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BuOE and tBHQ Reduces Growth and Viability of Castrate-Resistant Prostate Cancer

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BuOE and tBHQ reduces growth and viability of castrate-resistant prostate cancer

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Abstract

Prostate cancer is the second leading cause of cancer-related death in men. 10-20% of prostate cancer patients develop castration-resistant prostate cancer (CRPC) The median survival for CRPC patients is 14 months following diagnosis[1]. CRPC can spread to local tissues like the bladder, lymph nodes, and rectum but will also reach bone through distant metastasis. The metastasis of CRPC proves difficult for clinicians to treat. Redox active compounds can reduce growth and induce death of cancerous cells by the creation of ROS, which cannot be broken down by cancerous cells. This allows treatments like radiation and chemotherapy to be more effective in killing cancer.

Background

One treatment for prostate cancer is androgen deprivation therapy (ADT). Due to cancer's ability to use androgens as fuel for growth, the end goal of ADT is to decrease circulating testosterone with hope to reduce cancer cell proliferation and induce apoptosis. Cancer cells can become resistant to ADT and signs of progression are observed despite the "castrate" levels of androgen available. tert-butylhydroquinone, tBHQ, is an aromatic compound that is commonly used as a food preservative due to its antioxidative capacity. At higher concentrations, tBHQ becomes cytotoxic at the cellular level due to its ability to redox cycle into the volatile metabolite tert-butyl-p-benzoquinone, tBQ. This cycling between tBHQ and tBQ also creates superoxide, O2•– . BuOE is a SOD mimic manganese porphyrin that scavenges O2•– and produces hydrogen peroxide. By nature, cancer cells have a much lower tolerance to anti/pro-oxidant compounds compared to non-cancerous cells. Non-cancerous cells can breakdown H2O2 into H2O and O2, while cancerous cells are unable to perform this conversion; leading to reduced growth and cell death.

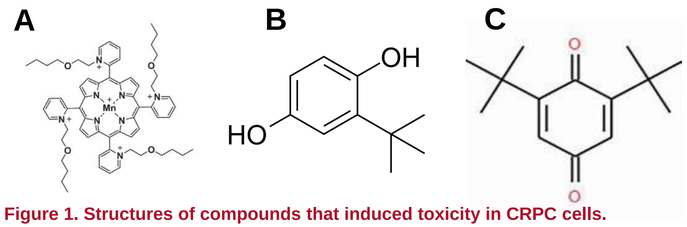

Figure 1. Structures of compounds that induced toxicity in CRPC cells. **A.** BuOE **B.** tBHQ **C.** tBQ

Hypothesis

We hypothesize that the combination of tBHQ and BuOE will reduce CRPC cell growth and viability via cytotoxicity produced by oxidation of tBHQ and production of H2O2.

Coupling BuOE with tBHQ reduces survivability of CRPC cells

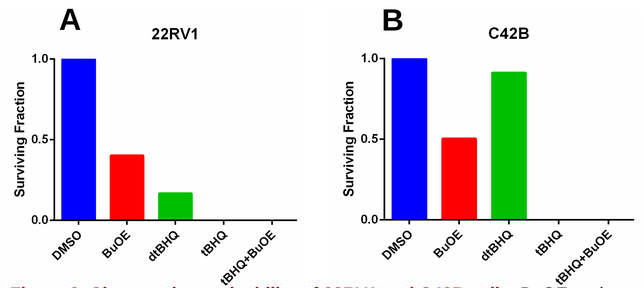

Figure 2. Clonogenic survivability of 22RV1 and C42B cells. BuOE reduces survivability by 50% while coupling tBHQ with BuOE induced 100% reduction.

