Pyroelectric Crystal Generated Very Low Dose X-rays Enhanced the Phytotherapeutic Effects of Beta-lapachone in Hormone Dependent Prostate Cancer Cells In Vitro

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ABSTRACT

Plethora of studies have demonstrated the phytotherapeutic potentials of beta-lapachone (β-lap), a phytochemical compound derived from the bark of the lapacho tree, native to South America. β-lap has been shown to exhibit its anti-cancer effects majorly by the futile cycling between the oxidized and the two electron reduction of β-lap mediated by NAD(P)H:quinone oxidoreductase (NQO1) using NADH or NAD(P) as electron source.

β-lap is known to selectively kill human cancer cells. NQO1 has been shown under-expressed in hormone dependent prostate cancer cells (LNCaP) compared to the hormone independent prostate cancer cells (PC3).

This study was aimed to investigate the enhancing effects of very low doses of x-ray radiation (VLDR (20mGy)) derived from a pyroelectric crystal generator on the phytotherapeutic activity of beta-lapachone on LNCaP cell line in vitro assessed by MTT and Trypan blue assays. The treatment-induced intracellular levels of ROS were also assessed using Nitro-blue tetrazolium assay. NQO1 activities in LNCaP cells were also investigated following treatment with VLDR and/or β-lap using Dicoumarol (a NQO1 inhibitor). Results indicate that LNCaP cells respond significantly to combined treatments compared to single treatments, and VLDR was found to therapeutically enhance the effects of β-lap in LNCaP cells dose-and time-dependently via a Nrf1-2/ARE/NQO1 pathway. Apoptosis seems to be the major mode of cell death.

INTRODUCTION

- In 2015, an estimated 220,800 new cases and 27,540 deaths are expected to occur due to prostate cancer (PCa) in US men, thus adding to the burden of the over 2.6 million men currently battling with the disease [1].
- Beta-lapachone (β-lap) is a promising anticancer bioreductive ortho-naphthoquinone found majorly in the bark of the South American Lapacho tree [2].
- Previous studies have shown that β-lap kills cancer cells independent of their androgen response, p53 status, and cell cycle phase, through a mechanism that targets the NAD(P)H:quinone oxidoreductase (NQO1) enzyme levels in cancer tissues [2].
- This means that the real lethality of β-lap can only be seen in prostate cancer cells that express an elevated level of NQO1 (i.e. NQO1+) via futile cycling between the oxidized and two electron reduction of β-lap.
- Its been shown that LNCaP cancer cells (like the normal prostate cells) are deficient in NQO1 (i.e. NQO1-) whereas aggressive PCa (PC3 cells) express high levels of NQO1 (i.e. NQO1+) [2].
- The principle of pyroelectric generation of very low doses of x-rays (VLDR) involves exploiting the pyroelectric effect of polarized pyroelectric crystals to generate low doses of low energy and high energy radiation by varying the temperature of the generator.
- The aims of this study include the following:

1. To assess the cytotoxic effects of VLDR (20mGy/hr.) & β-lap in both LNCaP and PC3 prostate cancer cell lines.
2. To test if VLDR can upregulate NQO1 and if NQO1 is actually needed for effective cell killing by β-lap in LNCaP.
3. To determine if the VLDR-induced NQO1 is capable of enhancing the therapeutic effects of β-lap in LNCaP.
4. To determine the molecular mechanism of VLDR-BL induced cell death in LNCaP cells.

RESULTS

Fig. 1 Effect of VLDR on PC3 vs LNCaP (MTT Assay)

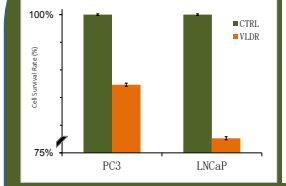


Fig. 2 Comparing Effects of VLDR + BL in LNCaP & PC3 cell lines (NQO1 dependent activity)

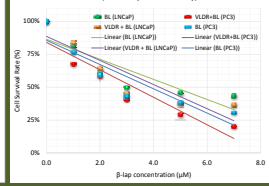


Fig. 3 Effect of BL vs VLDR+BL on LNCaP (MTT Assay)

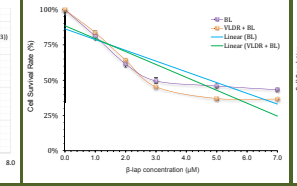


Fig. 4 Treatment-induced Cell viability and Cytotoxicity in LNCaP Cells (Trypan Blue Exclusion Assay)

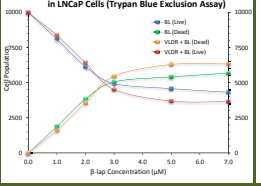


Fig. 5 Dose-dependent treatment-induced cellular ROS levels in LNCaP Cells (NBT Assay)

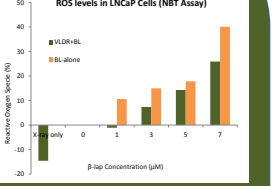


Fig. 6 Normalized correlation graph between the mean CSR and ROS in treated LNCaP Cells

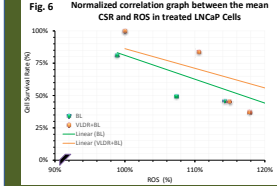


Fig. 7 Effect of NQO1 inhibitor (DIC) on LNCaP Cells exposed to VLDR and/or BL

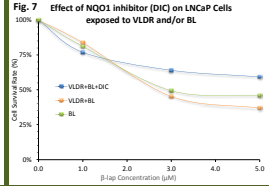


Fig. 8 Effect of Dicoumarol on VLDR exposed LNCaP Cells: Measuring VLDR-induced NQO1 Activity

