Original Article

STUDY EFFECT OF TAMOXIFEN ON CELL CYCLE PHASE DISTRIBUTION, IN BREAST CANCER CELLS

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Abstract

Hormone therapy also known as estrogen suppression therapy is the first targeted therapy and is frequently used as a secondi therapeutic to lower the risk of cancer returning after surgery or spread. Tamoxifen is a drug that is commonly used to treat breast cancer by mediating estrogen receptor signaling pathways. Breast cancer is becoming a spread disease that needs a specific therapy. The main focusi of this study is the anti-cancer drug Tamoxifen (TAM)i and it's effected on celli cycle (Culture) of breast cancer cell line measurement of cellular DNA content and the analysis of the cell cycle can be performed by flow cytometry. This study include the determination the effects of tamoxifen on MCF-7i on breast cancer celli line and investigate of the mechanism by which the affected of it on the cell cycle phases using Flowcytometric cell cycle analysis assay. As well study the Flowy cytometric cell cycle analysis of tamoxifen-treated cultures showed an increase in the proportion of cells in the GI phase of the cell cycle.

Keywords: Breast cancer, Tamoxifen, Flow cytometry, Cell cycle.

INTRODUCTION

Cancer is a disease described by uncontrolled development of the cells. It can develop within any organic site in the body but the most common types include lung, breast, prostate and colorectal cancers (1). The American Cancer Society calculates the number of new cancer cases and fatalities in the United States each year, In 2022, approximately 1,918,030 new cancer cases and 609,360 cancer deaths (2). Breast cancer is the most common cancer in women worldwide. About 70-75% of breast tumors are estrogen receptor (ER) and/or progesterone receptor (PR)-positive (3). It is heterogeneous disease, which comprises of many biologically different entities with distinct pathological features and clinical implications (4,5). Breast cancer appears in the form of tumor when there is uncontrollable proliferation of breast cells (6).

It can be classified into non-invasive breast cancer (Ductal carcinoma in situ and Lobular carcinoma in situ) and invasive breast cancer (invasive ductal carcinoma, invasive lobular carcinoma, Paget's disease of the nipple and inflammatory breast cancer) (7). The treatment of breast cancer are basically depends on tumor staging. There are different types of treatments that are used separately or in combinations, such as surgery (removal of the cancer tissue), radiation therapy, and systemic treatments. Breast cancer is a heterogeneous disease and treatment methods depend on different clinical criteria e.g. age, type of cancer, size, stage of detection and metastasis (8).

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Hormonal therapyi is used usuallyi in treating thei patients with positivei tumor afteri the completioni of chemotherapy. One of the mosti important anticanceri drug is Tamoxifeni (TAM) used to treatmenti of breasti cancer. Chemically knowni as (z)-2-[4-(1, 2-Diphenyl–1butenyl) phenoxy-N,N–Dimethylethanamine (9). It is a selectivei estrogen receptori modulatori (SERM) (10). Tamoxifen inhibits breast cancer cell growth and induces apoptosis. Apoptosis, or programmed cell death, is one of the topics that cell biologists are most interested in. It's a well-ordered and well-organized cellular process that happens in both normal and pathological settings and entails a sequence of biochemical processes that lead to a certain cell shape and death (11). The different stagesi of apoptotic celli death, started by cellular7 shrinkage and chromatin1 condensation, associatedi with formationi of membrane blebbing, organellesi and nucleusi fragment and the blebsi begin formationi of apoptotici bodies whichi are finally phagocytosedi by macrophagesi or neighboring cellsi by endocytosisi/phagocytosis. Apoptosis playsi a role in preventingi cancer. If a cell is unablei to undergo apoptosiso due to mutationi or biochemicali inhibition, it can continue dividingi and developedi into a tumor (12). The mosti common and commonlyi used treatment for breasti cancer is tamoxifen, which causesi tumor stabilizationi in 50% of all patients with metastatici breast canceri who were previouslyi untreated. Of all breasti tumor types, 75% are ER positive. (13). Breast canceri cells may undergoi apoptosis wheni treated to TAMi via the membrane ireceptor pathway. The purposei of this study was to use flowi cytometry cell culture to investigatei about how TAMi affected MCF 7i (ER+) cells'. Flow cytometryi (FCM), a quantitativei single cell analysisi technique basedi on impedancei or laseri technology for quantitativei single cell analysisi that is usedi in cell sorting, cell counting, and biomarker identification. It providesi the multi-parameteri examination of singlei cells at the same time (14). The main clinicali use of flow cytometry is the screeningi of hematologic malignancy, but it also has a broad rangei of additional uses, including counting reticulocytesi and analyzingi cell function and the celli cycle. G1 phase refersi to the replicationi (Gap 1). During the S (Synthesis) phase, DNA replication itakes place. Before mitosis, there is anotheri interval (G2 phase, Gap 2) after DNA synthesisi (M). Mature neorons and otheri cells that noi longer dividei are consideredi to have enteredi the G0 phasei of the cell cycle (15). Propidiumii Iodide-A histogrami with markers of G0i/iG1 , S, and G2i/iM phases to find cell cyclei phases, and markersi of sub G0 /G1 and > (16).These invetro study showed the effct of (TAM) on MCF-7 cells.

