



Comparative Study of Testosterone, Spironolactone, Docetaxel and Their Combination Effect on Prostate Cancer Cell Line LNCaP

Rawaa A. Alawi AL Khafaji^{1*}, Entisar J. Hamad AL-Mukhtar², Rana A. Ghaleb³

Abstract

Background: Prostate cancer is the (most frequent) type of cancer among males. Androgenic hormones have a big role in prostate cancer, which is a kind of glandular malignant neoplasia. Testosterone hormone play important role in the cancer cell propagation) and invasion into (surrounding tissue) by cell migration, which is the first stage in tumor metastasis.

Aim of the study: The aim of the study is to determine the effects of testosterone, spironolactone, docetaxel, combination of either testosterone plus spironolactone or testosterone plus spironolactone and docetaxel on the viability percentage of (LNCaP) cell line.

Materials and Methods: This study used the LNCaP (androgen-sensitive human prostate cancer) cell line, which was treated with various concentrations of testosterone, spironolactone, docetaxel, and a combination of testosterone plus spironolactone and testosterone plus spironolactone and docetaxel. After 48 hours of incubation, the MTT test was used to analyze their influence on the viability (proliferative or antiproliferative effect) of the LNCaP cell line.

Results: At low concentrations, testosterone increased the viability of LNCaP cells significantly ($p > 0.05$), whereas at high concentrations, it caused a (highly significant) ($p < 0.001$) decrease in viability. Spironolactone treatment increased the viability of the LNCaP cell line significantly ($p > 0.05$), but it decreased the viability of the LNCaP cell line at high concentrations. In a dose-independent way, docetaxel-treated cells demonstrated a highly significant ($p < 0.001$) decrease in viability. The viability of (LNCaP) cell line treated with the combination of testosterone plus spironolactone was high significantly ($P < 0.001$) decreased, and in the cells treated with the combination of testosterone plus spironolactone and docetaxel the viability was significantly ($p < 0.05$) decreased by low concentration and high significantly ($p < 0.001$) decreased at 250+250+250 and 500+500+500 $\mu\text{g/ml}$.

Conclusion: On the LNCaP cell line, high testosterone and spironolactone concentrations had an antiproliferative effect.

6683

Key Words: Testosterone, Spironolactone, LNCaP cell line, prostate cancer

DOI Number: 10.14704/nq.2022.20.6.NQ22673

NeuroQuantology 2022; 20(6): 6683-6689

Introduction

The second (most frequent) cancer in men and the (fifth most common) cause of death globally is prostate cancer. Incidence of (prostate cancer) and fatality rates are directly linked to age, with older men having the greatest incidence (more than 65 years old). [1] According to (GLOBOCAN) estimates, 1,276,106 new cases will be registered worldwide in 2020, with 449,761 in Europe and 64,955 in

France. Meanwhile, 358,989 men died of cancer (worldwide, with 107,315 in Europe and 9002 in France. [2] Because androgens play a role in a variety of illnesses, androgen receptor signaling is a critical factor in pathological states. The expression of genes involved in sexual development, prostate cell proliferation and survival, and, to some extent, cancer progression is regulated by the androgen receptor, a transcription factor that is activated by the testosterone metabolite 5-dihydrotestosterone.

Corresponding author: Rawaa A. Alawi AL Khafaji

Address: ¹University of Babylon College of Medicine Department of Pharmacology Iraq, ^{2,3}University of Babylon College of Medicine Department of Human Anatomy Iraq.

