Effect of combined exposure to dimethyl sulfoxide (DMSO) and nelfinavir on growth and migration of aggressive prostate cancer cell lines *in vitro*

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Abstract

Given the increasing resistance of prostate cancer cells to androgen therapies, there remains a need to develop more effective treatment combinations for this aggressive disease. We treated 22RV1 cell lines with dimethyl sulfoxide (DMSO) at concentrations of 1% and 3%, and Nelfinavir at concentrations of 1, 2.5, and 5 mM alone and in combination for 48 hours. Both drugs showed a dose-dependent effect on cell concentration and migration, with Nelfinavir suppressing proliferation more and DMSO suppressing migration more. Combined treatment appeared to show an increased effect on both. Further studies will be done at different concentrations with varying combinations to further show the dose-dependent effects of these drugs. This preliminary data shows promise for this combination to be effective in *in vivo* studies given that both drugs are FDA approved.

Introduction

Prostate cancer (PCa) is the second most common cancer diagnosed in men and is a leading cause of morbidity and mortality in the United States.[1] Current therapeutics for PCa involve androgen deprivation therapy (ADT). However, ADT becomes ineffective within a few years and enables the selection of highly aggressive and metastatic castrate-resistant prostate cancer cells (CRPC). Currently, there are no effective treatments against CRPC cells.[2-3] We hypothesized that the aggressive CRPC cells, which possess higher oxidative stress (Ox-stress) and endoplasmic reticulum stress (ER-stress) may be targeted using pharmaceutical agents that are known to alter these two critical cell signaling pathways.[4] Previous studies have shown that two clinically approved drugs, i.e. dimethyl sulfoxide (DMSO) can increase Ox-stress and nelfinavir increases ER-stress on cancer cells.[2-5] We investigated the effect of these two drugs, alone and in combination, on the proliferative and migratory abilities of a highly aggressive CRPC cell line, CWR22Rv1 (22Rv1).

Materials and Methods

Reagents: Tissue culture supplies, DMEM media and fetal bovine serum (FBS) were purchased from Fisher Scientific (Waltham, MA).

Cell culture and Treatment: The 22Rv1 cell line was purchased from American Type Culture Collection (ATCC). The CCK8 dye and Crystal violent stain were purchased from Sigma-Aldrich (St. Louis, MO). Both DMSO and nelfinavir-mesylate were purchased from Cayman chemicals (Ann Arbor, Michigan).

Proliferation and Migration Assays: 22Rv1 cells were cultured in 24-well culture plates to ~60% confluency and exposed to increasing concentrations of DMSO (1 and 3%) and nelfinavir (1, 2.5 and 5 mM) both alone and in combination for 48 hours. Following which, changes in their proliferation was measured by staining with CCK8 dye for 2 h and monitoring optical density (OD) at 450 nm using a HT-synergy spectrophotometer from BioTek (Winooski, Vermont). 22Rv1 cells were cultured in 24-well culture plates to ~95% confluency, and a vertical scratch was made using a p200 pipette tip. Cells were then exposed to increasing concentrations of DMSO and nelfinavir, both alone and in combination for 48 hours. Wells were stained with 30% crystal violet and washed with PBS, and images were captured using a light microscope attached to a camera. Changes in scratch width were measured in each well to determine their average migratory rates.

Results

We observed dose-dependent effects of both DMSO and nelfinavir on 22Rv1 cell proliferation. Interestingly, nelfinavir showed a higher suppressive effect on proliferation rate as compared to DMSO, as evident from decreased CCK8 OD values. However, even at these low doses, DMSO decreased cell migration more than nelfinavir, as evident from increased scratch width as compared to control wells. Most interestingly, combined treatment with DMSO and nelfinavir showed increased effects on both cell proliferation and migration of the 22Rv1 cells.