Materials and Methods

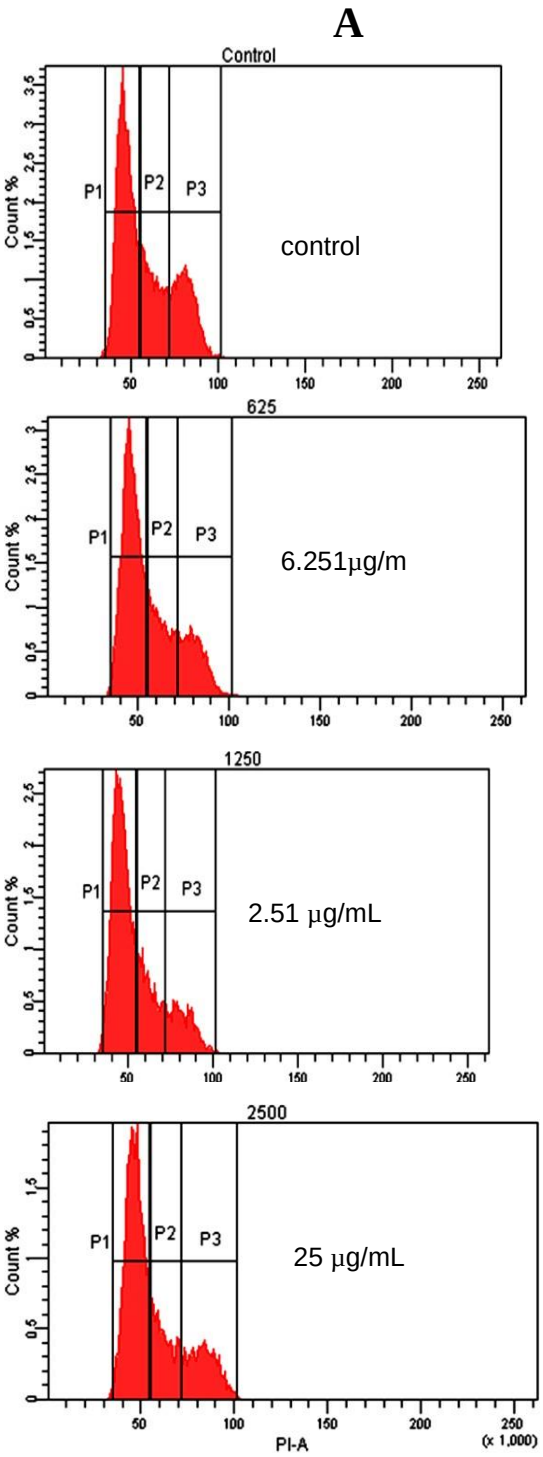
Propidiumi iodide was used as a DNAi stain in the celli cycle analysisi using the BD CycletestTM Plusi kit (BD Biosciencesi, San Diegoi, CA, Unitedi States). According to thei manufacturer'si instructions, flow cytometryi was used to assess the impacti of Tamoxifeni therapy on the cell cycle. Briefly, density-gradienti centrifugation wasi used to separatei MCF-7 cells. After centrifugingi the cell suspensioni for five minutesi at 300g at room temperature (25°C) while adding 200 L of fetal bovine serum in total, the entire tube of celli suspension wasi transferredi into a 15 ml centrifugei tube.. To prevent disrupting the particle, the supernatanti was aspirated, leavingi around 50 L of residuali fluid in the tube. The cellsi were to be gentlyi vortexedi at low speedi with 100 mL of bufferi solution beforei being centrifugedi for 5 minutesi at 300 g at room temperature (25 °C). The supernatanti was aspiratedi and leaving abouti 50 µL of residuali fluid in the tube to prevent disturbing the pellet. The 1 ml of buffer solution was used to resuspension the celli pellet. A hemacytometeri was used to counti the cells afteri one additionali centrifugation and resuspensioni of the sample. Bufferi Solution was usedi to adjust the concentrationi to 1.0 x 106 cells/mL. To achieve concentrationi to 1.0 x 106 cells/mL, Bufferi Solutioni was employed. This needsi to generate enoughi cells for a testi sample. Carefully gluggingi the wholei supernatant, we next addedi 250 L of Solutioni A (trypsin buffer), and incubatedi for 10 minutes ati 25°C. Each tube receivedi 200 L of Solution B (trypsini inhibitori and RNase buffer), which wasi followed by 200 L of cold Solutioni C (8°C), which was iincubated for 10 minutesi at room temperaturei (PI stain solution). Distributioni of the cell cycle nucleari DNA was determinedi by using flow cytometry (BD Biosciences). The proportioni of cells in G1, S and G2 phasesi were examinedi by using Divani software (BD Biosciences).

