THE EFFECTS OF ANDROGENS ON THE GROWTH AND MIGRATION OF PROSTATE CANCER CELL LINE LNCAP

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ABSTRACT

Background and Objectives: The most common type of cancer among men is prostate cancer. It is glandular malignant neoplasia, strongly affected by steroid hormones, especially androgens. Some of these hormones interfere with cell proliferation and invasion of cancer cells into surrounding tissue by cell migration that is an initial step in tumor metastasis. Cell migration is involved in numerous pathogenic processes, including cancer metastasis and inflammation. The aim of this in vitro study was to determine the effects of Testosterone, Progesterone, and Estrogen on the viability and migration of LNCaP.

Materials and Methods: Androgen-sensitive human prostate cancer line (LNCaP) used; these cells were treated with different concentrations of Testosterone, Estrogen, and Progesterone hormones to assess the impact of hormonal treatment on cells proliferation and migration. Cell viability determines by MTT assay. For the determination of the effect of sex hormones on LNCaP cells migration, mitomycin used to inhibit cells growth, and then scratches made in the wells, exposed the cell to Testosterone 250 ng/ml, Estrogen 50 pg/ml and Progesterone 50 ng/ml. The migration rate measured by measuring the diameters of the scratches after 24 hours.

Results: Testosterone and Estrogen shows a significant increase in cell viability of LNCaP after 24 and 48 hours while Progesterone shows a significant decrease in cell viability after 24 and 48 hours. Estrogen increase cell migration of LNCaP (P<0.05).

Conclusion: Testosterone and Estrogen increase the proliferation of prostate cancer cells. Progesterone has an anti-proliferative effect on the LNCaP cell line. Estrogen Promotes cancer cell migration, while Progesterone inhibit cell migration.

Keywords: Testosterone, Progesterone, Estrogen, docetaxel, LNCaP cell line, prostate cancer.

I. INTRODUCTION:

Males are more likely to develop prostate cancer. It is the second-leading cause of cancer mortality in males in the United States. Adenocarcinomas account for almost all prostate cancers. The majority of prostate cancers develop slowly, but some grow rapidly⁴. Over 80% of the cases diagnosed after the age of 65 years. Despite this, only 10% of men who develop prostate cancer die from it. Prostate cancer is found in 39% of men aged 70–79 years old and in 43% of men aged 80 years old⁵. The risk of prostate cancer believed influenced by sex hormones. Androgens, in particular, implicated in the pathogenesis of prostate cancer due to their critical function in the growth and proliferation of normal and cancerous prostate cells through androgen receptor signaling. Higher levels of circulating Testosterone and lower levels of sex hormone-binding globulin (both within normal endogenous ranges) were found to be associated with an elevated prostate cancer risk⁶. Testosterone is the most significant androgen secreted by the testis. Daily, males produce about 8 mg of Testosterone. The Leydig cells make 95% of the hormone, while the adrenals produce about 5%⁷. Increased 5α-Reductase (5α-R) activity leads to more Testosterone being converted to Dihydrotestosterone (DHT), which stimulates cell proliferation and prostate growth. Poorly grown prostates in men with congenital (5α-R) deficiency and a reduction in prostate
volume and Prostate Specific Antigen production in men taking 5α-R inhibitors, such as finasteride or dutasteride, are the most consistent effects of Testosterone and DHT on the prostate\textsuperscript{[5]}. Stromal Progesterone Receptor (PR) prevents the growth and progression of prostate cancer by preventing stromal cells from differentiating into cancer-associated fibroblasts and decreasing the tumor-favoring microenvironment. PR restricted the transcriptional release of cytokines such as stromal cell-derived factor-1 (SDF-1) and interleukin-6 (IL-6). SDF-1 can interact with the C-X-C chemokine receptor type 4 (CXCR4) on epithelial cells' membranes to promote cancer invasion, tumor angiogenesis, Resistance to drug-induced apoptosis, cell growth, and metastasis\textsuperscript{[6]}. By activating the Janus kinase/signal transducer and activator transcription 3 pathway, IL-6 promotes cancer growth and resistance. In addition, both cytokines can activate Androgen Receptor signaling in Prostate cancer even at low androgen levels, enabling the progression and development of Castration resistance prostate cancer (CRPC)\textsuperscript{[7]}.

The aim of this in vitro study was to determine the effects of Testosterone, Progesterone, and Estrogen on the viability and migration of LNCaP.

