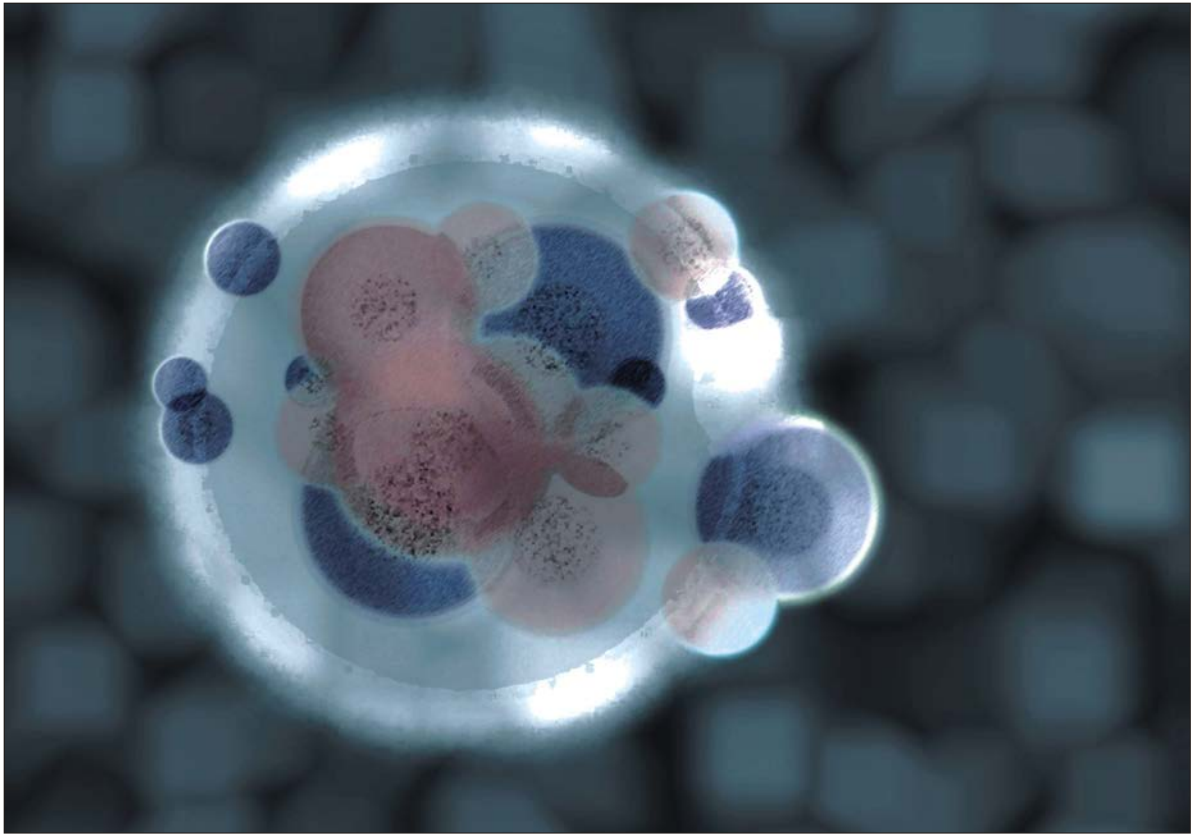

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Effects of Tamoxifen and Glucose on Breast Cancer T47D Cells

[1]Shamileh Fouladdel, [2]Bagher Larijani and [1,3]Ebrahim Azizi
[1]Laboratory of Molecular Research, Department of Pharmacology and Toxicology, Faculty of Pharmacy,
[2]Endocrinology and Metabolism Research Center, Shariati Hospital,
[3]National Center of Excellence of Toxicology,
Tehran University of Medical Sciences, Tehran, Iran

Abstract: Due to controversial reports on the relationship between diabetes and cancer, we decided to look at the effects of Tamoxifen (TAM) in the presence and absence of high glucose concentrations on proliferation of human breast cancer T47D cells and its Tamoxifen resistant subline (TAMR-6 cells). Both cell types were separately grown in 24-well culture plates for up to 4 weeks in the RPMI1640 culture medium containing TAM, different concentrations of glucose, combination of TAM and glucose and control (RPMI). Trypan blue dye exclusion method was used to evaluate the *in vitro* cytotoxicity of test compounds. Data indicated a time and dose dependent antiproliferative effect of glucose on T47D cells at studied concentrations. Results also showed that the cytotoxicity of glucose was significantly greater on parent T47D than TAMR-6 cells ($p<0.01$). A significant synergistic effect was also observed between TAM and glucose on both cell types ($p<0.01$). In conclusion, present results indicate that high glucose concentrations similar to diabetic conditions exert antiproliferative effects in breast cancer T47D cells.