E-mail: ¹Rawaaadil281991@gmail.com, ³Rana.a.ghaleb@gmail.com



[3] Spironolactone is a nonselective aldosterone receptor antagonist. Its antiandrogenic action lowers testosterone, (androstenedione, and dehydroepiandrosterone) levels in (castrated males) with prostate cancer, making it a potentially effective medicine in the treatment of prostate cancer. Its antiandrogenic properties are supported by a report of a clinical and (prostate specific antigen (PSA) response) in a man with prostate cancer treated with spironolactone. [4] Spironolactone prevents cancer stem cells from growing by inhibiting the DNA damage response. Spironolactone activates the ataxia-telangiectasia mutant checkpoint pathway ATM-Chk2-mediated checkpoint pathway in (colon cancer) cell lines, upregulating the expression of a set of major histocompatibility complex class I-like molecules known as the Natural Killer Group 2D (NKG2D) ligands. [5] Docetaxel (Taxotere®), a cytotoxic taxane with antineoplastic action against a variety of cancer cells, has shown synergistic effect with a number of other anticancer drugs. It is a cytotoxic antimicrotubular drug that inhibits normal cell division by promoting and stabilizing microtubule assembly while also preventing microtubule depolymerization. [6] For metastatic prostate cancer, docetaxel is the first-line chemotherapeutic drug. The emergence of resistance, on the other hand, reduces its efficacy and decreases the survival advantage. [7]

Materials And Methods

The (androgen-dependent) human prostate (adenocarcinoma) cells derived from lymph node metastases that make up the LNCaP cell line were cultured in RPMI-1640 media with the addition of (penicillin) (100 U/ml), (streptomycin) (100 g/ml), and 5% (fetal bovine serum) at (37°C) in 5% CO₂. (LNCaP cells) were seeded in tissue culture 96-well plates at a density of 5*10⁵ cells/ml before 24 hours of the treatment with either testosterone (Testopel) 100mg/1ml, spironolactone(Aldacton) 25mg, docetaxel (Taxotere®) 120mg/6ml, combination of testosterone plus spironolactone or testosterone plus spironolactone and docetaxel. Distilled water 5ml was used to dissolve spironolactone and placed in sonicator at 37 C. Sonication accelerates the dissolution of a solid into a liquid by agitating particles in a solution with sound waves [8], which

is then diluted with complete growth media to obtain final concentrations of (1000, 500, 250, 125, 62.5, 31.25 µg/ml) for spironolactone, also similar concentrations were prepared for both testosterone and docetaxel. The combination of spironolactone plus testosterone was prepared by adding equal concentrations of each agent (500+500, 250+250, 125+125, 62.5+62.5, 31.25+31.25, 15.625+15.625 µg/ml), similarly the combination contain spironolactone plus testosterone and docetaxel was prepared by adding equal concentration of each agent (500+500+500, 250+250+250, 125+125+125, 62.5+62.5+62.5, 31.25+31.25+31.25, 15.625+15.625+15.625 µg/ml). Following that, 200 µl of each concentration was poured into each well and left to incubate for another 48 hours. After the 48-hour exposure period, the wells were cleaned with 200 µl of (sterile PBS). The MTT assay was used to investigate the effects of testosterone, spironolactone, docetaxel, and combinations of testosterone plus spironolactone or testosterone plus spironolactone and docetaxel on the growth of the (LNCaP) cell line. The (MTT assay) determines how quickly a (tetrazolium salt) is converted into a (formazan product) in the cell (purple color). The opacity of the purple color is (directly proportional) to the number of live cells; this may be evaluated using (spectrophotometry) and gives a relative estimate of cell viability. Three replicates for each concentration was considered. Microsoft Office Excel 2010 was used to collect and analyze all of the data. A one-way Anova test was used to examine the differences between each treated group and the (control group). P-values (≤ 0.05) and (≤ 0.001) were considered statistically (significant) and (highly significant) respectively.

6684

Results

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation testosterone caused a significant increase ($p < 0.05$) in the viability of LNCaP cell line at the concentrations 125 and 62.5 µg/ml, while it caused a significant ($p < 0.05$) and a (highly significant) ($p < 0.001$) decrease in the viability of these cells at the concentration 250 µg/ml and at the concentrations 500 and 1000 µg/ml respectively as shown in (figure. 1).



