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 second 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 focus of this study is the anti-cancer drug Tamoxifen (TAM) and its effect on cell 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 cell 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 Flow 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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How to cite this article: Ibrahim Waleed, Firas Hassan, Raghad.Z. Atta, STUDY EFFECT OF TAMOXIFEN ON CELL CYCLE PHASE DISTRIBUTION, IN BREAST CANCER CELLS, J PHARM NEGATIVE RESULTS 2022;13: 608-611.
Hormonal therapy is used usually in treating their patients with positive tumor after the completion of chemotherapy. One of the most important anticancer drug is Tamoxifen (TAM) used to treat patients of breast cancer. Chemically known as (z)-2-[4-(1, 2-Diphenyl-1-butenyl) phenoxy-N,N-Dimethylethanamine (9). It is a selective estrogen receptor modulator (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 stages of apoptotic cell death, started by cellular7 shrinkage and chromatin condensation, associated with formation of membrane blebbing, organelles and nucleoli fragment and the blebs begin formation of apoptotic bodies which are finally phagocytosed by macrophages or neighboring cells by endocytosis/phagocytosis. Apoptosis plays a role in preventing cancer. If a cell is unable to undergo apoptosis due to mutations or biochemical inhibition, it can continue dividing and developed into a tumor (12). The most common and commonly used treatment for breast cancer is tamoxifen, which causes tumor stabilization in 50% of all patients with metastatic breast cancer who were previously untreated. Of all breast tumor types, 75% are ER positive. (13). Breast cancer cells may undergo apoptosis when treated to TAM via the membrane receptor pathway. The purpose of this study was to use flow cytometry cell culture to investigate about how TAM affected MCF 7i (ER+) cells. Flow cytometry (FCM), a quantitative single cell analysis technique based on impedance or laser technology for quantitative single cell analysis that is used in cell sorting, cell counting, and biomarker identification. It provides the multi-parameter examination of single cells at the same time (14). The main clinical use of flow cytometry is the screening of hematologic malignancy, but it also has a broad range of additional uses, including counting reticulocytes and analyzing cell function and the cell cycle. G1 phase refers to the replication (Gap 1). During the S (Synthesis) phase, DNA replication takes place. Before mitosis, there is another interval (G2 phase, Gap 2) after DNA synthesis (M). Mature neurons and other cells that no longer divide are considered to have entered G0 phase of the cell cycle (15). Propidium iodide-A histogram with markers of G0i/iG1 , S, and G2i/M phases to find cell cycle phases, and markers of sub G0/G1 and > (16). These invetro study showed the effect of (TAM) on MCF-7 cells.

Materials and Methods
Propidium iodide was used as a DNA stain in the cell cycle analysis using the BD CycleTestTM Plusi kit (BD Biosciencesi, San Diego, CA, Unitedi States). According to thei manufacturer'si instructions, flow cytometry was used to assess the impact of Tamoxifen therapy on the cell cycle. Briefly, density-gradient centrifugation was used to separate MCF-7 cells. After centrifuging the cell suspension for five minutes at 300g at room temperature (25°C) while adding 200 L of fetal bovine serum in total, the entire tube of cell suspension was transferred into a 15 ml centrifuge tube. To prevent disrupting the particle, the supernatant was aspirated, leaving around 50 L of residual fluid in the tube. The cells were to be gently vortexed at low speed with 100 mL of bufferi solution before being centrifuged for 5 minutes at 300 g at room temperature (25°C). The supernatant was aspirated and leaving about 50 μL of residual fluid in the tube to prevent disturbing the pellet. The 1 ml of buffer solution was used to resuspension the cells pellet. A hemacytometer was used to counti the cells afteri one additional centrifugation and resuspension of the sample. Bufferi Solution was used to adjust the concentration to 1.0 x 106 cells/mL. To achieve concentration to 1.0 x 106 cells/mL, Bufferi Solution was employed. This needi 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 Solutioni B (trypsin inhibitor and RNase buffer), which was followed by 200 L of cold Solutioni C (8°C), which was incubated for 10 minutes at room temperaturei (PI stain solution). Distributioni of the cell cycle nucleai DNA was determinedi by using flow cytometryi (BD Biosciencesi). The proportioni of cells in G1, S and G2 phases were examini by using Divani software (BD Biosciencesi).