Conclusion

The combined drug efficacy of DMSO and nelfinavir could have a profound effect in decreasing the aggressive properties of CRPC cells. Finding a way to combat the cancer's aggressiveness and resistance could help develop novel strategies to reducing PCa mortality. Since both drugs are clinically approved and were used at clinically achievable concentrations, our *in vitro* findings may represent how our findings will translate in a human model *in vivo*.

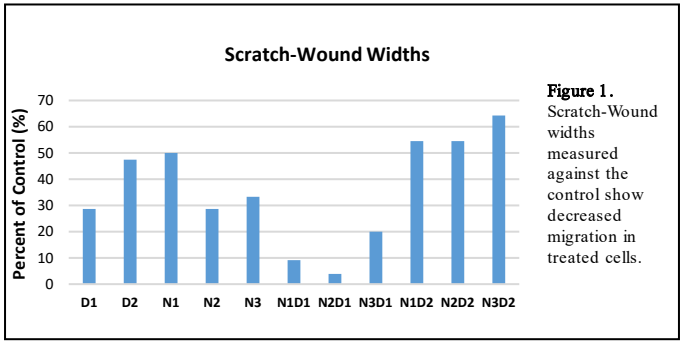


Figure 1. Scratch-Wound widths measured against the control show decreased migration in treated cells.

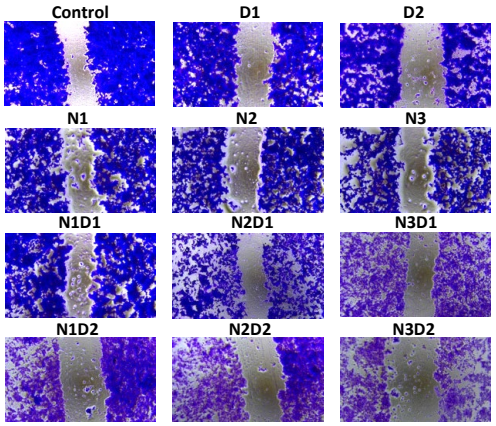
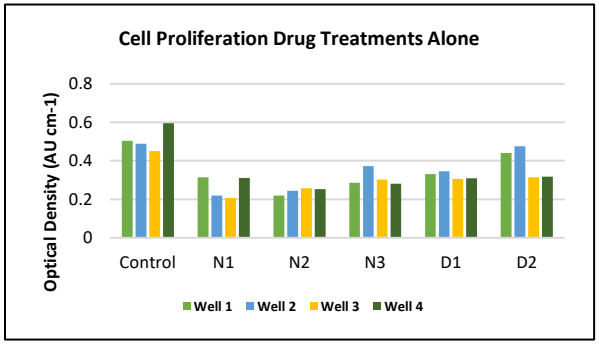
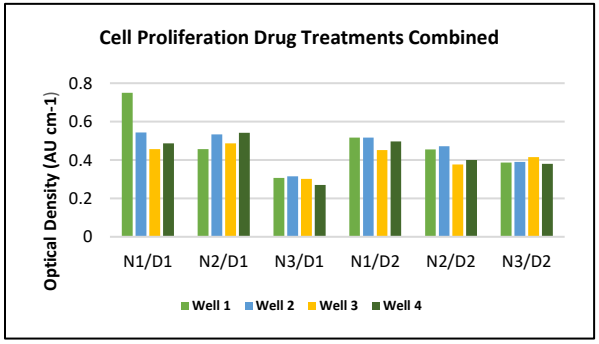


Figure 2. Crystal violet stained 22RV1 cells show the anti-migratory and anti-proliferative effects of DMSO and Nelfinavir against a control with scratch-wound assays. DMSO 1% (D1), DMSO 3% (D2), Nelfinavir 1, 2.5, 5 mM (N1, N2, N3).





Figures 3 and 4. Cell proliferation of 22RV1 with drug treatments alone and in combination. Proliferation is shown via Optical Density (OD) at 450 nm after the addition of CCK8 dye (2 hours).

References

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