II. METHOD:

LNCaP cells [androgen-dependent, prostate adenocarcinoma cells derived from lymph node metastasis were kindly obtained from a cancer research laboratory/ college of medicine/ university of Babylon and grown in RPMI-1640 medium with penicillin (100 U/ml), streptomycin (100 g/ml), and 5% fetal bovine serum at 37°C in 5% CO\textsubscript{2}. LNCaP cells were seeded into tissue culture 96-well plates at a density of 5*10\textsuperscript{4} cells/ml before 24 hours of hormonal treatments. Ethanol was used to dissolve hormones (ethanol concentrations not exceeding 0.02%) then diluted with complete growth medium to get final concentrations of (1000, 500, 250, 125, 62.5, 31.25 ng/ml) for Testosterone, (500, 50, 5 ng/ml) for progesterone and (5000, 500, 50, 5 pg/ml) for Estrogen. Following that, 200 µl of each combination was poured into each well and incubated for an additional 24 or 48 hours. The wells rinsed with 200 µl of sterile PBS once the exposure was completed. The effect of these hormones on the growth of the LNCaP line assessed by MTT assay. MTT assay measures the cellular conversion of a tetrazolium salt into a formazan product (purple color). The number of live cells is directly proportional to the opacity of the purple color, this may be determined using spectrophotometry and yields a relative estimate of cell proliferation\textsuperscript{[8]}. Cells were treated with combinations of docetaxel (32μg/ml) with either Testosterone (250ng/ml), Progesterone (50ng/ml), or Estrogen (50 pg/ml) and incubated for 48 hours. The vitality of the cells was determined using the MTT assay after the treatment was completed. Cells migration were assessed using the surface scratching method\textsuperscript{[9]}. In this method, cells in 48 wells plates allowed to grow and reach a confluence of about 95%. Confluent monolayers scratched using a sterile 2 mm diameter pipette, washed twice with phosphate buffer then the diameter of the scratches imaged and measured using a digital microscopic camera and specific software. Wells plates containing the scratches incubated for 48 h in the presence of the testing hormones at specific concentrations. After the end of the incubation period, the diameters of the scratches imaged and measured again under the same lens power and the difference in diameter between the two periods represents the cell's migration and measured on the images by pixels. Hormones concentrations used were as following: Testosterone (250ng/ml), Progesterone (50 ng/ml) and Estrogen (50 pg/ml). Four replicates for each concentration considered. Image J version 1.46r was used to analyzed and calculate the wound diameters. All data collected and analyzed by Microsoft Office Excel 2011 and Sigma plot version 12.5 software. The differences between the means of the sex hormones and chemotherapy concentrations analyzed using a one-way Anova test. P-values ≤0.05 and ≤0.001 considered statistically significant and highly significant, respectively.

III. RESULTS:

Cell Proliferation Assays

Figure (1) shows the effects of different concentrations of Testosterone on the viability of LNCaP prostate cancer cell line. Comparing to the control group (untreated cells), Testosterone caused a significant (P<0.05) increase in cell viability at concentrations (1000, 125, 62.5 ng/ml) after incubation for 24hr. Incubation for 48 hours showed that the Testosterone at all concentrations tested causes a significant (P<0.05) increase in cell viability when compared with the control group.
Figure (1): Viability of LNCaP cells treated with different concentrations of Testosterone and incubated for 24 and 48 hours.

Figure (2) illustrates the effect of Progesterone on cells viability. Progesterone significantly (P<0.05) decreased cells viability at the concentrations (50, 500 ng/ml), but there were no significant (P<0.05) differences between the control group and the concentration of (5 ng/ml) after the incubation periods of 24 and 48 hours.

Figure (2): Viability of LNCaP cells at different concentrations of Progesterone after incubation for 24 and 48 hours.

Estrogen hormone at the concentrations (50, 500, and 5000 pg/mL) causes a significant (P<0.05) increase in cell viability in comparison with the control group after 24 hours of incubation. All concentrations were significantly (P<0.001) increased the viability of the cells after an incubation period of 48 hours, Figure (3).
Effect of Testosterone, Progesterone, and Estrogen on cell migration of LNCaP cell line

In this experiment, cells treated with the three hormones each with a particular concentration similar to that used for viability testing. As shown in figure 4, there was a significant increase in cell migration after 48 hours of incubation with Estrogen hormone at the concentration (50 pg/mL) compared with the control group, while there were no significant differences regarding Progesterone (50 ng/ml) and Testosterone (250 ng/ml) compared to the control group.

**Effect of docetaxel on the stimulatory effects of hormones:**

This experiment aimed to evaluate the effect of the presence of Docetaxel on the viability of cells treated with the three hormones. As shown in (Figure 5), Docetaxel at the concentration of (32 μg/ml) added to the cells alone or in combination with each hormone at a specific concentration. Results indicated an increase in cell viability induced by the three hormones, whereas Docetaxel caused a significant decrease in cells viability in comparison with the control group. Combination groups (Docetaxel 32 μg/ml with Testosterone 250 ng /mL, Progesterone 50 ng /mL, Estrogen 50 pg/mL) showed no significant (P<0.001) difference than DOX group, indicating that Docetaxel inhibits the stimulatory effects of the hormones.

