alpha-santalol, a chemopreventive agent induces apoptosis in human prostate cancer cells by causing caspase-3 activation

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ABSTRACT

The anticancer effects of α-santalol, a major component of sandalwood oil, have been reported against the development of certain cancers such as skin cancer both in vitro and in vivo. The primary objectives of the current study were to investigate the cancer preventive properties of α-santalol on human prostate cancer cells PC-3 (androgen independent and P-53 null) and LNCaP (androgen positive and P-53 wild-type), and determine the possible mechanisms of its action. The effect of α-santalol on cell viability was determined by trypan blue dye exclusion assay. Apoptosis induction was confirmed by analysis of cytoplasmic histone-associated DNA fragmentation using both an apoptotic ELISA kit and a DAPI fluorescence assay. Caspase-3 activity was determined using caspase-3 (active) ELISA kit. PARP cleavage was analyzed using Immunoblotting. α-santalol at 25-75 μM decreased cell viability in both cell lines in a concentration and time dependent manner. Treatment of prostate cancer cells with α-santalol resulted in induction of apoptosis as evidenced by DNA fragmentation and nuclear staining of apoptotic cells by DAPI. α-santalol-induced apoptotic cell death and activation of caspase-3 was significantly attenuated in the presence of pharmacological inhibitors of caspase-8 and caspase-9. In conclusion, the present study reveals the apoptotic effects of α-santalol in inhibiting the growth of human prostate cancer cells.

RESULTS

Treatment of prostate cancer cells with α-santalol resulted in a concentration and time-dependent reduction in cell viability.

α-santalol treatment resulted in apoptosis in human prostate cancer cells, as evidenced by caspase-3 activity and DNA fragmentation caused by α-santalol.

Apoptosis induction was confirmed by analysis of cytoplasmic histone-associated DNA fragmentation using both an apoptotic ELISA kit and a DAPI fluorescence assay. Caspase-3 activity was determined using caspase-3 (active) ELISA kit. PARP cleavage was analyzed using Immunoblotting. α-santalol at 25-75 μM decreased cell viability in both cell lines in a concentration and time dependent manner. Treatment of prostate cancer cells with α-santalol resulted in induction of apoptosis as evidenced by DNA fragmentation and nuclear staining of apoptotic cells by DAPI. α-santalol-induced apoptotic cell death and activation of caspase-3 was significantly attenuated in the presence of pharmacological inhibitors of caspases.

CONCLUSIONS

α-santalol treatment resulted in apoptosis in human prostate cancer cells irrespective of androgen or p53 status.

Treatment of prostate cancer cells with α-santalol resulted in activation of caspase-3 and PARP cleavage.

Apoptotic DNA fragmentation caused by α-santalol was significantly attenuated in the presence of caspase inhibitors.

This study was supported by AACP(American Association of Colleges of Pharmacy)-New faculty Research Award and Mentoring grants from Wilkes University.

METHODOLOGY

Cell Morphology assay: (5x10⁴ cells for each cell line) were seeded in culture dishes and incubated for overnight attachment. Following treatments with various concentrations of alpha-santalol for different time points, images (20x) were taken using bright field microscopy to analyze morphology of the cells.

DAPI assay: (5x10⁴ cells for each cell line) were seeded on cover slips in 12-well plates and incubated for overnight attachment. Following 24h treatment with alpha-santalol and DMSO control, cells were fixed in 3% formaldehyde and permeabilized with 1% Triton x-100. Cells were incubated with 1μg/ml DAPI for 15 minutes. Cells stained with DAPI were captured using Nikon Fluorescence microscope (60x magnification).

Trypan Blue assay: Standard protocol for trypan blue assay was used for determining the cell viability in control and treatment groups.

Caspase activity assay: The effect of α-santalol on caspase-3 activity in PC-3 and LNCaP cells was determined using a commercially available Caspase-3 (active) ELISA kit (Invitrogen Corporation, Camarillo, CA). Active caspase-3 (mg/mg total protein of cell lysate) was determined and results were expressed as folds of caspase-3 activity in α-santalol-treated cells relative to DMSO-treated control.

Apopotic DNA Fragmentation assay: Histone-associated DNA fragment release into the cytosol, a well-accepted technique for quantitation of apoptotic cell death was determined using a kit from Roche Diagnostics. Briefly, 1x10⁴ cells in 1 ml of complete medium were plated in 24-well plates, and allowed to attach overnight. The next day, cells were treated with desired concentrations of α-santalol, and the plates were incubated for 24 or 48 h at 37°C. ELISA assay was carried out per manufacturer’s protocol.

Statistical analysis: Each experiment was performed at least twice with triplicate measurements for quantitative comparisons. Statistical significance of difference in measured variables between control and treated groups was determined by one-way ANOVA. Difference was considered significant at P < 0.05.