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 were analyzied 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 was using 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).
**Table 1: Tamoxifen effect on cell cycle in MCF-7 cells comparing with control**

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>G1 % ±SD MCF-7</th>
<th>S % ±SD MCF-7</th>
<th>G2 % ±SD MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52.7 ±0.424</td>
<td>22.4 ±0.141</td>
<td>23.55 ±0.636</td>
</tr>
<tr>
<td>6.25</td>
<td>57.2 ±0.566</td>
<td>22.55 ± 0.636</td>
<td>18.1 ±0.283</td>
</tr>
<tr>
<td>12.5</td>
<td>62.95 ±0.636</td>
<td>20.65 ±0.495</td>
<td>14.75 ±0.354</td>
</tr>
<tr>
<td>25</td>
<td>58.85 ±0.354</td>
<td>19.85 ±0.212</td>
<td>19.35 ±0.212</td>
</tr>
</tbody>
</table>

**Discussion**

Cell cycle phase distribution of MCF-7 cells study was performed to determine whether or not tamoxifen suppression, of MCF-7 cell viability was related to cell cycle arrest (14). Flow cytometry was used to detect the DNA contents after cell staining with propidium iodide. A and B showed that at G1 phase the percentage of cells was significantly increased by increasing the concentration of tamoxifen in a dose-dependent manner starting from slight change at 6.25 µg/mL (57.2%) to maximum increasei...
at 12.5 μg/mL (62.95%) as compared with the control. The increase in G1 phase was accompanied with no significant differences in S phase at 6.25 μg/mL except at 12.5 and 25 μg/mL that shown in table (1). On the other hand an apparent decrease G2/Mi population was observed in a concentration dependent manner after 24 hrs in comparison to the control. Induction of cell cycle arrest and apoptosis was considered as the most important targets for developing anti-cancer drugs. Arresting the tumor cell cycle will dramatically inhibit the cell proliferation and resulting in programmed cell death. These studies showed flow cytometric analysis revealed that tamoxifen cause accumulation of MCF-7i cells at G1i phase in a dose-dependent pattern which suggested that the transition from G1 to S phase was blocked by tamoxifen which was mainly observed at 12.5 μg/mL. This results agrees with Robert L. Sutherland when used Flow cytometric cell cycle analysis of tamoxifen-treated cell cultures showed an increase in the proportions of cells in the G1 phase of thei cell cycle and also cellsi were not only arrested in the G1 phase but also in the G2i phase of the cell cycle (18). This study revealed that tamoxifen is a potent inhibitor of estrogeni and induces apoptosisi is ER+ cells; however at concentrations, higheri than 12.5 μg/ml thei level of G1 phasei was decreased. At 25 μg/mL concentration, of tamoxifen, showed lessi, effect, on celli cycle, indicating, that the estrogeni reversible, effectsi of tamoxifen, in MCF-7 cells, is mediatedi, via the estrogeni, receptori mechanismi., these, agreementi, with Lykkesfeldti, AE indicating, that in highi, concentrationi of, tamoxifen the estrogeni, reversiblei, effectsi, of it in MCF-7i cells, is mediatedi, via the estrogeni, receptori, mechanismi (19).

Conclusion
Flow cytometric, cell, cycle analysis of tamoxifen, cultures showedi, an increasei, in the proportioni, of cells, in the G1i phase, of thei cell cyclei, (but no in veryi, highi concentrationi). Tamoxifen, from, flow cytometrici, resultsi; showed, celli cycle, arresti toward, MCF-7. It, showed, dose-dependent, manneri the maximum, concentrationi, at (12.5 μg/mL), that morei, effecti, on G1i phase, accumulationi, in MCF-7 cell cycle.

References