Key words: Diabetes, glucose, tamoxifen resistance, breast cancer, T47D cells

INTRODUCTION

Breast cancer remains a leading cause of women morbidity and mortality worldwide[1]. At present despite early diagnosis, a significant percentage of patients with breast cancer die from disease progression and metastasis. Therefore, systemic treatment is required to prevent disease progression. Since in most cases progression of breast cancer is due to hormones such as estrogens, hormone therapy plays a significant role in breast cancer management[2,3]. Antiestrogens, especially Tamoxifen (TAM), are the drugs of choice for the treatment of all stages of breast cancer[4-7]. Tamoxifen as a classical anti-estrogen and its new congeners as well as compounds known as Specific Estrogen Receptor Modulators (SERMs) are regarded as competitive inhibitors of estrogen receptors[4,8,9]. As a result of hormonal therapy, gene transcription, DNA synthesis and cellular proliferation are altered or prevented[10,11]. Unfortunately, in the systemic treatment of patients with advanced breast cancer, the occurrence of drug resistance in patients is a major problem. Therefore, despite the tremendous therapeutic and commercial success of TAM since its introduction, intrinsic or acquired resistance in almost all patients during prolonged treatment[12-14] mandates more elaboration of anticancer effects of TAM in different conditions. On the other hand, there are reports indicating an apparent increase in the prevalence of breast cancer in diabetic patients[15-17]. It has also been reported that glucose concentration may be an important factor in breast cancer cell proliferation. However, the relationship of hyperglycemia to cell proliferation and differentiation is not completely understood[18]. In one study, an apparent link observed between high glucose-induced PKC isozyme down regulation with concomitant acceleration of cell cycle progression in MCF-7 breast cancer cells[19]. However, same study showed that the cell proliferation and DNA synthesis in drug resistant subline of MCF-7 breast cancer cells (MCF-7/ADR) were unaffected by the increase in glucose concentration. Therefore, the aim of the present study was to investigate the effect of glucose concentrations similar to diabetic conditions on antiproliferative effects of TAM on breast cancer T47D cells and its TAM-resistant subline.

Corresponding Author: Dr. Ebrahim Azizi, Director of Molecular Research Laboratory,
National Center of Excellence of Toxicology, Tehran University of Medical Sciences,
Tehran, Iran Tel/Fax: (+9821) 6959100

MATERIALS and METHODS

Cell line and culture conditions: The human breast cancer T47D cell line (ATCC HTB-133) was obtained from Pasteur Institute Cell Bank of IRAN (Tehran, IRAN). During the study (2004-2005) cells were maintained in RPMI-1640 (Gibco, UK) culture medium supplemented with 10% Fetal Bovine Serum (Gibco, UK) and 100 U mL^{-1} of penicillin and 100 ng mL^{-1} of streptomycin (Sigma, UK) at 37°C in 5% CO_2 incubator.

The Tamoxifen-resistant subline was isolated *in vitro* and characterized in our lab[20] by continuous exposure of T47D cells to TAM at concentrations starting from $1x10^{-8}$ M and increasing in a stepwise manner to $1x10^{-6}$ M within 6 months. Cells that were capable of sustained growth in medium containing 1 μM of TAM were considered to be resistant to Tamoxifen and are referred to TAMR-6 cells here after.

The T47D and TAMR-6 cells were seeded in 24-well plates ($5x10^4$ cells in each well) for 3 and 6 days and 4 weeks in the RPMI 1640 culture medium containing TAM (1 μM), different concentrations of glucose (15-50 mM), combination of TAM and glucose and control (RPMI). Culture medium was changed accordingly every 3 days throughout the experiments. Cells in quadruplicate wells were then counted using Trypan blue dye exclusion method to evaluate the *in vitro* cytotoxicity of test compounds. Data are presented as mean±SE of the average determination of 4 wells in three independent experiments.