Neoplasia of hormone-associated tissues presently accounts for more than a third of all newly diagnosed cancers in men and women in the united states [10]. Prostate cancer is one of these types of cancers, especially influences.
by sex androgens. Results of viability documented by this study indicated a significant increase in the viability of the prostate cancer cells at all Testosterone concentrations tested after 48 hours of incubation. These effects are dose and time-dependent, in which the proliferation of cancer cells found to increase by many folds after 48 hours comparing with the effect after 24 hours for the same doses. Morrissey et al found that Cell proliferation increased significantly by day 2 of Testosterone treatment and reach its peak on day 8. Testosterone treatment significantly increased Prostate-specific antigen and mRNA expression, and was rapidly metabolized by LNCaP cells compared with other types of androgens [11]. Testosterone promotes the proliferation of LNCaP cells and drives prostate cancer growth via ligand-mediated activation of the androgen receptor [12]. Androgens enhance LNCaP proliferation through PI3K/Akt-independent activation of mTOR and consequent post-transcriptional increases in cyclin D protein expression. [13]. At low concentrations, cell proliferation grew gradually with increasing Testosterone concentrations, then plateaued with no more response despite logarithmically increased Testosterone concentrations[14].

Progesterone normal ranges are 1 to 20 ng/mL in women and less in men. In women, it inhibits the proliferation of endometrial [15]. In this study, concentrations of (50, 500 ng/ml) which are above the normal levels were found to suppress the proliferation of prostate cancer cells whereas the concentration of 5 ng/ml did not cause any effect on cell proliferation. Previous research found that progesterone promotes cell differentiation while inhibiting cellular proliferation via the nuclear progesterone receptor (nPR). It was shown that progesterone-mediated growth inhibition was frequently preceded by decreased cyclin expression and/or activation of the cyclin-dependent kinase [16]. Progesterone Receptor protein levels were increased by castration therapy but decreased when tumors progressed to the castration-resistant stage to pre-castration levels. In vitro, increased PR expression in prostate stromal cells suppressed cell proliferation and delayed cell cycling throughout the development of prostate cancer [17]. In this study, we tried to figure out the effect of Estrogen at different concentrations on the viability of LNCaP prostate cancer cells. At the concentrations (5, 50, 500, 5000 pg/mL) there was a highly significant increase in cell viability that appear after 48 hours of administration. Arnold et al, 2004, previously described this time-dependent effect. They documented that Estrogen stimulatory effect appears after 3 days and peaked on the 8 days. They suspect that Estrogen acts as a weak androgen in these cells by binding directly to the mutant Androgen Receptor (AR). The presence of Estrogen receptors in prostate tissue implies that Estrogens may play a role in prostate cancer development. Eα expression is reported to be increased during Prostate cancer progression [18]. Despite the proliferative effects of Testosterone at (250 ng/ml), it showed a limited effect on cells migration (Figure 4). This result described by previous studies that confirmed that the treatment of prostate cancer cells with testosterone reduces their migration rate. This inhibitory effect has been attributed to a membrane AR (mAR) [19]. Despite this, numerous other research has found that Testosterone/ZIP9 interactions, rather than Testosterone/AR interactions, activate the migratory machinery of a metastatic prostate cancer cell line[20]. The same effect seen for Progesterone at (50 ng/ml). Progesterone affect cell migration in different ways depending on tissue type. It was confirmed to inhibit migration in vascular smooth muscle cells, but to enhance migration in breast cancer cells and lung adenocarcinoma cells [21]. Previous research in different cancer cells suggests that progesterone regulates cell motility via regulating cadherins and other cell adhesion molecules. Cadherin expression has been shown to increase cell motility, and elevated levels of progesterone produced a substantial shift in cadherin and laminin expression in mammary gland epithelial cells, resulting in a disordered basement membrane and reduced cell to cell adhesion and motility [22]. Estrogen showed the most significant effect on cells migration. Estrogen Promotes Prostate Cancer Cell Migration through Paracrine Release of Alpha-Enolase (ENO1) from Stromal Cells, Estradiol promotes ENO1 secretion from stromal cells, ENO1 protein stability increased in prostatic stromal cells in an Estrogen receptor-dependent manner. ENO1, which is generated by prostate stromal cells, attaches to the surface of prostate cancer cells as a plasminogen receptor, therefore promoting prostate cancer cell motility [23]. Another research show the ERβ5 and its predictive usefulness in prostate cancer. Both ERβ2 and ERβ5 increased prostate cancer cell invasion, but only those expressing ERβ5 migrated faster [24]. Docetaxel is a powerful anticancer medication. Docetaxel inhibits androgen transport upon ligand activation via microtubule stabilization; therefore, its action can be ascribed in part to its effect on androgen receptor transport. These findings imply that androgen suppression and microtubule targeting have complimentary effects [25].

V. CONCLUSION:
Testosterone and Estrogen increase the proliferation of prostate cancer cells. Progesterone has an anti-proliferative effect on the LNCaP cell line. Estrogen Promotes cancer cell migration, while Progesterone inhibit cell migration,
REFERENCES: