**ABSTRACT**

Alpha-santalol, a constituent of sandalwood oil, is a known inhibitor of prostate cancer in vitro, within both androgen-dependent (LNCaP) and androgen-independent (PC-3) cell lines. Our research evidence suggests alpha-santalol induces autophagy through altering the AKT-mTOR axis. Because mTOR is an inhibitor of autophagy and AKT is an activator of mTOR, as well as an inhibitor of pro-apoptotic proteins like FoxO and BAX, the reduced expression of both mTOR and AKT yields insight into alpha-santalol's currently unknown mechanism(s) of anti-tumor effects. In conclusion, our study generates support for alpha-santalol inducing autophagy in human prostate cancer cells, specifically through close association with the AKT-mTOR pathway.

**PATHWAY**

- **Detection of acidic vesicular organelles:** PC-3 and LNCaP cells (2x10^5) were plated on cover slips in a 12 well plate and allowed to attach by overnight incubation. Following treatment with DMSO (control) or alpha-santalol for 24h, cells were stained with acridine orange in PBS, washed with PBS, and then examined under a fluorescence microscope at high power magnification for acidic vesicular organelles (i.e. autophagosomal structures).

- **Immunoblotting:** PC-3 and LNCaP cells (7.5x10^5 cells for each cell line) after attachment were treated with different concentrations of α-santalol (20 and 40 μM) or DMSO for different time periods (6 or 9h). Immunoblotting was performed using standard procedures using LICOR-Odyssey infrared scanner with actin as a loading control.

- **Trypan Blue Assay:** PC-3 cells (5x10^4 cells) after attachment was pre-treated for 22h with 3-MA and co-treated with alpha-santalol (20 and 40 μM) or DMSO for 24h. Standard protocol for trypan blue assay was used for determining the cell viability in control and treatment groups.

**RESULTS**

- Based on our results from acridine orange staining and increased LC3 expression shown via immunoblotting, induction of autophagy was evident in both LNCaP and PC-3 cells.
- Cell viability in the presence of known autophagy inhibitor, 3MA, was significantly lower compared to alpha-santalol treated cells.
- Expression of phosphorylated (i.e. activated) AKT and mTOR proteins was reduced in cells treated with alpha-santalol compared to control-treated cells.

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**CONCLUSIONS**

**Figure 1:** Autophagy induction in prostate cancer cells, as supported by acridine orange staining

**Figure 2:** Effects of alpha-santalol on expression of LC3 in PC3 and LNCaP cell lines

**Figure 3:** PC-3 cell viability in the presence of alpha-santalol and/or 3-MA

**Figure 4:** Effect of alpha-santalol on cellular expression of AKT-mTOR axis intermediates