Original Article

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In Vitro Evaluation of the Effects of Tamoxifen on Prostate Cancer Cells

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ABSTRACT

BACKGROUND AND OBJECTIVE: Considering the high prevalence of prostate cancer and the effect of androgens on its progression, this study was conducted to investigate the inhibitory effects of tamoxifen as an anti-androgen on prostate cancer.

METHODS: In this experimental study, the human cell line (PC3) was purchased from the Pasteur Institute. The effect of tamoxifen at concentrations of 0, 3.25, 7.5, 15, 30 and 60 µM on cells was evaluated, and the tests of viability, migration, colonization and cell morphological changes were respectively performed using MTT, wound healing, colonization, and giemsa staining methods.

FINDINGS: IC50 dosage of tamoxifen of 15 µM with a regression coefficient of 0.90 was obtained within 24 hours. The results showed that tamoxifen significantly inhibited proliferation with 7.3±0.6 colonies compared with 100 colonies of control ($p<0.03$) and migration with 278.4±1.5 µm groove diameter compared with 89.68 ± 0.9 µm of control ($p<0.01$) at the dose of 15 µM. Treatment of cells with a dose of 15 µM also causes changes in the nucleus and cytoplasm and causes apoptosis in comparison with the control group.

CONCLUSION: The results of this study showed that tamoxifen has significant inhibitory effects on PC3 prostate cell and can be considered as an appropriate way for the treatment of prostate cancer.

KEY WORDS: *Prostate Cancer, Tamoxifen, Cell Migration, Cell Proliferation*.

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Introduction

Nowadays, cancer is the second leading cause of death after cardiovascular disease (1). Prostate cancer is one of the six most common and deadly cancer in developed countries. One in six people over the age of 65 is estimated to develop this cancer (2). Hereditary factors, nutrition, sexual behavior patterns, alcohol consumption, and exposure to ultraviolet radiation play important role in the incidence of prostate cancer (3). One way to escape from cancer cells is to enhance apoptosis, or programmed cell death as a way to prevent cell growth, migration and metastasis by destroying cancer cells. The incidence of prostate cancer may be associated with age and hormonal changes such as increased testosterone levels. Endocrine studies have shown that the reduction of androgens results in atrophy of prostate cancer cells (4).

The tamoxifen is a nonsteroidal triphenylethylene antiestrogen and a selective estrogen receptor modulator (SERM) (5). Tamoxifen is metabolized by the cytochrome P450 system into its active metabolites, including 4–hydroxytamoxifen, and N–desmethyltamoxifen in the liver (6). Considering the high prevalence of prostate cancer in recent years and the anti-cancer effects of tamoxifen, this study was conducted to investigate the cytotoxic effects, proliferation and morphological changes of tamoxifen on prostate cancer cell line (PC3).

Methods

Materials: PC3 cell line was purchased from Pasteur Institute, tamoxifen and DMSO from Sigma (Steinheim, Germany) and the RPMI 1640, FBS, Penicillin-Streptomycin and Trypsin-EDTA from Gibco Co. (Gibco, Life Technologies, USA).

Methods: In order to determine the IC50, 104 cells were cultured in 96-well plates in 100 µl culture medium using MTT method. Cells were treated with 50 µl of different doses (3.25, 7.5, 15, 30 and 60 µM) of tamoxifen. After 12, 24 and 36 hours, 15 µl of MTT solution was added, incubated for four hours, and then 100 µl of DMSO solution was added and read with an ELISA device at a wavelength of 570 nm (7). For migration test, a scratch was created by crystal sampler and then, doses of 3.25, 7.5 and 15 µM were treated in 0, 6 and 12 hours and stained by Giemsa stain (8). Lower doses were used due to the detachment of cells. To examine the morphology of the cells, doses of 0, 3.25, 7.5 and 15 µM were treated for 24 hours and were then examined by microscope (9). In colony-forming assay, 100 cells were cultured in 6-well plates, which increased the space between the colonies, were treated with doses of 0, 3.25, 7.5 and 15 µM, and were fixed and stained with 0.5% crystal violet powder and methanol for 45 minutes at room temperature (10).

Statistical tests: Statistical analysis was performed using SPSS 16 software (Chicago, USA) and the results were compared with one way ANOVA test and Tukey test. $P<0.05$ was considered significant.

Results

The results showed that within 24 hours, cell proliferation in all treatment groups decreased with a regression coefficient of 0.90 (Fig. 1).

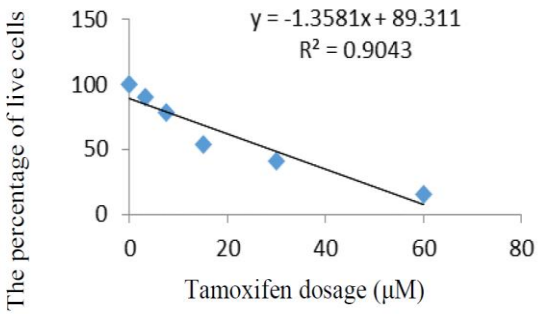

Figure 1. Effect of tamoxifen on cell viability within 24 hours. The scatter plot is presented with linear equation and regression.

Cell migration showed a significant difference between the groups treated with a dose of 15 µM; within 24 hours and a dose of 15 µM, migration significantly decreased compared with control group (89.68 ± 0.9 µm) with a groove diameter of 278.4±1.05 µm ($p<0.01$) (Fig. 2). The colony-forming ability of cancer cells showed a significant difference in all treated groups; at 15 µM, it inhibited cell proliferation with 7.3±0.6 colonies compared to 100 control colonies ($p\leq0.05$) (Fig. 3). The nuclei were normal in the control group, but in tamoxifen receiving groups, as the dose increased, the nuclei swelled, the cytoplasm became congested and the cell was wrinkled (Fig 4).

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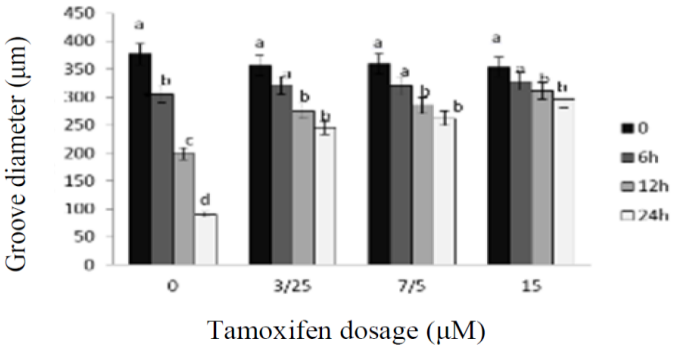

Figure 2. Effect of tamoxifen on migration at different doses. The groove diameter in the control group decreased significantly as time increased, but this migration did not significantly decrease in the 15 µM group within 24 hours. The letter a indicates lack of significance and the letters b, c, and d indicate significance ($p<0.05$) compared with the previous groups.

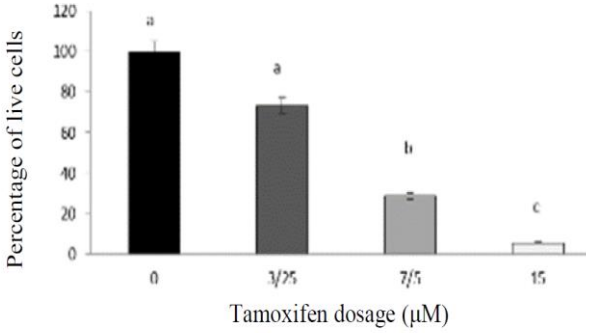

Figure 3. Effects of tamoxifen on colony formation in PC3 cells. Tamoxifen at high doses, especially at a dose of 15 µM, has a significant inhibitory effect on colony formation. The letter a indicates lack of significance and the letters b, and c indicate significance ($p < 0.05$) compared with the previous groups.

