Antiproliferative effects of Dutasteride and Bardoxolone-methyl (CDDO-me) combination in Prostate Cancer (PCa) cells

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Abstract

Introduction: Prostate cancer (PCa) is one of the most commonly diagnosed cancer amongst men worldwide and a leading cause of cancer-associated morbidity and mortality in men in the United States (US). Androgens plays a pivotal role in prostate cancer's growth via regulating androgen receptor (AR) signaling. Hence, several clinically approved drugs are available to target different stages of AR signaling and to suppress tumor growth. However, monotherapy has often resulted in the selection of androgen resistant tumors and recurrence of highly aggressive PCa. Therefore, in this study we investigated the combined effects of these agents towards maximum tumor suppression. The three drugs used in our studies were, Enzalutamide, which suppresses androgen binding to AR; Dutasteride, which suppresses the conversion of androgen to a more potent DHT; and CDDO-me, which suppresses AR gene and protein expression.

Objectives: Our objective was to see whether a combination treatment regimen using Enzalutamide, Dutasteride, and CDDO-me may act synergistically to reduce PCa growth, as compared to control or each agent alone.

Methodology: Effect of drugs, alone or in combination, on PCa cell proliferation was carried out using the C4-2B cell line. Cells were cultured into 24-well culture plates (~0.25 x 106 cells/well) overnight and then exposed to Enzalutamide (0-50 μM), CDDO-Me (0-500 nM) or Dutasteride (0-62.5 μM) either individually or in different two/three-drug combinations of varying concentrations. After 48 h of drug exposure/s, changes in cell viability were measured using a colorimetric dye, CCK8. The optical density (OD) in control and treated samples were enumerated using a spectrophotometric plate reader (HT Synergy). In addition, at the end of the experiments, cells were washed with phosphate buffered saline (PBS) and images were captured using a digital camera (Leica ICC50) mounted on an inverted microscope (Olympus). Percent change in cell proliferation was calculated and statistical analysis was done to observe the effects of drugs alone or in combination on C4-2B cell growth, as compared to untreated cells.

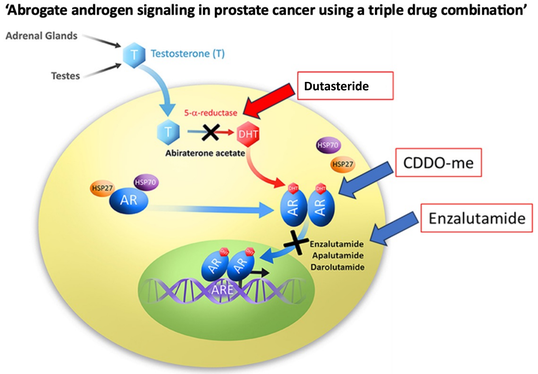
Results: Enzalutamide, even at the highest dose used, did not show significant suppression in cell growth. However, a dose-dependent effect in suppressing cell growth was evident for both CDDO-me and Dutasteride, which were significantly cytotoxic at the highest dose used. Therefore, subsequent proliferation assays were carried out using lower doses of each of these two agents. Ultimately, our findings showed that a combination of CDDO-Me at 500 nM and Dutasteride at 31.25 μM was optimal in suppressing C4-2B cell growth, as compared to each drug alone. Furthermore, the captured images clearly showed a precipitous decrease in both cell numbers and altered cell morphologies, indicative of increased apoptosis.

Conclusion: Our in vitro experiments showed that two clinically approved anti-cancer agents that target AR signaling, may be used at lower doses to achieve significantly anti-proliferative effects in PCa cells. This combined targeting strategy may decrease the side-effects associated with each drug and suppress the selection of drug resistant prostate tumors.

Introduction

Prostate cancer is the second most common cancer in men [1]. The disease is also very resilient and is resistant to many common forms of therapy on the market. Early screening has led to a decrease in the mortality rates. Current therapies look to deprive the cancer of androgen, androgen deprivation therapy (ADT). However, these cancers can lead to castration resistant prostate cancer (CRPC), which ADT is ineffective at treating. There have been drug therapies tested to block the androgen binding to the androgen receptor (AR), yet the cancer cells have been shown to alter the binding proteins and to contain point mutations that lead to resistance to these drugs [2,3]. A novel method is to downregulate the androgen receptors so there are less available. CDDO-ME has been shown to increase oxidative stress on cancer cells while Enzalutamide blocks the androgen binding to AR in the cancer cells. It has been shown that when given together, CDDO-ME enhances the effects of Enzalutamide [4]. In addition, Dutasteride blocks the conversion of testosterone to DHT, a more potent form of testosterone [5]. The combination of these agents is hypothesized to lead to better outcomes regarding PCa cell proliferation. Please see Schematic below:

'Abrogate androgen signaling in prostate cancer using a triple drug combination'



Methods

Cell Culture: The LNCaP (androgen-dependent) and C4-2B cells (androgen-independent) were purchased from ATCC (Rockville, MD, USA). Cells were cultured in DMEM media supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals) and 1% antibiotic (Sigma) in a humidified incubator containing 5% CO2 at 37 °C. Cells were also cryogenically frozen in media containing 10% dimethyl sulfoxide (DMSO).