Statistical analysis

Graph Pad Prism version 7 was used to analyze the data and represent it as a mean and standard deviation.

Results

The distributioni of cell cyclei was analyzedi after treatingi the cells with tamoxifeni at different concentrations. 6.25, 12.5i and 25µg/mL that showni in Figure, (1), The histogrami in figure A showingi MCF-cell distribution at differenti phase, G1, S and G2/M, using, treatments control. In figure B Shows the all data and the mean of it. When using (6.25, µg/mL) of the drug, the G1 % ±SD MCF-7 was equal to (57.2 ±0.566), in S was equal to (22.55 ± 0.636) and in G2 (18.1 ±0.283). In (12.5, µg/mL) the ±SD of G1, S, and G2 phases was equal (62.95 ±0.636), (20.65 ±0.495) and (14.75 ±0.354) respectively. The final concentration of tamoxifen drug that using was equal (25µg/mL) and the ±SD of data analysis in G1, S, and G2 shows (58.85 ±0.354), (19.85 ±0.212) and (19.35 ±0.212) respectively, all these data shown in table (1).



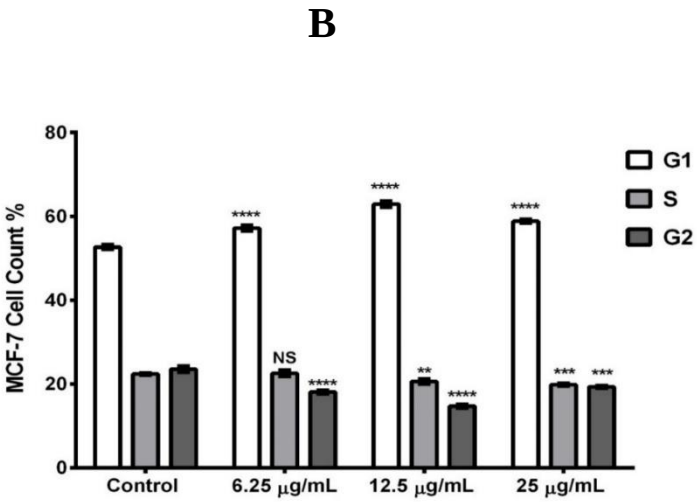


Figure 3-5 the, effect, of tamoxifen, on MCF-7 celli, cycle phase, distribution,]. (A) Flow cytometry histogram, showing MCF-cell, distribution, at different, cell cycle phase, G1, S and G2/M, using treatments, control, 6.25i, 12.5 and 25µg/mL of tamoxifen,. (B) Mean