Combination of tBHQ and BuOE reduces growth in CRPC cells

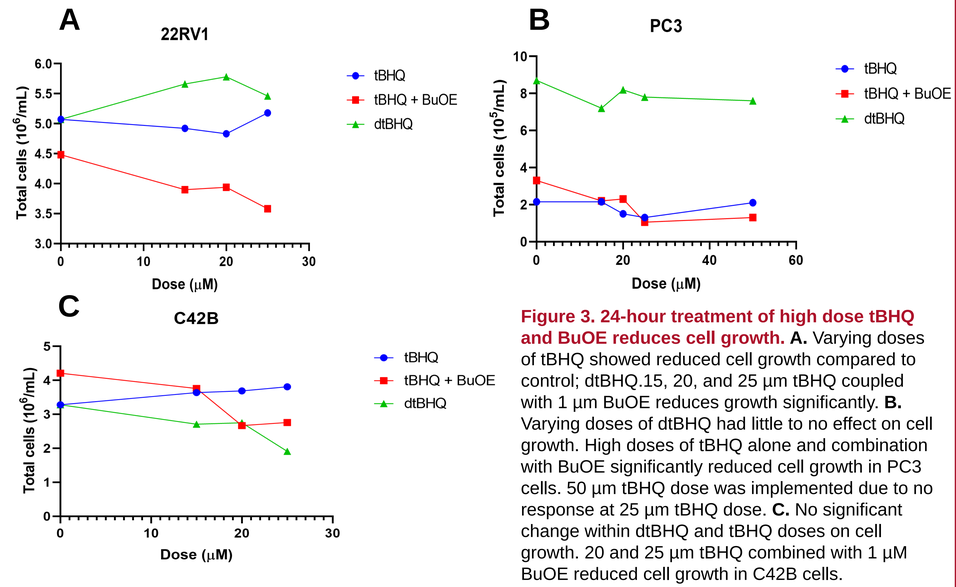

Figure 3. 24-hour treatment of high dose tBHQ and BuOE reduces cell growth. **A.** Varying doses of tBHQ showed reduced cell growth compared to control; dtBHQ.15, 20, and 25 μm tBHQ coupled with 1 μm BuOE reduces growth significantly. **B.** Varying doses of dtBHQ had little to no effect on cell growth. High doses of tBHQ alone and combination with BuOE significantly reduced cell growth in PC3 cells. 50 μm tBHQ dose was implemented due to no response at 25 μm tBHQ dose. **C.** No significant change within dtBHQ and tBHQ doses on cell growth. 20 and 25 μm tBHQ combined with 1 μM BuOE reduced cell growth in C42B cells.