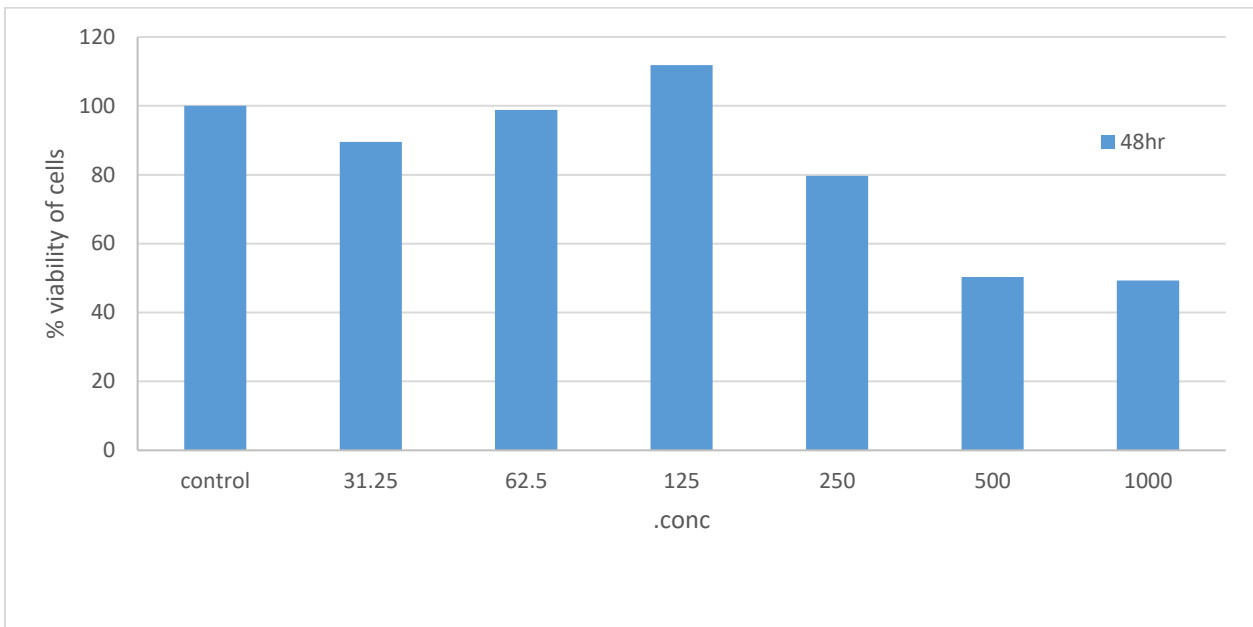


Figure 1: Effect of (Testosterone) on the (Viability) Percentage of LNCaP cell line after 48 hours of incubation

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation spironolactone caused (a significant) ($p < 0.05$) increase in the viability of LNCaP cell line at the concentration 62.5 µg/ml. While at the

concentrations 31.25, 125 and 1000 µg/ml it caused significant decrease in the viability of these cells. Differences between the control group and 250 and 500 µg/ml treated group was insignificant ($p > 0.05$) as shown in (figure. 2). 6685

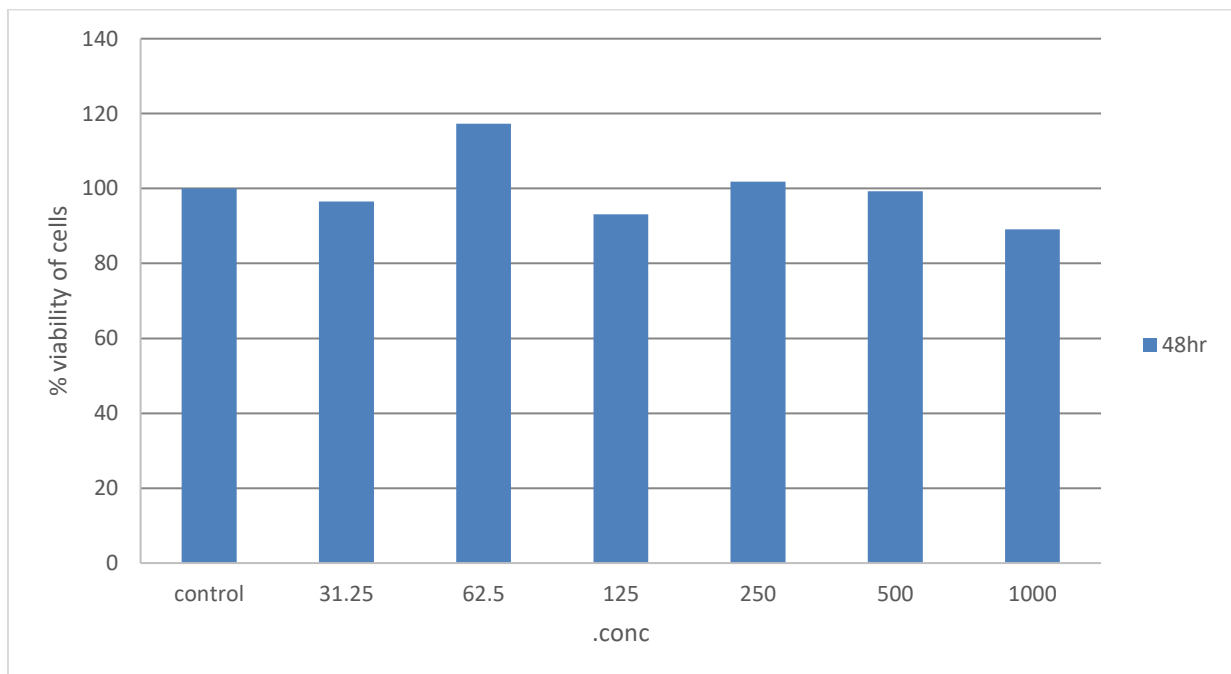


Figure 2: Effect of Spironolactone on the Viability Percentage of LNCaP Cell line after 48 hours of incubation.

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation docetaxel caused a highly significant ($p < 0.001$)

decrease in the viability of LNCaP cell line at (all concentrations) as shown in (figure 3)



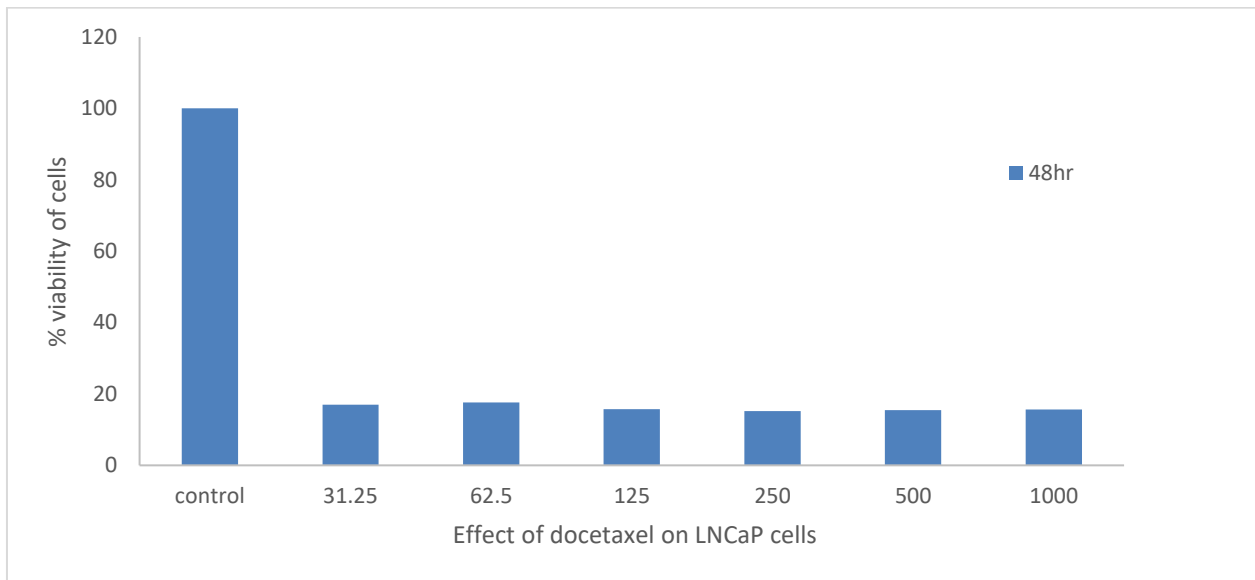


Figure 3: Effect of Docetaxel on the Viability Percentage of LNCaP Cell line after 48 hours of incubation.

Comparing to the control group (untreated cells), result showed that after 48 hours of incubation the combination of spironolactone plus testosterone

caused significant ($P < 0.05$) decrease in cell viability at concentration 31.25+31.25 $\mu\text{g/ml}$ while it caused insignificant ($p > 0.05$) in the viability of these cells at all other concentrations as shown in (figure. 4).

6686

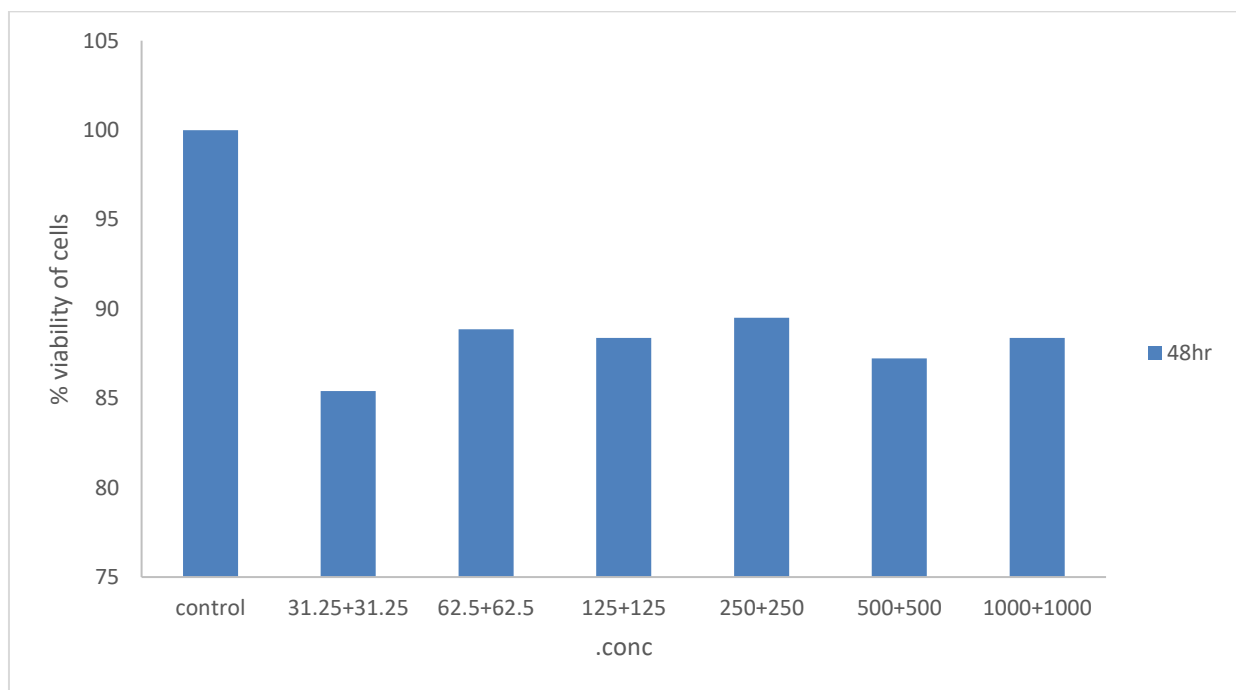


Figure 4: Effect of spironolactone plus testosterone combination on the viability percentage of LNCaP cell line after 48 hours of incubation.

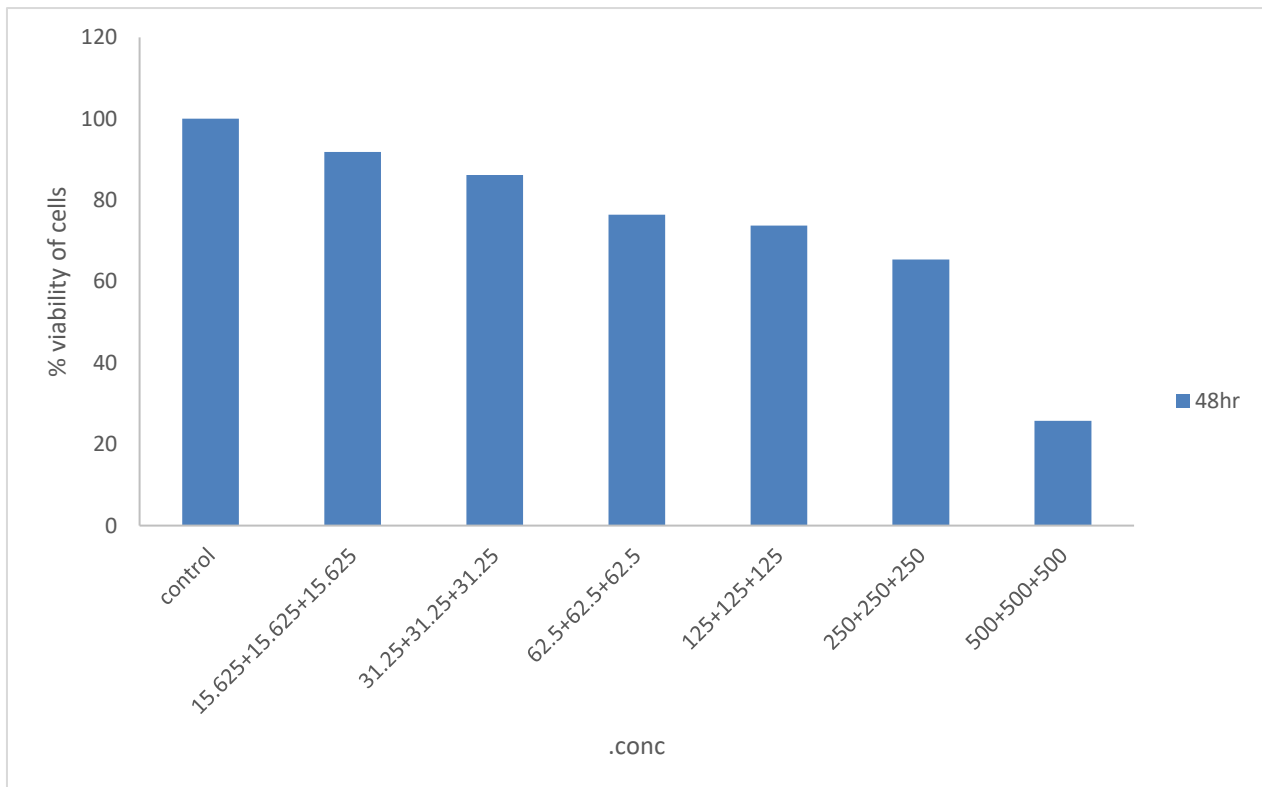
Comparing to the control group (untreated cells), result showed that after 48 hours of incubation the viability of LNCaP cell line was significantly ($P < 0.05$) decreased after treatment with the combination

containing 62.5 $\mu\text{g/ml}$ of each component and that containing 125 $\mu\text{g/ml}$ of each component. Difference between the control group and 15.625 $\mu\text{g/ml}$ and 31.25 $\mu\text{g/ml}$ treated groups was insignificant



($p > 0.05$). Also the combination containing 250 $\mu\text{g/ml}$ and that containing 500 $\mu\text{g/ml}$ of each component decreased the viability of LNCaP cell line high significantly ($p < 0.001$). The combination

containing 500 $\mu\text{g/ml}$ of each component showed the maximum cytotoxic effect against the LNCaP cell line (figure 5).



6687

Figure 5: Effect of spironolactone plus testosterone and docetaxel combination on the viability percentage of LNCaP cell line after 48 hours of incubation.

Discussion

Regarding the role of testosterone in promoting or surpassing prostate cancer, evidences are inconsistent. Testosterone is not a tumor-promoting factor for prostate cancer, but it is possible that it caused prostate epithelial cells to develop hypersensitivity to androgens, giving them a selective growth advantage that led to a few of those cells progressing to malignancy (Bosland, 2014). A study by Anagnostopoulou et al., (2013) stated that in animal models, testosterone has been shown to inhibit cancer cell proliferation by counteracting the proliferative effects of endogenous hormones like DHEA. [9]

Because very high androgen levels inhibit the proliferation of prostate cancer cells, this effect could be used in prostate cancer treatment. However, these pathways have largely remained undiscovered at the molecular and cellular levels. As a result, the purpose of this study is to see how

different testosterone concentrations affect the viability of (prostate cancer) cells. High testosterone has an inhibitory effect on cell viability in the LNCaP cell line due to androgenic receptor (AR) downstream signaling or non-genomic AR activity. Furthermore, hormonal activation of the androgen receptor causes the receptor to self-stabilize, resulting in an increase in AR activity. As a result, in clinical practice, a therapeutic reduction in androgen levels is a clinical target that would result in a drop in AR activity and, as a result, a decline in AR-driven prostate cancer progression. [10]

The viability of the (LNCaP) cell line increased after treatment with a low dosage of spironolactone, but decreased significantly when the concentration was increased to 1000 $\mu\text{g/ml}$. These results agree with Isla, (2016) who found that taking spironolactone was linked to a decreased risk of prostate cancer in a retrospective, matched cohort study. [11] Also, this result agrees with Walsh, (1975) who found that spironolactone can suppress the level of plasma



androgens by spironolactone. These findings could be attributed to the effect of spironolactone as an inhibitor of androgen synthesis, as found in a Walsh study in which spironolactone was given to castrated men with metastatic prostate cancer. This study found that spironolactone decreased the amounts of the androgens produced by the adrenal glands, including testosterone, androstenedione, and dehydroepiandrosterone. [12]

The decreased in the viability of the (LNCaP) cell line after treatment with all concentrations of docetaxel agrees with Yang et al., (2019) study that docetaxel suppresses the development of cultured prostate cancer cells in a dose-independent way. These findings are connected to the function of docetaxel as a microtubule inhibitor, which prevents microtubule disintegration by binding to -tubulin. [13] As a result, by arresting cells in the (G2/M phase) of the cell cycle, docetaxel causes cell death, which peaked at 24 and 48 hours. Docetaxel inhibited PI3K/Akt activation, lowered Bcl-2 levels, and enhanced caspase-3/9 activation, and it had a higher (inhibitory effect) on (AR-dependent LNCaP cells) than on AR-independent PC3 cells on growth suppression and apoptosis in prostate cancer cells. [14]

After 48 hours of incubation, the testosterone and its combination with spironolactone was significantly ($P < 0.05$) reduced the viability of the LNCaP cell line. These findings agree with Jeffrey (2015), who found that despite historical evidence that suggests testosterone is generally hazardous for men with active malignancies, testosterone therapy to (castrate-resistant patients) may help in restoring (hormone sensitivity) and hence aid in transforming bad tumours into a (less aggressive phenotype). Prostate cancer cell development is suppressed by high testosterone concentrations, whereas cell growth is stimulated by low testosterone doses. Therefore, the researcher believe it is dangerous to use excessive doses of testosterone in men who are hormone resistant. It may be particularly wrong to give testosterone to males who have significant prostate tumors. [15]

The viability of the LNCaP cell line was significant ($P < 0.001$) reduced in a time-dependent and concentration dependent fashion after treatment with the mixture containing 250 $\mu\text{g}/\text{ml}$ and that containing 500 $\mu\text{g}/\text{ml}$ of each of testosterone, spironolactone and docetaxel after 48 hours of incubation. These findings agree with Paula et al., 2021 they found that docetaxel-induced decrease of free testosterone blood levels in patients with

metastatic prostate cancer plays a predictive significance. Docetaxel affects androgen receptor signaling, whereas testosterone reduces docetaxel absorption by cells and prevents microtubule stability. As a result, it was suggested that testosterone levels be reduced while receiving docetaxel chemotherapy. Although blood androgens (testosterone, androstenedione, and DHEA) decrease following docetaxel treatment, its effect on lowering testosterone levels is less evident. The majority of testosterone 6 β - and 16 β -hydroxylation is catalyzed by CYP3A4, which is also primarily responsible for the metabolism of docetaxel. Docetaxel has been demonstrated to induce CYP3A4. Through inactivation through 6 β - and 16 β -hydroxylation, CYP3A4 upregulation can reduce testosterone levels. [16]

Conclusion

All high concentrations of testosterone have an antiproliferative rather than proliferative effect on the LNCaP cell line. Spirolactone has an antiproliferative impact.