Proliferation Assay: Both cells were cultured in 24 well plates (1x10^6 cells/well). Treatment was initiated the day after. Proliferation was measured after 72 hours by utilizing the CCK8 dye [Sigma]. Briefly, two different PCa cell lines, LNCaP and C4-2B cells, were cultured in 24-well tissue culture plates and exposed to different doses of either Dutasteride alone or CDDO-me alone, or combination of these two agents at different concentrations. Changes in the number of live cells were measured after 72 hour incubation by using the CCK8 colorimetric dye and measurement of the optical density (OD) values using a HT-synergy plate reader. Data on the effects of drugs, alone or combination, on PCa cell proliferation at 72 h post-exposure are shown in the Results section [Fig.3A & 3B]. In addition, we documented microscopic changes on the morphology and confluency of both control and treated cells by using a digital camera mounted on a Leica inverted microscope. Representative images are provided in [Fig.4, A, B & C].

Statistical Analysis. The GraphPad Prism (version-6) Software was used for the statistical analyses. Results were expressed as the standard error of the mean (± SEM).

Results

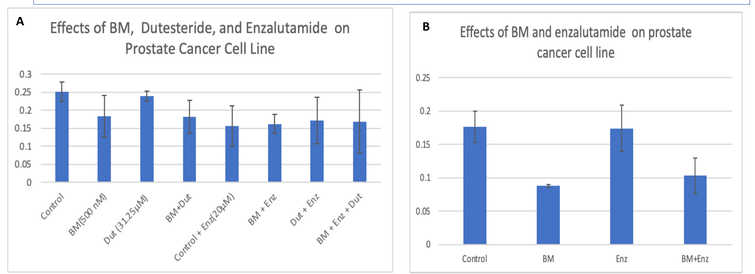


Figure-1: Effect of individual drugs at the highest concentrations, on LNCaP (A) and C4-2B (B) cell proliferation using the CCK8 assay are shown from two independent experiments (n=2) in triplicate wells. Results in LNCaP (**panel-A**) showed that low dose of CDDO-me (500 nM) was slightly toxic, but Dutasteride (31.25 μM) was not. However, Enzalutamide (20 μM) was toxic alone in these androgen-dependent cell line. Results in C4-2B cells (**panel-B**) showed that CDDO-me (BM) at a 1 μM dose was toxic (>50%) to cells. However, Enzalutamide (20 μM) was not and did not further increase the effect of CDDO-me when combined in these androgen-independent (CRPC) cell line.

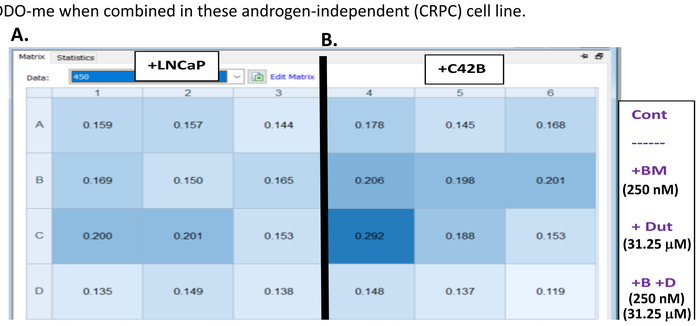


Figure-2: Efficacy of CDDO-me (BM) and Dutasteride (Dut) on LNCaP (panel-A) and C4-2B (panel-B) cell lines. A representative image of the data obtained from the HT-synergy plate reader is shown in Control (untreated) and BM (250 nM), Dut (31.25 μM) and BM + Dut combination treatment are shown. Data shows numbers obtained in triplicate wells in each treatment group. low dose CDDO-me (250 nM) did not affect LNCaP cell growth and had a slight proliferative effect on C4-2B cells. Exposure to low dose Dutasteride (31.25 ⍰M) alone also did not show significant decrease in either LNCaP or C4-2B cell proliferation, and in fact, showed a slight increase in cell growth. Interestingly however, when combined together, these two agents were able to significantly decrease the growth of both LNCaP and C4-2B cells, as compared to the controls.

