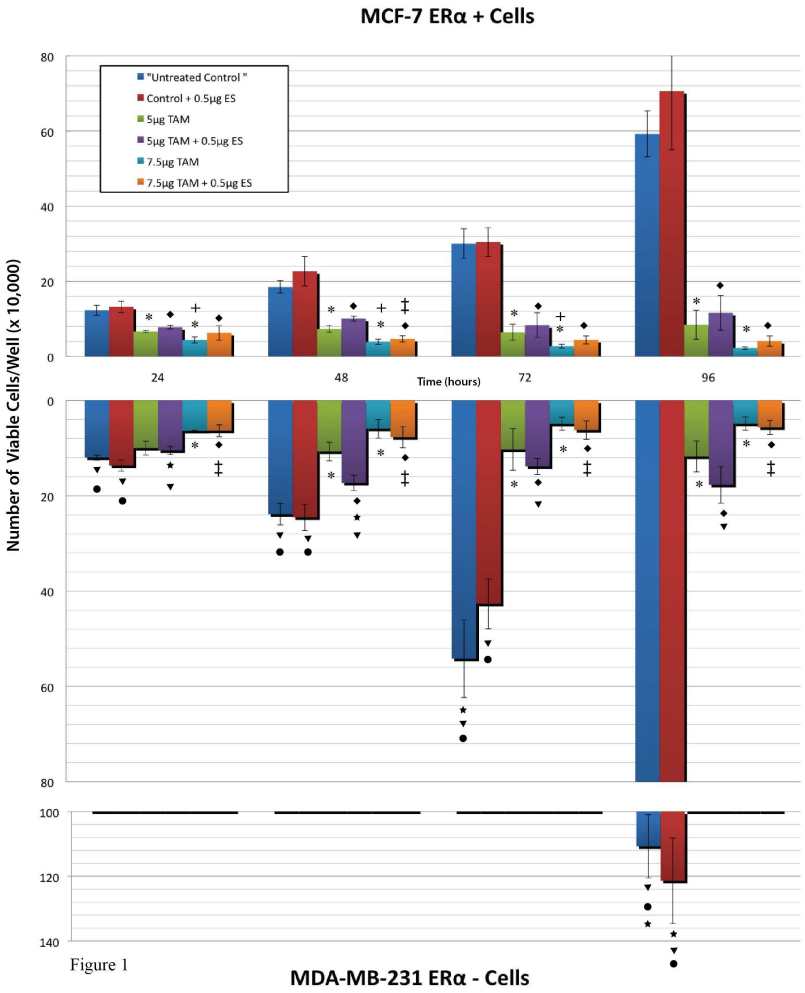

Figure 1

Figure 1

Average cell proliferation of MCF-7 and MDA-MB-231 cells exposed to 5 and 7.5µg/mL Tamoxifen (TAM) alone or in combination with 0.5µg/mL Estradiol (ES). Compared to controls, TAM significantly inhibited proliferation in both cell lines. With the addition of ES, the antagonistic effect of TAM was mitigated to some degree. (*) different from untreated control; (◆) different from control +ES, (+) different from 5µg/mL TAM alone, and (‡) significantly different from cells treated with 5µg/mL TAM with ES. (⋆) different from MCF-7 based on Student's T-Test, (▼)different from MCF-7 by one-way ANOVA, and (□) different from MCF-7 by Tukey's Test. Bars represent +/- 1 Standard Error of Mean.

Cell morphology of both cell lines was adversely affected in the presence of Tamoxifen with or without ES compared to untreated controls. Exposure of MCF-7 cells to TAM alone showed many detached cells and cell debris in the medium. The cell size shrank, lamelliopedia shortened, and cell surface exhibited many protrusions. These effects were more prominent in 7.5µg/mL treatment groups. Combination treatment groups with ES reduced these effects to some extent. In contrast, MDA-MB-231 cells treated with 5µg/mL TAM exhibited less damaging signs, but at 7.5µg/mL, the cell line manifested similar characteristic as those found in MCF-7 cells. In combination with ES, MDA-MB-231 and MCF-7 cells showed improved cell growth displaying more adherent and normal looking cells. Similar to previous results obtained for cell replication and viability, both cell lines showed distinct sensitivity to TAM treatments in their cloning ability (Table 2). Compared to MDA-MB-231, the cloning ability of ER+ MCF-7 cells was significantly reduced at both concentrations and all durations. As before, 7.5µg/mL of drug exerted more inhibitory effects as the 5µg/mL concentration especially in MCF-7 cells. The addition of ES to 5µg/mL TAM improved the clonability to some degree in MCF-7 which, however, was not significant for the 5µg/mL TAM treatment, but significantly different from the 7.5µg/mL treatment groups. In contrast, ES in combination with TAM reduced clonability significantly at both time periods in MDA-MB-231.

Table 2

Clonability of MCF-7 and MDA-MB-231 cells presented as percent of control. (+) different from Control, (^) different from Control with ES, (++) different from 7.5 µg/mL TAM (*) different from 7.5 µg/mL TAM combined with ES(▼) different from MCF-7 by one-way ANOVA, (■)different from MCF-7 by Tukey's Test, and (□)different from MCF-7 by Student's T-Test.

