Chemoprevention by α-Santalol on UVB Radiation-induced Skin Tumor Development in Mice

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Abstract. *Studies have shown the chemopreventive effects of α-santalol on chemically and UVB-induced skin cancer in mice. The objective of the present investigation was to find the lowest effective concentration of α-santalol for the chemopreventive effects on UVB-induced skin tumor development in mice and to determine antiperoxidant effect of α-santalol in order to elucidate its possible mechanism of action. Female SKH-1 mice were divided into different groups receiving either vehicle alone or different concentrations of α-santalol. Mice in all the groups were initiated and promoted with UVB radiation for skin tumor development. The promotion phase continued for 30 weeks. Skin tumors were counted once a week for 30 weeks. Lipid peroxidation was assayed in skin and liver microsomes by measuring malonaldehyde formed using thiobarbituric acid method. Topical administration of α-santalol reduced UVB-induced skin tumor development in a concentration-dependent manner. Application of α-santalol (5%) significantly ($p<0.05$) delayed skin tumor development for 25 weeks and reduced tumor multiplicity. α-Santalol also inhibited in vitro lipid peroxidation in skin and liver microsomes. α-Santalol application prevents UVB-induced skin tumor development possibly by acting as an antiperoxidant.*

Nonmelanoma skin cancer is the most frequently occurring malignant neoplasm, accounting for over 1.2 million new cases each year in the United States (1). Epidemiological, clinical and laboratory studies have implicated solar ultraviolet (UV) radiation as a tumor initiator, tumor promoter and complete carcinogen, and excessive exposure can lead to the development of various skin disorders, including melanoma and nonmelanoma skin cancers (2).

Developing novel strategies to overcome cancer development has become the goal for decreasing the risk of cancer. Chemoprevention is one of the approaches used to reduce the risk of developing cancer, either by preventing the disease entirely, or slowing down or reversing the carcinogenic progression (3). Research studies using phytochemicals, naturally occurring antioxidants, minerals and vitamins has increased at an astonishing rate over the past few years (4). Recent studies from our laboratory using one such naturally occurring agent have provided evidence that α-santalol (5% v/v, in acetone) has chemopreventive effects against chemically and UVB-induced skin cancer in mice (5, 6). Studies from our laboratory also showed chemopreventive effects of α-santalol in a concentration-dependent manner on chemically induced skin tumor development in female CD-1 mice (7).

The purpose of the present investigation was to study the concentration response to determine the lowest effective concentration of α-santalol which would provide chemopreventive effects on UVB-induced skin tumor development and also study the *in vitro* effects of α-santalol on lipid peroxidation in female SKH-1 mice to elucidate possible mechanisms of action.

Materials and Methods

Chemicals. Sandalwood oil (SW oil) was purchased from a local store that was distributed by NOW Foods (Glendale Heights, IL, USA). Pyridoxal phosphate, ethylenediaminetetraacetic acid (EDTA), disodium salt, dithiothreitol, ornithine, ethanolamine and methoxy ethanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All routine chemicals were obtained from Fisher Scientific (Hanover Park, IL, USA). α-Santalol was isolated from sandalwood oil by distillation under reduced pressure and characterized as reported elsewhere (5).

Animals. Female SKH-1 mice (five-weeks-old) were purchased from Charles River Laboratories (Wilmington, MA, USA). Institutional guidelines were followed in the handling and care of the animals.