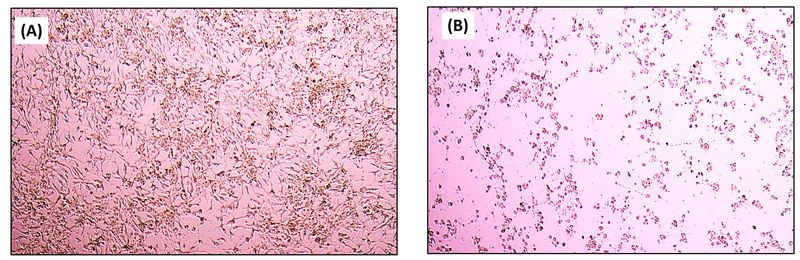


Figure-3: Representative images of C4-2B cells (40X) in control untreated (panel-A) and in those exposed to the CDDO-me (250 nM) and Dutasteride (31.25 μM) combination for 72 hours. The images were digitally captured using a camera mounted on an inverted Leica microscope. Results clearly show that combined treatment of low doses of CDDO-me and Dutasteride can have drastic effects in decreasing cell proliferation and cell numbers in this Pca cell line.

Discussion

Discussion: As shown in the above image and data from the plate-reader data: low dose CDDO-me (250 nM) did not affect LNCaP cell growth and had a slight proliferative effect on C4-2B cells. Exposure to low dose Dutasteride (31.25 μM) alone also did not show significant decrease in either LNCaP or C4-2B cell proliferation, and in fact, showed a slight increase in cell growth. Interestingly however, when combined together, these two agents were able to significantly decrease the growth of both LNCaP and C4-2B cells, as compared to the controls. Images clearly showed a lot less cells in the drug treated plates as compared to control cultures. Our proof-of-concept studies suggest that combined targeting of both DHT conversion and AR expression by using Dutasteride and CDDO-me, respectively, may have translational value, especially since Dutasteride is already clinically approved for BPH in patients and since CDDO-me is in several late-stage clinical trials..

Limitations: First of all, this is preliminary data carried out in 1-2 experiments. Secondly, due to the formation of Dutasteride crystals, we do not know whether the concentration used is sufficient in our combination experiments with CDDO-me. Last but not the least, these are in vitro cell culture studies using PCa cell lines and may not translate to what may be seen in patients in vivo.

Future Studies: Reproducible experiments should be performed using this three-drug combination. We hope to test both the anti-metastatic and anti-proliferative capabilities of these agents using different PCa cell lines.

References

1. Sekhoacha M, Riet K, Motloung P, Gumenku L, Adegoke A, Mashele S. **Prostate Cancer Review: Genetics, Diagnosis, Treatment Options, and Alternative Approaches.** Molecules. 2022 Sep 5;27(17):5730. doi: 10.3390/molecules27175730. PMID: 36080493.

2. Mondal D, Narwani D, Notta S, Ghaffar D, Mardhekar N, Quadri SSA. **Oxidative stress and redox signaling in CRPC progression: therapeutic potential of clinically-tested Nrf2-activators.** Cancer Drug Resist. 2021 Mar 19;4(1):96-124. doi: 10.20517/cdr.2020.71. PMID: 35582006.

3. Mansinho A, Macedo D, Fernandes I, Costa L. **Castration-Resistant Prostate Cancer: Mechanisms, Targets and Treatment.** Adv Exp Med Biol. 2018;1096:117-133. doi: 10.1007/978-3-319-99286-0_7. PMID: 30324351.

4. Khurana N, Chandra PK, Kim H, Abdel-Mageed AB, Mondal D, Sikka SC. **Bardoxolone-Methyl (CDDO-Me) Suppresses Androgen Receptor and Its Splice-Variant AR-V7 and Enhances Efficacy of Enzalutamide in Prostate Cancer Cells.** Antioxidants (Basel). 2020 Jan 12;9(1):68. doi: 10.3390/antiox9010068. PMID: 31940946.

5. Andriole G, Bruchovsky N, Chung LW, Matsumoto AM, Rittmaster R, Roehrborn C, Russell D, Tindall D. **Dihydrotestosterone and the prostate: the scientific rationale for 5alpha-reductase inhibitors in the treatment of benign prostatic hyperplasia.** J Urol. 2004 Oct;172(4 Pt 1):1399-403. doi: 10.1097/01.ju.0000139539.94828.29. PMID: 15371854.

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