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α-Santalol, a derivative of sandalwood oil, 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 dependent 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 treatment also resulted in activation of caspase-3 activity and PARP cleavage. The 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.

Introduction

Prostate cancer continues to be one of the most commonly diagnosed diseases and the second leading cause of cancer related deaths among men in the United States (Siegel et al. 2011). It is typically diagnosed in the 6th and 7th decades of life, and hence a modest delay in disease progression could offer a significant impact on disease related morbidity, mortality and quality of life. Molecular mechanisms underlying onset and progression of prostate cancer are not fully understood, but the factors implicated in pathogenesis of this disease include age, race, diet, androgen secretion and metabolism, and activated oncogenes (Ross and Henderson 1994; Whittemore et al. 1995; Nelson et al. 2003). Options exist to treat localized disease including surgery, radiation therapy, and hormonal therapy, but clinical management of advanced prostate cancers has been challenging (Ramakrishnan and De Weese 1999; Gilligan and Kantoff 2002; Walsh et al. 2007). Androgen ablation is a frequently suggested treatment option for prostate cancer but this treatment selection is palliative and has a limited scope for hormone refractory cancers (Laufer et al. 2000). Moreover, chemotherapy and radiation therapy are largely ineffective against advanced prostate cancer (Ramakrishnan and De Weese 1999; Gilligan and Kantoff 2002; Walsh et al. 2007).

A continuous increase in cancer incidence and failure of conventional chemotherapy against advanced prostate cancer warrants development of novel agents to treat and prevent this malignancy. In the last few decades, chemoprevention involving naturally occurring compounds has been identified as a promising and cost-effective approach to reduce incidence and morbidity of prostate cancer by inhibiting the precancerous events before the occurrence of clinical disease (Surh 2003). Prostate cancer which is usually diagnosed in the 6th and 7th decades of life provides a large window of opportunity for intervention to prevent or slow its progression. Therefore, development of agents which offer significant protection against the development of human prostate cancer is highly desirable. These chemopreventive agents could have a significant impact on disease-related costs, morbidity, and mortality for a large

Abbreviations: DMSO, dimethyl sulfoxide; ELISA, enzyme linked immunosorbent assay; PARP, poly ADP ribose polymerase; DAPI, 4′-6-diamidino-2-phenylindole; PBS, phosphate buffered saline; PVDF, polyvinylidene fluoride; PMSF, phenyl methane sulfonyl fluoride.

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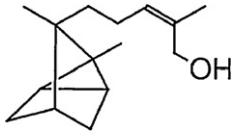

Fig. 1. Structure of α-santalol.

segment of population. Prostate cancer in many ways remains as an ideal candidate for chemoprevention because of its high incidence and long latency.

Many naturally occurring chemopreventive agents, including silibinin, inositol hexaphosphate, decursin, apigenin, grape seed extract, curcumin, and epigallocatechin-3 gallate have been identified in laboratory studies, which could be useful in the management of prostate cancer (Singh and Agarwal 2006). Similarly compounds such as PEITC and sulforaphane have also been shown to have prostate cancer preventive properties in both *in vitro* and *in vivo* models (Singh et al. 2005; Xiao and Singh 2010; Xiao et al. 2010; Bommareddy et al. 2009). The present study focuses on investigating the anticancer properties of α-santalol (Fig. 1), a major component of sandalwood oil (Santalum album Linn, Indian sandalwood) which has been traditionally used in the treatment of various skin disorders (Zhang and Dwivedi 2011). In addition to prevention of chemically-induced (Dwivedi et al. 2003) and UVB-induced skin carcinogenesis (Dwivedi et al. 2006; Bommareddy et al. 2007; Arasada et al. 2008) in various animal models, α-santalol has also been shown to suppress proliferation of non-melanoma and melanoma skin cancer cells in culture by causing cell cycle arrest and/or apoptosis induction (Zhang et al. 2010; Kaur et al. 2005). α-Santalol used at concentrations of 2.5 and 5% (w/v) in acetone in animal models did not result in any noticeable side effects indicating the concentrations used in these studies are well tolerated by normal cells (Dwivedi et al. 2006; Bommareddy et al. 2007; Arasada et al. 2008). Studies employing vinblastine and taxol as positive controls examined the mechanism of action of α-santalol in UACC-62 melanoma cells and concluded that α-santalol exhibited microtubule depolymerization similar to that of vinblastine (Zhang et al. 2010). Despite these studies on skin cancer models, however, the efficacy of α-santalol against hormonal cancers, such as prostate cancer, has not been investigated. Here, we show for the first time that α-santalol effectively suppresses growth of both androgen-dependent, i.e., p-53 wild type (LNCaP) and androgen-independent, i.e., p-53 null (PC-3) human prostate cancer cells by causing caspase-3 activation, and inducing apoptosis. Furthermore, we provide evidence to indicate that α-santalol-induced cell death in prostate cancer cells may partially be regulated by both intrinsic and extrinsic apoptotic pathways.