Statistical analysis: SPSS 12 was used to perform statistical analysis of data. The ANOVA and students t-test were used to examine the differences among treatments. Mean differences with $p<0.05$ considered significant.

RESULTS

Cytotoxicity of different concentrations of glucose on T47D cells: The antiproliferative effects of glucose on T47D cells were determined using trypan blue dye exclusion method as described in the methods. A time and dose dependent antiproliferative effects of glucose was observed on T47D cells compare to control RPMI (Fig. 1). This effect was highest at 50 mM concentration of glucose at all time points.

Effect of combination of glucose and TAM on proliferation of T47D and TAMR-6 cells: Effects of glucose at 25 mM (G25), TAM at 1 μM (TAM-6M) and their combination (TAM+G) on proliferation of both cell types were determined using trypan blue dye exclusion method as described in the methods. A time dependent antiproliferative effects of all treatments was observed on

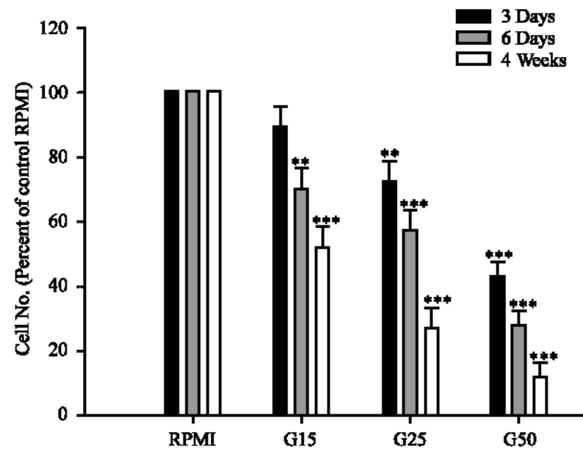

Fig. 1: Antiproliferative effects of different concentrations of glucose on T47D cells. The T47D breast cancer cells were exposed to glucose concentrations (G15, G25, G50 mM) for up to 4 weeks to determine cell viability using Trypan blue as explained in the methods. Data are presented as mean±SE of the average determination of 4 wells in 3 independent experiments. **Significant at $p<0.01$ and ***significant at $p<0.001$ compare to control RPMI

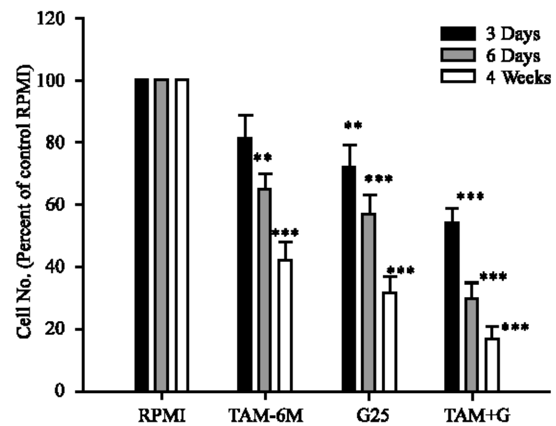

Fig. 2: Antiproliferative effects of glucose and TAM combination on T47D cells. The T47D breast cancer cells were exposed to glucose (25 mM; G25), TAM (1 μM; TAM-6M) and their combination (TAM+G) to determine cell viability using Trypan blue as described in the methods. Data are mean±SE of the average determination of 4 wells in 3 independent experiments. **Significant at $p<0.01$ and ***significant at $p<0.001$ compare to control RPMI

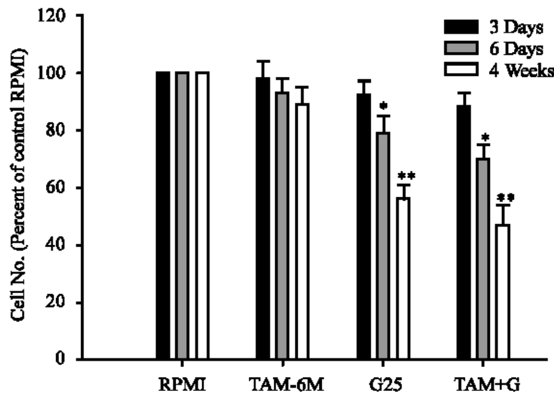

Fig. 3: Antiproliferative effects of glucose and TAM combination on TAMR-6 cells. The TAMR-6 breast cancer cells were exposed to glucose (25 mM; G25), TAM (1 μM; TAM-6M) and their combination (TAM+G) to determine cell viability using Trypan blue as described in the methods. Data are mean±SE of the average determination of 4 wells in 3 independent experiments. *Significant at $p<0.05$ and **significant at $p<0.01$ compare to control RPMI

T47D cells compare to control RPMI (Fig. 2). Effect of glucose (25 mM) on proliferation of T47D cells was comparable to TAM (1 μM) at all time points. Unlike T47D cells, glucose (G25) had significantly more growth inhibitory effect on TAMR-6 cells than TAM at 1 μM concentration (Fig. 3). Synergistic inhibitory effect of glucose and TAM combination was significantly more on T47D cells than TAMR-6 cells (Fig. 2 and 3).

DISCUSSION

The benefits of hormonal therapy of breast cancer have been widely accepted. Nevertheless, almost half of the tumors are less likely to respond effectively to anti-hormonal treatment. Similarly, some of the patients do not respond to chemotherapy[4-7]. In the systemic treatment of patients with advanced breast cancer, the occurrence of drug resistance in some patients is a major problem. Possibilities to overcome TAM resistance have been reported by using newly developed antiestrogens, progestins or aromatase inhibitors[21,22]. However, none of these agents could completely replace TAM so far from first-line clinical use[23,24]. In addition to necessity to further elucidate the molecular mechanisms underlying TAM resistance, it is quite important to address effectiveness of TAM in patients with breast cancer and other diseases such as diabetes. It has been reported that diabetes is a risk factor for developing breast cancer in certain conditions[17-19]. Despite controversial data in this regard, none of them could clearly demonstrate the effectiveness of hormonal therapy in diabetic patients with breast cancer. In addition, it is not clearly known that following development of drug resistance in tumor cells, diabetic patients would better respond to hormonal therapy or not. In one study, cell proliferation and DNA synthesis only increased in MCF-7 human breast cancer cell line but not in its multidrug resistant variant (MCF-7/ADR cells) when glucose concentration in the culture medium was increased from normal (5.5 mM) to high (25 mM) levels[19]. In another study, unlike MCF-7 breast cancer cells, there was no significant effect of leptin and high glucose on cell proliferation, DNA synthesis, levels of cell cycle proteins, PKC isozymes, or PPAR subtypes in multidrug-resistant human breast cancer NCI/ADR-RES cells[18]. Similar to these studies, proliferation of TAMR-6 cells in our study was not affected significantly by increasing glucose concentration to 25 mM in short time up to 6 days. Unlike above mentioned reports, in our study, increase in glucose concentration significantly reduced proliferation of T47D breast cancer cells at all time points. This inhibitory effect of 25 mM glucose was also observed in TAMR-6 cells at 4 weeks time point. We also observed a synergistic inhibitory effect between high glucose concentrations and TAM. These differences in results could be due to differences in cell lineage, experimental design, levels of drug resistance or other yet unknown possible factors that need to be thoroughly investigated. In addition to above mentioned studies, it has also been reported that increased activity of aldose reductase, the rate-limiting polyol pathway enzyme that converts glucose into sorbitol, mediates pathologies associated with diabetes and is thought to be involved in increased resistance to chemotherapeutic drugs[25]. Results of a cohort study also showed that circulating insulin levels were not predictive of future breast cancer incidence, but there may be a weak association with type 2 diabetes, perhaps modulated via increased adiposity[26]. It can be concluded that depending on the cell line and culture conditions, glucose may decrease and or increase proliferation of breast cancer cells. Therefore, more studies are needed to clearly determine the relationship between high glucose conditions similar to diabetes and breast cancer cell proliferation.

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