Reference

- Rawla, Prashanth, *Epidemiology of Prostate Cancer*, World J Oncol. 2019;10(2):63-89.
- Wendy Bijoux, Emilie Cordina-Duverger, Soumaya Balbolia, Pierre-Jean Lamy, Xavier Rebillard, Brigitte Tretarre, Sylvie Cenee and Florence Menegaux. *Occupation and prostate Cancer risk: results from the epidemiological study of prostate cancer (EPICAP)* Journal of Occupational Medicine and Toxicology. 2022; 17(5).
- Sebastian Student, Tomasz Hejmo, Aleksandra Poterała-Hejmo, Aleksandra Leśniak and Rafał Bułdak. *Anti-androgen hormonal therapy for cancer and other diseases*, European Journal of Pharmacology. 2020; 866 (172783).
- Santhanam Sundar and Peter D Dickinson , *PMC PubMed Central@ Spironolactone, a possible selective androgen receptor modulator, should be used with caution in patients with metastatic carcinoma of the prostate* , 2012, doi: 10.1136/bcr.11.2011.5238.
- Rajanna A. *Novel approach to target cancer stem cells for therapy*. Med Hypotheses. 2016;88:83-5.
- Katherine A. Lyseng-Williamson and Caroline Fenton , *Docetaxel: a review of its use in metastatic breast cancer* 2005, 65 (17).
- Lu, Xinxing , Yang, Feiya ,Chen, Dexi ,Zhao, Qinxin ,Chen, Dong ,Ping, Hao Xing, Nianzeng , *Quercetin reverses docetaxel resistance in prostate cancer via androgen receptor and PI3K/AKT signaling pathways* , International Journal of Biological Sciences, 2020, 16(7) .
- Claire Gillespie, *sciencing. How Does Sonication Work?* 2018.
- Vasilias angnostopoulou , Losif Pediaditakis , Saad AL Kahtani , Saud A.Alarafi, Eva mari. et al. *'Differential effects of dehydroepiandrosterone and testosterone in prostate and colon cancer cell apoptosis: The role of nerve growth*



factor (NGF) receptors', *Endocrinology*. 2013; 154(7), pp. 2446–2456.

Tiziana Siciliano, Ulrich Sommer, Alicia-Marie K. Beier, Matthias B. Stope, Angelika Borkowetz , Christian Thomas and Holger H. H. Erb, *Current Issues in Molecular Biology* , The Androgen Hormone-Induced Increase in Androgen Receptor Protein Expression Is Caused by the Autoinduction of the Androgen Receptor Translational Activity. 2022; 44, 597–608.

Isla S. Mackenzie, Steven V. Morant, Li Wei, Alastair M. Thompson and Thomas M. MacDonald, *Spironolactone use and risk of incident cancers: a retrospective, matched cohort study*, *British pharmacological society journals*. 2016; <https://doi.org/10.1111/bcp.13152> Citations: 28.

P C Walsh and P K Siiteri, *Suppression of plasma androgens by spironolactone in castrated men with carcinoma of the prostate*, *National Library of medicine*. 1975; 114(2):254-6. doi: 10.1016/s0022-5347(17)67001-0.

Chongyi Yang , Weijie Zhang , Jie Wang, Pengpeng Chen and Jiangjiang Jin, *Effect of docetaxel on the regulation of proliferation and apoptosis of human prostate cancer cells*, *National Library of Medicine*. 2019; 19(5):3864-3870. doi: 10.3892/mmr.2019.9998. Epub.

Nehmé , P.Varadarajan , Q Zhang, M Gerhold , X Lin. et al. 'Modulation of docetaxel-induced apoptosis and cell cycle arrest by all-trans retinoic acid in prostate cancer cells', *British Journal of Cancer*. 2001; 84(11), pp. 1571–1576

Jeffrey Turner and Marina Is testosterone the new therapy for prostate cancer ? prostate cancer research institute 2015, Is. 18 Vol. 4.

Paula Kappler, Michael A. Morgan, Philipp Ivanyi, Stefan J. Brunotte, Arnold Ganser and Christoph W. M. Reuter, *Prognostic role of docetaxel-induced suppression of free testosterone serum levels in metastatic prostate cancer patients*, *Scientific Reports* volume 11, Article number: 16457 (2021)

6689