Materials and methods

Reagents

α-Santalol, isolated from sandalwood oil (Now Foods, Glendale HTS, IL) by column chromatography using n-hexane:ethyl acetate 3:1 as a solvent system was provided by Dr. Chandradhar Dwivedi (South Dakota State University). Cell-culture reagents, including RPMI 1640 medium, F-12K medium, FBS, and penicillin/streptomycin antibiotic mixture were purchased from Invitrogen-Life Technologies (Carlsbad, CA). A kit for quantitation of histone-associated apoptotic DNA fragment release into the cytosol was purchased from Roche Diagnostics (Mannheim, Germany). ELISA kit for caspase-3 activity was purchased from Invitrogen (Camarillo, CA). Cleaved PARP antibody was from Cell Signaling (Danvers, MA). Caspase inhibitors were from BD Pharmingen (San Diego, CA). All other chemicals and reagents were purchased in their highest purity available.

Cell lines

Cell lines PC-3 and LNCaP which were authenticated earlier by Research Animal Diagnostic Laboratory (University of Missouri, Columbia, MO) to test for interspecies contamination and alleles for short tandem repeats identifiable in the ATCC database were a generous gift from Dr. Shivendra V. Singh (University of Pittsburgh Cancer Institute, Pittsburgh). PC-3 cells were cultured in F-12K Nutrient Mixture supplemented with 7% (v/v) FBS and 1% penicillin/streptomycin antibiotic mixture. The LNCaP cells were maintained in RPMI 1640 medium supplemented with 1 mM/l sodium pyruvate, 10 mM/l HEPES, 0.2% glucose, 10% (v/v) FBS, and 1% penicillin/streptomycin antibiotic mixtures. Normal human prostate epithelial cell line PrEC was maintained in PrEBM (Cambrex). Each cell line was maintained in an atmosphere of 95% air and 5% CO_2 at 37 °C. A stock solution of α-santalol was prepared in dimethyl sulfoxide (DMSO) and an equal volume of DMSO (final concentration <0.5%) was added to the controls.

Cell viability assay

The effect of α-santalol on cell viability in PC-3 and LNCaP cells was determined using Trypan blue dye exclusion assay. Cell viability was expressed as % of DMSO control. Briefly, 1×10^4 cells in 1 ml of complete medium were plated in 12-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. At the end of the incubation, both floating and adherent cells were collected and centrifuged at 5000 rpm for 5 min. The pellet was resuspended in 30 μl of 0.4% trypan blue solution in PBS, and live (white) cells were counted using a hemacytometer under an inverted microscope.

Apoptosis assays

Apoptosis induction by α-santalol was assessed by (i) fluorescence microscopic analysis of cells with condensed and fragmented DNA following staining with DAPI, and (ii) quantification of cytoplasmic histone-associated DNA fragmentation. For DAPI assay, cells (2×10^4) were plated on coverslips, allowed to attach overnight and exposed to DMSO or α-santalol for the desired time period at 37 °C. Cells were washed with PBS, and fixed with 3% paraformaldehyde for 1 h at room temperature. The cells were washed three times with PBS, permeabilized with 0.1% Triton X-100 for 15 min, washed again with PBS and stained by incubation with 200 ng/ml DAPI for 20 min. Cells with condensed and fragmented DNA (apoptotic cells) were counted under a fluorescence microscope. Apoptosis induction by α-santalol was analyzed through cytoplasmic histone-associated DNA fragmentation using a commercially available ELISA kit (Roche diagnostics, Mannheim, Germany). Cytoplasmic histone associated DNA fragmentation was determined and results were expressed as enrichment factor of cytoplasmic histone-associated DNA fragmentation in α-santalol-treated cells relative to DMSO-treated control as described by us previously (Bommareddy et al. 2009). Briefly, 1×10^4 cells in 1 ml of complete medium were plated in 24-well plates, and allowed to attach overnight. The next day, cells were treated and incubated with desired concentrations of α-santalol for 24 h or 48 h at 37 °C. ELISA assay was carried out per manufacturer's protocol.