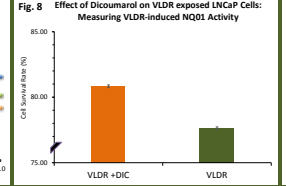
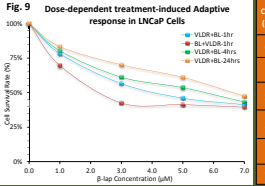


Fig. 9 Dose-dependent treatment-induced Adaptive response in LNCaP Cells



CONC (μM)	BL alone (CSR(%))	VLDR + BL (CSR(%))	DGIR (%)	DGIR ranking	BER by VLDR	BER ranking
0.0	100.00	100.00	0.00	N	1.00	N
1.0	81.13	83.74	-2.60	5 (-)	0.97	5 (-)
2.0	61.50	64.17	-2.67	4 (-)	0.96	4 (-)
3.0	49.43	45.30	4.13	3	1.09	3
5.0	45.81	36.98	8.83	1	1.24	1
7.0	43.20	36.56	6.64	2	1.18	2

Fig. 11

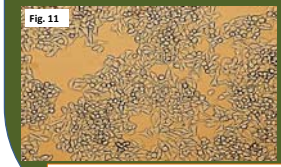


Fig. 12

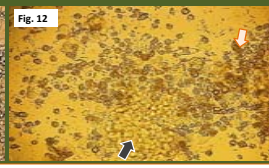


Fig. 13

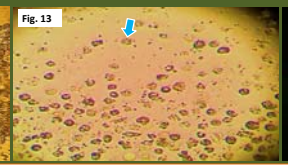


Fig. 14

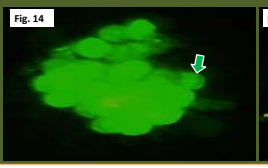
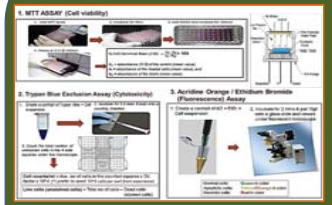
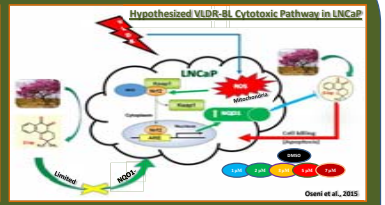


Fig. 15

In Figure 1-9: Data points represent the means ± standard deviation (SD) of three independent experiments performed in triplicate. P < O.O5. Fig. 11-15 are showing the histomicrographs of the following: LNCaP cell culture at 85 % confluence prior to exposure to VLDR+BL (Fig. 11); LNCaP cells 24hrs. following exposure to BL5 (Fig. 12); LNCaP cells 24 hrs. following exposure to VLDR + BL5 (Fig. 13); AO/EB fluorescently stained untreated LNCaP cells (Fig. 14); AO/EB fluorescently stained LNCaP cells following exposure to VLDR (20mGy) + BL5.

MATERIALS AND METHODS





Hypothesized Pharmacodynamic Interactions between VLDR-BL in LNCaP In Vitro:

- Beta-lapachone Enhancement Ratio (BER) by very low dose x-radiation (VLDR):
  BER = CSR (BL) ÷ CSR (VLDR(20mGy/hr.)+BL Eq-1
- Differential Growth Inhibition Rate (DGIR) between the single (BL) and combination (VLDR+BL) treatments:
  DGIR = CSR (BL) – (CSR (VLDR(20mGy/hr.)+BL Eq-2
- Adaptive Stress Response Ratio (ASRR) by VLDR at 4hrs and 24hrs drug treatment intervals:
  ASR =(CSR (VLDR(20mGy/hr.)+BL)^24hrs) ÷ (CSR (VLDR(20mGy/hr.)+BL)^4hrs)..... Eq-3
- Compusyn® software was used to determine the potency (IC50) of VLDR+BL in LNCaP.

DISCUSSION & CONCLUSION

- A previous study have already demonstrated that NQO1 is a highly inducible detoxifying and antioxidant enzyme that is regulated by the Keap1/Nrf2/ARE pathway [4]
- Investigating the activation or up-regulation of NQO1, which is located downstream of the signaling pathway serves as a dosimeter to assess the ability of VLDR to activate Nrf1/2, which binds to the antioxidant response element (ARE) in the nucleus of LNCaP cells to increase the expression of NQO1.
- This study further demonstrate that the initial increase in the ROS levels by VLDR may be responsible for the activation of this pathway followed by a VLDR induced rise in NQO1 level, being an antioxidant enzyme, may be responsible for the scavenging of the ROS and hence cytotoxicity.
- We also observed a dose- and time-dependent adaptive stress responses by LNCaP to VLDR & BL.
- This in vitro study have been able to demonstrate the potential therapeutic benefit of pyroelectric crystal generated (@20mGy/hr.) in Cancer treatment.
- Our fluorescence assay results suggested that the major mode of cell death by this combination therapy is via Apoptosis.
- In conclusion, our results confirm that VLDR-induced NQO1 level is capable of enhancing the phytotherapeutic effects of β-lap.
- This study has further reiterated the conclusion from our previous findings that pyroelectric crystal generators (a non-radioisotope source of x-rays) has potential benefits in cancer radiotherapeutic intervention [3].
- These findings offer a rationale for the assessment of pyroelectric crystal generated VLDR as a chemo-enhancer in prostate cancers that have low or deficient expression of NQO1, thus offering a potentially synergistic target by exploiting its NQ01 upregulation activity.

ACKNOWLEDGEMENTS

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