	MCF-7			MDA-MB-231		
Time (hours)	0	96	120	0	96	120
Control (DMSO)	100	100	100	100	100	100
Control DMSO +0.5 µg ES	100	85	87	100	102	100
5 µg TAM	100	25 $^{+}$	25 $^{+}$ *	100	82 $^{+}$■ ▼ ◆	69 $^{+}$■ ▼ ◆
5 µg TAM + 0.5 µg ES	100	29.5 ^ $^{++}$	22 ^ $^{++}$	100	69 ^■ ▼ ◆	32 ^■ ▼ ◆
7.5 µg TAM	100	5.68 $^{+}$	4 $^{+}$	100	85 $^{+}$■ ▼ ◆	30 $^{+}$■ ▼ ◆
7.5 µg TAM + 0.5 µg ES	100	2.27 ^	2 ^	100	41 ^ *■ ▼ ◆	27 ^■ ▼ ◆

DISCUSSIONS

This study reveals that Tamoxifen alone at a concentration of 5 and 7.5 µg/mL significantly reduced the proliferation, viability, and clonability in both ERα+ MCF-7 and ERα- MDA-MB-231 human breast cancer cells compared to untreated controls. As expected, 7.5µg/mL TAM exerted more cytotoxic effects. In addition, two cell lines exhibited differential cytotoxic response and differences between the two TAM concentrations were evident at many instances. In combination with 0.5µg/mL Estradiol, the cytotoxic impact of TAM on cell multiplication and viability was lessened through the course of treatment in both cell lines, but more noticeable in MDA-MB-231 cells. Tamoxifen treatment alone altered cell morphology in both cell lines, but more pronounced in MCF-7 at 5µg/mL. The effects were ameliorated when the cells were exposed only to 5µg/mL TAM with 0.5µg/mL ES. Clonability was substantially reduced in the presence of TAM especially in MCF-7 cells. Addition of ES to TAM did not improve the frequency of clone formation in either cell line.

Similar to our study, Salami et al[15] reported a differential cytotoxic response in ERα positive and ERα negative breast cancer cells to TAM and observed the effects of TAM on cell viability and proliferation were time dependent. Another study conducted by Koutsilieris et al[9] on MCF-7 and MDA-MB-231 cells found altered cytotoxic and cytostatic effects when Adriamycin, a chemotherapeutic drug was used in combination with osteoblast-derived growth factors. In their investigation, Reddel et al[10] reported that ES did not reverse the toxic effects of TAM when used in combination on MCF-7 and MDA-MB-231 cells which is in contradiction with our study as both cell proliferation and viability were improved when TAM was used in combination with ES 0.5µg/mL. However, this inhibitory effect of ES on TAM was not obvious at 7.5µg/mL, possibly due to the drug's severe toxicity at this concentration. Zhang et al[16] suggested that TAM and ES in combination altered expression of bcl-2 genes associated with resistance to apoptosis in MCF-7 cells. An increase in bcl-2 expression in relation to apoptosis resistance might explain why viability and proliferation in the groups treated with TAM and ES combination were higher than TAM treatments without ES. In another study, Taylor et al[17] reported in comparing Tamoxifen effects on the growth of ER+ and ER- breast cancer cells that multiplication and growth of ER- breast cancer cells improved in presence of ES at concentrations higher than 10^{-8} M. Paquette et al[18] observed that metabolites of ES improved growth of MDA-MB-231 *in vitro*. This might explain the significant improvement in viability and clonability of MDA-MB-231 compared to MCF-7 ER+ cells in presence of TAM and ES together observed in the present study. Furthermore, Kallio et al[19] observed a slower death rate in MDA-MB-231 cells compared to MCF-7 cells in the presence of TAM alone. They found that the time at which half of MCF-7 cells were dead was earlier compared to MDA-MB-231cells treated with TAM alone Their observation could possibly explain why MDA-MB-231 cells multiplication rates and viability were higher than MCF-7.

Both 5 and 7.5 µg/mL concentrations of TAM altered the cell morphology and cell colony characteristics severely in MCF-7 cells. The effects on MDA-MB-231 cells were less pronounced especially in 5µg/mL group. The 7.5µg/mL TAM however, produced almost similar morphological alterations in both cell lines. Additionally, ES reversed some of the alterations made by TAM in both cell lines. Although clonability of both cell lines was significantly reduced compared to untreated controls, the MDA-MB-231 demonstrated significantly higher colonies compared to MCF-7 cells in all treatments at all studied intervals. Similar morphological alterations and reduction in colony formation were observed by Xanthopolous et al[13] in Page

B4T1 mouse breast cancer cell, an analog to human MCF-7 ERα+ cells. Hassan et al[20] noted that cloning ability of 4T1 ERα+ mouse breast cancer cells was significantly reduced in dose-dependent manner similar to what was found in this report. In another study, Diel et al[21] treated MCF-7 cell with 10^{-7} M TAM and observed shrunken cells as observed in this report. Mandlekar et al[22] reported that 5μM TAM produced condensed nuclei in MDA-MB-231 ERα- human breast cancer cells. The above studies along with the present investigation on TAM's toxicity with and without ES provide evidence that MCF-7 ERα+ and MDA-MB-231 ERα- human breast cancer cell lines differ in their sensitivity to TAM toxicity and the ability of ES in inhibiting the intensity of cytotoxic properties of Tamoxifen.

CONCLUSION

The purpose of this study was to evaluate cytotoxic effects of Tamoxifen with and without Estradiol in human breast cancer MCF-7 ER+ and MDA-MB-231 ER- cells. Cell proliferation and viability indicate that MCF-7 cells are more sensitive to 5 and 7.5μg/mL of TAM alone than MDA-MB-231 cells. Furthermore, it was found that ES lessened the cytotoxic effects of TAM in both cell lines, but this effect was more pronounced in MDA-MB-231 cells compared to MCF-7 cells. Clonability assay found that TAM alone inhibited the cloning ability of both MCF-7 and MDA-MB-231, but MDA-MB-231 clonability was significantly higher in TAM alone or in combination with ES. It is suggested that a differential sensitivity exists between ERα+ MCF-7 and ERα- MDA-MB-231 human breast cancer cell lines to Tamoxifen treatment with or without Estradiol.

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