Combination of tBHQ and BuOE reduces viability in CRPC cells

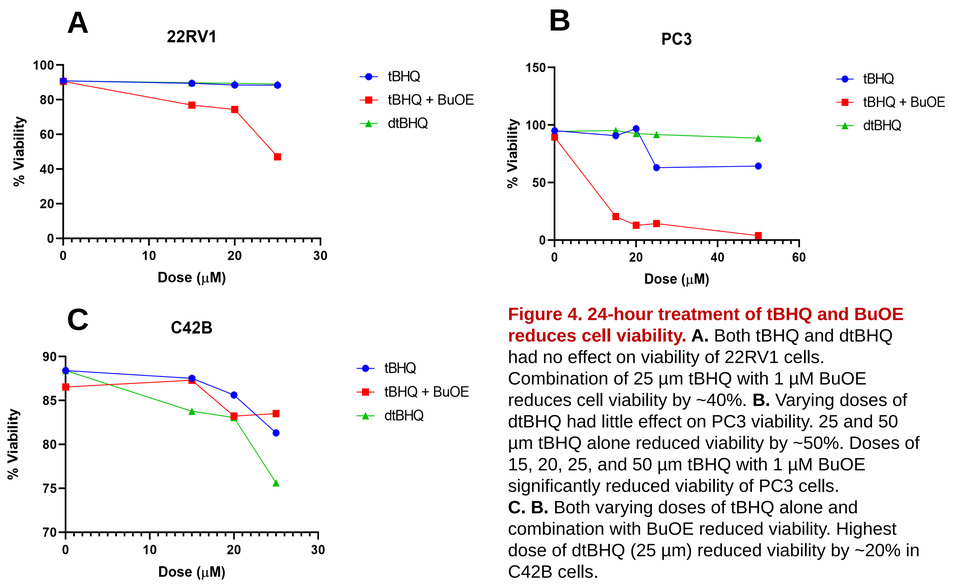

Figure 4. 24-hour treatment of tBHQ and BuOE reduces cell viability. **A.** Both tBHQ and dtBHQ had no effect on viability of 22RV1 cells. Combination of 25 μm tBHQ with 1 μM BuOE reduces cell viability by ~40%. **B.** Varying doses of dtBHQ had little effect on PC3 viability. 25 and 50 μm tBHQ alone reduced viability by ~50%. Doses of 15, 20, 25, and 50 μm tBHQ with 1 μM BuOE significantly reduced viability of PC3 cells. **C. B.** Both varying doses of tBHQ alone and combination with BuOE reduced viability. Highest dose of dtBHQ (25 μm) reduced viability by ~20% in C42B cells.

Discussion

Braeuning et al. have shown that tBHQ exerts cytotoxicity to cells via redox cycling and production of superoxide molecules[2]. Previous studies have shown that MnTE-2-PyP, an extremely similar SOD mimic manganese porphyrin, suppresses prostate cancer growth via intracellular H2O2 production[3]. We speculate that BuOE follows the same mechanism of H2O2 production in CRPC cells. We speculate that BuOE can assist in cycling of tBQ back to tBHQ by reductases to induce constant toxicity. We observed 2 odd interactions during this project. Firstly, tBHQ interacted with MTT and created formazan yielding false positive results in heavily damaged cells. Secondly, we saw reduced survivorship of dtBHQ control wells that neighbored tBHQ treated wells. Braeuning et al. observed this in an experiment and discovered small amounts of tBQ in control wells neighboring tBHQ treated wells. This phenomenon might be explained by the effect of tBHQ on the Nrf2 signaling pathway[2]. An example of this interaction can be seen in the dtBHQ treated 22RV1 clonogenic survival data in **Figure 2.**

Conclusion and Future Directions

- tBHQ doses alone have slightly reduced cell growth and viability in 22RV1, PC3, and C42B cells
- Coupling high dose tBHQ (25 and 50 μm) with 1 μM BuOE significantly reduces growth and viability in 22RV1, PC3, and C42B cells.
- Redox biology is crazy!
- Future implications: Identifying an optimal dose of tBHQ for PC3 cells, adding radiation dosing after treatment of tBHQ and BuOE to see effect on viability, and starting in vitro models.

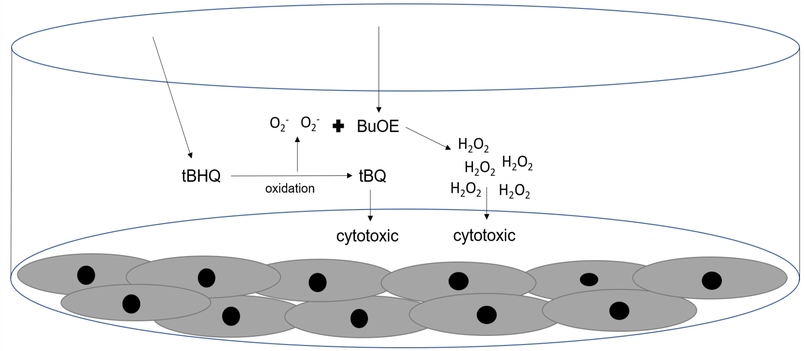

Figure 5. Proposed mechanism that induces reduced growth and viability in CRPC. Highly toxic tBQ is created by the oxidation of tBHQ, this process also produces superoxide radicals. BuOE utilizes the superoxide radicals and produces hydrogen peroxide molecules that are cytotoxic to cancerous cells.

Acknowledgements

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References

1. Mansinho, A., Macedo, D., Fernandes, I., and Costa, L. "Castration-Resistant Prostate Cancer: Mechanisms, Targets, and Treatment." *Advances in Experimental Medicine and Biology,* 1096, 117-133 (2018). doi: 10.1007/9783-319-99286-0_7.
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