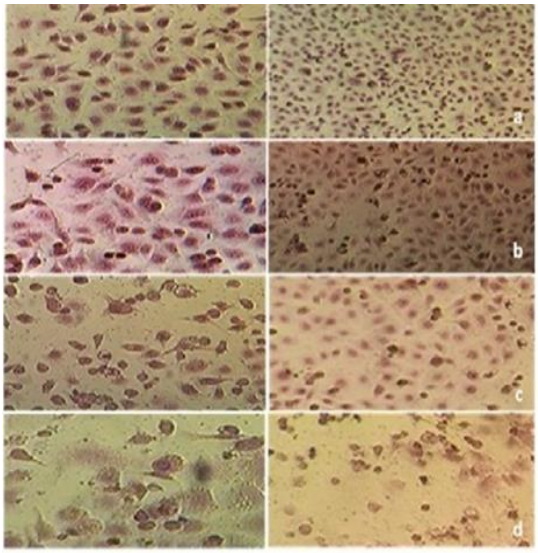

Figure 4. Effect of tamoxifen on cell morphology.

The control groups, and 3.25, 7.5 and 15 µM groups are shown with letters a, b, c, and d, respectively. As the dosage increases, the nuclei become denser and the cytoplasm transforms, becomes apoptotic and breaks down.

Discussion

This study showed that tamoxifen has dose-dependent toxic and anti-proliferative effects on prostate cancer cells. Similar to our results, Norris et al. found that tamoxifen was able to show cytotoxic effects through inhibition of TGFβ signaling (11). Tamoxifen, similar to TGFβ, inhibits the growth and proliferation of prostate cancer by inhibiting the activity of PKC and increasing the activity of P21 (12). Moreover, Kalachaveedu et al. showed that similar to tamoxifen, raloxifene induced apoptosis in breast cancer cells by increasing caspase-9 (13). Our results showed that the nuclei are oval, cytoplasm is dense and cells are wrinkled in the treated groups, which indicate the incidence of apoptosis and is consistent with the findings of others. Tamoxifen can inhibit the growth of breast cancer cells by inhibiting PKC, increasing P21 and stopping the cell cycle in the G1/S phase (14). Considering the role of cell migration and metastasis in cancers, it is important to inhibit these pathways. The results regarding migration show that, similar to other studies, tamoxifen can significantly reduce the rate and power of cancer cell migration in treated groups (15). A study by Koka et al. showed that MAPK and AKT pathways play an important role in regulating cell migration (16).

The inhibitory effects of tamoxifen on other cancers, such as colon and lung, also confirm their potential inhibitory effects by suppressing the signaling pathways of AKT and ERK1/2 (17). It has been shown that androgen receptor antagonists reduce the effects of androgens and inhibit the progression of prostate cancer (18), or analogues of luteinizing hormone releasing hormone (LHRH) reduces testosterone levels (19). Thus, it seems that tamoxifen also inhibits the progression of breast cancer by inhibiting estrogen receptor alpha (ERα) (20), and inhibits these receptors and reduces the signaling pathways for the growth of testosterone prostate cancer through binding to androgen receptors. In addition to anti-cancer effects, tamoxifen has been shown to lower the risk of developing cardiovascular disease by lowering cholesterol, and low-density lipoprotein (LDL) (21). The beneficial effects of tamoxifen on bone tissue and the treatment of diseases associated with this tissue have also been proven (22). It has also been observed that herbal compounds with effects similar to tamoxifen, such as soy-derived genistein, also have anticancer effects on colon and gastric cancers (23). Tamoxifen seems to play an inhibitory role as an analogue androgen

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in different pathways. According to these results, tamoxifen has significant inhibitory effects on PC3 prostate cells and can be a good option for improving cancer.

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