Tumorigenesis protocol. A skin cancer protocol as described by Dwivedi *et al* (6) was used. Five-week-old female SKH-1 mice were divided into four groups of 30 mice to study the concentration response during the tumorigenesis protocol. Tumorigenesis was initiated and promoted by UVB radiation (180 mJ/cm^2, Daavlin

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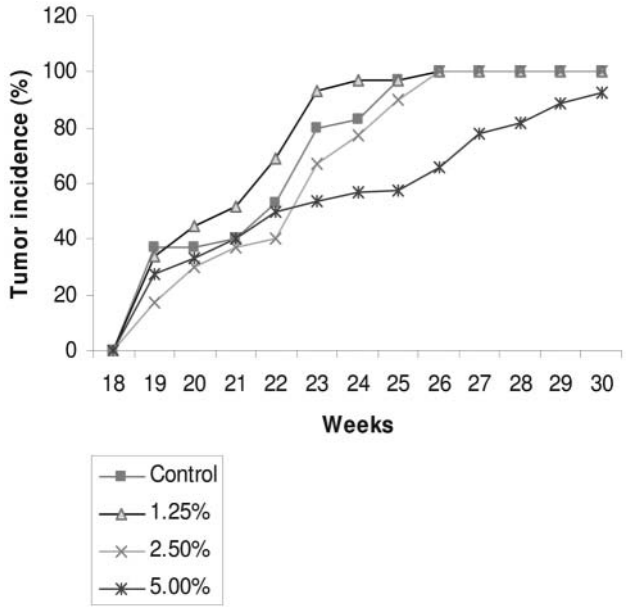

Figure 1. *Effects of α-santalol on tumor incidence in UVB-initiated and promoted skin cancer in SKH-1 mice. Incidence in the 5% α-santalol group was significantly ($p<0.05$) lower when compared to other groups.*

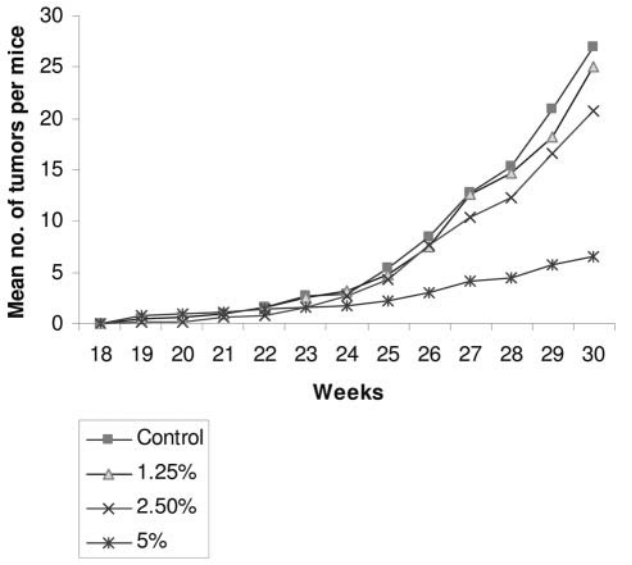

Figure 2. *Effects of α-santalol on tumor multiplicity in UVB-initiated and promoted skin cancer in SKH-1 mice. Mice from 2.5% and 5% α-santalol groups had significantly ($p<0.05$) fewer tumors when compared to other groups.*

Spectra® Bryan, OH, USA). Initiation was carried out for 10 days. On the 10th day after initiation, promotion was started where the mice were irradiated with UV radiation (180 mJ/cm^2) twice a week. Mice in group 1 served as control and were pretreated with 100 μl acetone (vehicle for α-santalol) 1 h prior to UVB exposure. Mice in groups 2, 3 and 4 were pretreated with 100 μl of 1.25%, 2.5% and 5% α-santalol in acetone respectively 1 h prior to UVB exposure. Skin tumors were counted once weekly for 30 weeks and tumor incidence and multiplicity were calculated. Mice were also weighed once weekly.

Lipid peroxidation assay. Five female SKH-1 mice were fasted overnight and sacrificed by decapitation. The dorsal epidermis was removed, rinsed and homogenized in ice-cold 1.15% KCl using an Omni GLH homogenizer (Omni International, Inc., Warrenton, VA, USA). The homogenates were then centrifuged at 10,000 xg for 15 min in a J2-21 centrifuge (Beckman Instruments, Inc., Fullerton, CA, USA). The supernatant obtained was recentrifuged in an Optima LE-80K preparative ultracentrifuge (Beckman Instruments) at 105,000 xg for 60 min under refrigeration. The microsomal pellet was washed twice and resuspended in potassium phosphate buffer (15 mM, pH 7.4). Microsomal lipid peroxidation was induced enzymatically (NADPH-induced) or nonenzymatically (ascorbate-induced). α-Santalol was dissolved in dimethylsulfoxide (DMSO) and was added to the incubation mixture at a final concentration of 11 mM. The thiobarbituric acid assay for malonaldehyde was used to measure lipid peroxidation (8).

Statistical analysis. The INSTAT software (Graph Pad, San Diego, CA, USA) was used for the data analysis. Chi-square was used for the comparison of papilloma incidence and ANOVA, followed by a Tukey post test, was used to determine significance for tumor multiplicity. Significance was set at $p<0.05$.

Results

The effects of α-santalol treatment on UVB-induced tumor incidence are given in Figure 1. Tumors appeared in all the groups in the 19th week of promotion. By the end of 30 weeks, all mice in control, 1.25% and 2.5% groups, had at least one tumor, but in the 5% group 92% mice had at least one tumor (Figure 1). There was no significant difference in tumor incidence until the 23rd week of promotion among the four different groups. There was a significant decrease in tumor incidence with the 5%-treated group when compared to other three groups after 23 weeks of promotion. There was no significant difference in the tumor incidence among all the groups at the end of the experiment.

Figure 2 illustrates the data on UVB-induced tumor multiplicity in SKH-1 mice which shows that pretreatment with α-santalol significantly reduced the mean number of tumors by the end of thirty weeks in a concentration-dependent manner. α-Santalol 2.5% and 5.0% treatment significantly reduced tumor multiplicity throughout the 30-week period. The mean number of tumors were 26.9, 25.07, 20.5 and 6.5 in control, 1.25%, 2.5% and 5.0% α-santalol-treated groups, respectively. There was no significant difference in tumor multiplicity between control and 1.25% α-santalol groups but there was a significant decrease in tumor multiplicity in the 2.5% and 5% α-santalol groups, indicating that pretreatment with α-santalol of 2.5% and 5% concentrations lowered the number of tumors formed in

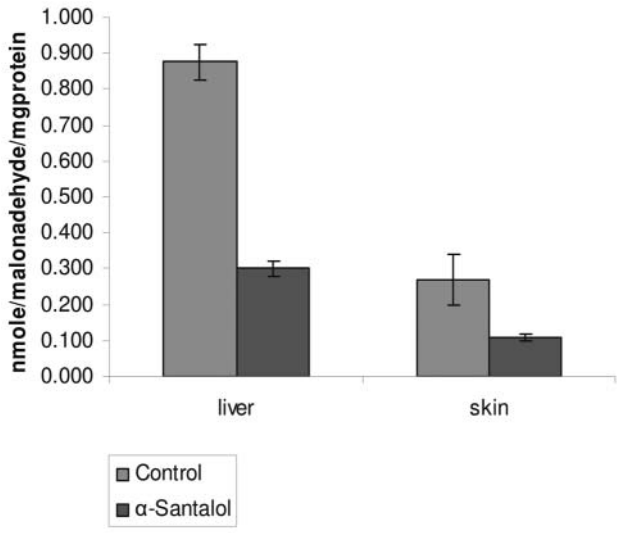

Figure 3. *Effects (in vitro) of α-santalol on ascorbate-induced lipid peroxidation. Numbers are derived from incubations carried out in triplicate and from at least five mice.*