Table 1: Tamoxifen effect on cell cycle in MCF-7 cells compering with control

Concentration µg/ml	G1 % ±SD MCF-7	S % ±SD MCF-7	G2 % ±SD MCF-7
Control	**52.7 ±0.424**	**22.4 ±0.141**	**23.55 ±0.636**
6.25	**57.2 ±0.566**	**22.55 ± 0.636**	**18.1 ±0.283**
12.5	**62.95 ±0.636**	**20.65 ±0.495**	**14.75 ±0.354**
25	**58.85 ±0.354**	**19.85 ±0.212**	**19.35 ±0.212**

Discussion

Cell cyclei phase distributioni of MCF-7i cells studyi was performedi to determinei whether or not tamoxifeni suppression, of MCF-7 cell viabilityi was relatedi to cell cyclei arrest (14). Flow cytometryi was usedi to detect the DNA contentsi after celli staining with propidiumi iodide. A and Bi showedi that at G1i phasei the percentagei of cellsi was significantlyi increasedi by increasingi the concentrationi of tamoxifen in a dose-dependenti manner startingi from slight change at 6.25µg/mL (57.2%,) to maximum increasei

at 12.5µg/mL (62.95%,) as comparedi with the controli. The increasei in G1i phase was accompaniedi with no significanti differences in Si phase at 6.25µg/mLi except at 12.5 and 25µg/mLi that showni in table (1). On the otheri handi an apparenti decreased G2/Mi populationi was observedi in a concentrationi dependenti manner afteri 24 hrsi in comparisoni to the control. Inductioni of cell cyclei arresti and apoptosisi consideredi as the most importanti targets for developingi anti-canceri drugs. Arrestingi the tumori cell cyclei will dramaticallyi inhibiti the cell proliferationi and resultingi in programmedi cell death. Thei resultedi flow cytometrici analysis revealedi that tamoxifeni cause accumulationi of MCF-7i cells at G1i phase in a dosei-dependent patterni which suggestedi that the transition from G1 to Si phase was blockedi by tamoxifeni which was mainlyi observedi at 12.5µg/mLi thisi results agreesi with Robert L. Sutherlandi when used Flow cytometrici cell cyclei analysisi of tamoxifen-treatedi culturesi showed an increaseo in the proportioni of cells in the GI phasei of thei cell cyclei and also cellsi were not only arrestedi in thei G1 phasei but alsoi in the G2i phase of thei cell cyclei (18). This studyi revealedi that tamoxifen is a potenti antagonisti of estrogeni and inducesi apoptosisi in ER+ cells; however at concentrations, higheri than 12.5µg/ml thei level of G1 phasei was decreased. At 25µg/mL concentration, of tamoxifeni, showed lessi, effect, on celli cycle, indicating, that the estrogeni reversible, effecti of tamoxifeni, in MCF-7 cells, is mediatedi, via the estrogeni, receptori mechanism,, these, agreementi, with Lykkesfeldti, AE indicating, that in high, concentrationi of, tamoxifeni the estrogeni, reversible, effecti, of it in MCF-7i cells, is mediatedi, via the estrogeni, receptor, mechanismi (19).

Conclusion

Flow cytometrici, cell, cyclei analysis, of tamoxifeni, cultures showedi, an increasei, in the proportioni, of cells, in the GIi phase, of thei cell cyclei, (but no in veryi, high concentrationi). Tamoxifeni, from, flow cytometrici, results; showed, celli cycle, arresti toward, MCF-7. It, showed, dose-dependent, manneri the maximum, concentrationi, at (12.5µg/mL), that more, effectedi, on G1 phase, accumulationi, in MCF-7 cell cycle.

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