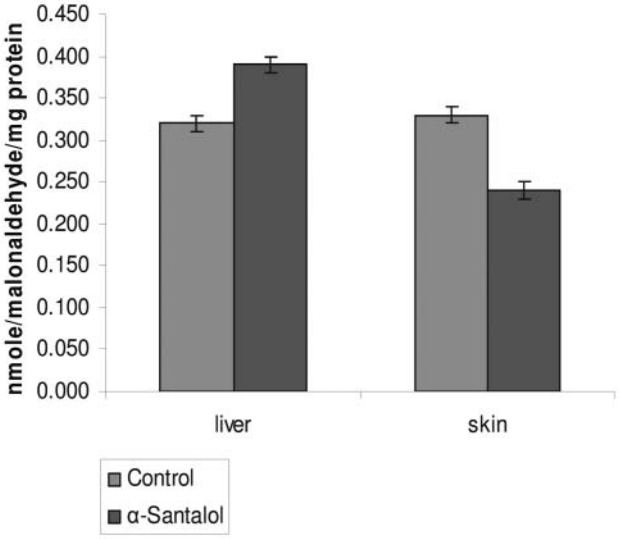

Figure 4. *Effects (in vitro) of α-santalol on NADPH-induced lipid peroxidation. Numbers are derived from incubations carried out in triplicate and from at least five mice.*

SKH1 mice to a greater extent. α-Santalol pretreatment (5%) provided significantly higher ($p<0.05$) protection against UVB-induced tumor development than α-santalol (2.5%) pretreatment.

There was no significant difference in weight gain between the groups (data not shown). Topical application of α-santalol in SKH-1 mice did not influence the weight gain. In addition to this, the mice skins treated with α-santalol appeared healthy without any apparent toxic symptoms.

Antiperoxidant effects (*in vitro*) of α-santalol are presented in Figures 3 and 4.

α-Santalol, at a final concentration of 11 mM, inhibited ascorbate-induced lipid peroxidation in both liver and skin microsomes (Figure 3). However, α-santalol, at a final concentration of 11 mM, inhibited NADPH-induced lipid peroxidation only in skin microsomes (Figure 4).

Discussion

Chemoprevention as a serious and practical approach to intervention in order to arrest or reverse the process of carcinogenesis has been enhanced within the past few years (9). To combat a disease as complicated and multifactorial as cancer, a putative chemoprotective agent must elicit diverse mechanistic properties to modulate the carcinogenic process leading to cell cycle arrest and apoptosis induction (4, 10).

α-Santalol is extracted from the root and heartwood of the sandalwood tree (*Santalum album*) by steam distillation (10). The essential oil, emulsion and paste of sandalwood have been used as an Ayurvedic medicinal agent in India for centuries for the treatment of inflammatory and eruptive skin diseases (11, 12). The major constituent (90% or more) of the oil is santalol, which is available as a racemic mixture of two isomers α- and β-santalol. NMR and GC-MS analysis have identified α-santalol, a naturally occurring terpenoid constituting about 61%, as a major component (5). Our present study showed that α-santalol inhibits UVB-induced skin tumor development in a concentration-dependent manner. α-Santalol (2.5% and 5%) pretreatment significantly reduced skin tumor development. However, 1.25% α-santalol did not have any significant effect on UVB-induced skin tumor development. Thus, the minimum possible concentration of α-santalol in this study which could potentially reduce skin tumor development was 2.5%. However, 5% α-santalol provided an optimal chemo-prevention.

The skin is constantly exposed to UV irradiation and the lipids, proteins and DNA in skin are extremely sensitive to UV-induced damage (13). The damaging effects of UV light are mediated *via* the generation of free radicals (14). Lipid peroxidation is thought to play a significant role in the development of cancer. Polyunsaturated fatty acids are abundant in cellular membranes and in low-density lipoproteins. The finding that a number of antioxidants can reduce the development of UV irradiation-induced skin cancer in mice suggests that oxidative reactions are important in this process and can serve as targets for skin cancer prevention. UV irradiation-induced immune suppression also predisposes animals and humans to the development of skin cancer (15).

The results of the present investigation indicated that α-santalol treatment inhibited UVB-induced skin tumor development in SKH-1 mice and also inhibited lipid peroxidation (*in vitro*). Thus, antiperoxidant effects of α-santalol may play a role in preventing UVB-induced skin tumor development. However, other mechanisms such as induction of apoptosis and inhibition of cell proliferation also contribute to the chemopreventive effects of α-santalol (16).

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