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Time and dose-response effects of honokiol on UVB-induced skin cancer development

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ABSTRACT: Honokiol has shown chemopreventive effects in chemically-induced and UVB-induced skin cancer in mice. In this investigation, we assessed the time-effects of a topical low dose of honokiol (30 µg), and then the effects of different honokiol doses (30, 45, and 60 µg) on a UVB-induced skin cancer model to find an optimal dose and time for desirable chemopreventive effects. UVB radiation (30 mJ/cm^2, 5 days/week for 25 or 27 weeks) was used to induce skin carcinogenesis in SKH-1 mice. For the time-response experiment 30 µg honokiol in acetone was applied topically to the animals before the UVB exposure (30 min, 1 h, and 2 h) and after the UVB exposure (immediately, 30 min, and 1 h). Control groups were treated with acetone. For the dose-response study, animals were treated topically with acetone or honokiol (30, 45, and 60 µg) one hour before the UVB exposure. In the time-response experiment, honokiol inhibited skin tumor multiplicity by 49-58% while reducing tumor volumes by 70-89%. In the dose-response study, honokiol (30, 45, and 60 µg) significantly decreased skin tumor multiplicity by 36-78% in a dose-dependent manner, while tumor area was reduced by 76-94%. Honokiol (60 µg) significantly reduced tumor incidence by 40% as compared to control group. Honokiol applied in very low doses (30 µg) either before or after UVB radiation shows chemopreventive effects. Honokiol (30, 45, and 60 µg) prevents UVB-induced skin cancer in a dose-dependent manner. Honokiol can be an effective chemopreventive agent against skin cancer.

Keywords: Honokiol, nonmelanoma skin cancer, UVB, SKH-1 mice, dose-response, time-response

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1. Introduction

In recent years the number of skin cancer cases has increased dramatically, accounting for over 3.5 million cases each year in the United States alone. Some scholars propose that there is an unrecognized skin cancer epidemic in the United States (*1*). American Cancer Society estimates indicated 12,190 deaths from skin cancer in 2012 (*2*). Ultraviolet (UV) radiation exposure is the major risk factor for most skin cancers (*3*). Sunlight and tanning lamps are major sources of UV radiation.

UV radiation is composed by UVA, UVB, and UVC rays. UVA and UVB rays damage skin and can cause skin cancer, UVC rays are filtered by the atmosphere and do not reach the Earth's surface. Exposure to UVB rays can induce skin cancer faster than exposure to UVA rays. However, studies have proven that experimentally, UVA rays can cause skin cancer with long term exposure (*4*). The amount of UV exposure depends on the strength of the rays, the length of time the skin is exposed, and whether the skin is protected with clothing or sunscreen (*5*). UV rays cause DNA damage to skin cells (*6*), induction of signal transduction pathways that lead to cell proliferation, and induction of inflammatory responses and immunosuppression. All these effects caused by UV radiation are necessary for tumor development. UV radiation acts as a complete carcinogen on skin causing cancer initiation, promotion and progression (*7*).

Chemoprevention of skin cancer by natural compounds has gained importance in recent years (*8,9*). One phytochemical that is being extensively investigated against different models of cancer is honokiol (Figure 1), whose effects are investigated for the prevention of skin cancer in this study. Honokiol (HNK, $C_{18}H_{18}O_2$, MW 266.33) is a naturally occurring biphenol isolated from the bark and seed cones of *Magnolia officinalis* and other plants of the genus *Magnolia*. The stem bark of *Magnolia officinalis* is known as Houpo in traditional Chinese medicine, and it

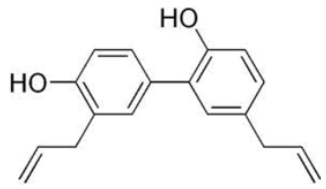

Figure 1. Molecular structure of honokiol.

has been used for relieving neurosis and gastrointestinal complaints (*10*).

Studies have demonstrated that honokiol has multiple pharmacological properties such as antioxidant (*11*), anti-inflammatory (*12*), and central nervous system depressant effects (*10,13*). It has been reported that honokiol delayed the formation of papillomas in mouse skin initiated by 7,12-dimethylbenz(a)anthracene (DMBA) and promoted by 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) (*14*). Honokiol has also shown anticancer effects against melanoma (*15*), pancreatic cancer (*16*), breast cancer (*17*), head and neck squamous cell carcinoma (*18*), and squamous cell skin cancer (*19*).

Our laboratory and other groups have reported the chemopreventive effects of honokiol on UVB-induced skin cancer development in mice. Honokiol induced apoptosis by both extrinsic and intrinsic pathways, inhibited UVB-induced inflammation and inflammatory mediators, reduced cell survival signals and proliferation markers, up-regulated cell cycle inhibitor proteins, and down regulated cell cycle promoter proteins in skin tumors (*20,21*). Honokiol also proved to cause apoptosis and cell cycle arrest in A431 squamous carcinoma cell line by increasing the activation of pro-apoptotic proteins and the expression of cell cycle inhibitor proteins p21 and p27. Honokiol decreased the expression of cyclins and CDKs protein promoters of the cell cycle (*19*).

This study was designed to investigate: *i*) The chemopreventive effects of honokiol when applied topically at low dose (30 µg) either before or after UVB exposure (time-response) and *ii*) The effects of honokiol on UVB-induced skin carcinogenesis when applied topically at different doses 30, 45, and 60 µg (dose-response) to find the optimal dose and time for desirable effects.

2. Materials and Methods

2.1. *Chemicals and reagents*

Honokiol 98% (HPLC) was purchased from Nacalai tesque (Kyoto, Japan). All other reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. *Animals*

Five to six-week-old female SKH-1 hairless mice were purchased from Charles River Laboratories (Wilmington, MA, USA). Institutional Animal Care and Use Committee (IACUC) approvals were obtained for all experimental protocols. The IACUC oversees animal programs, facilities, and procedures and provides assurance to federal agencies that South Dakota State University is in compliance with federal regulations on the humane care and use of animals in research. Mice were housed in a climate-controlled environment with a 12 h light/dark cycle and were provided with free access to food and water during the experiment.

2.3. *UVB light source*

Four FS-40-T-12 UVB lamps were used as UVB light source. The dose of UVB exposure was controlled by integrating dosimeters manufactured by Daavlin Corporation (Bryan, OH, USA).

2.4. *UVB-induced skin tumor development protocol*

Carcinogenesis was initiated and promoted by exposing the backs of six-week-old female SKH-1 mice to a UVB dose of (30 mJ/cm^2), 5 days a week (Monday-Friday) for 25 to 27 weeks. The UVB exposure and treatments were performed in the morning throughout the whole experiment, in order to keep consistency. This UVB induced skin cancer protocol has been described in detail elsewhere (*21,22*), this UVB dose is relevant to the human UVB exposure causing cancer development (*23*). This skin cancer induction scheme was used for both experiments.

For the time response experiment, six groups of animals (n = 20) randomly selected were used for the honokiol treatment: 30 µg in 200 µL of acetone. The difference among the groups was the time at which they received the topical honokiol treatment: 30 min, 1 h, or 2 h before UVB exposure (30 mJ/cm^2) and immediately, 30 min, or 1 h after UVB exposure. The control groups had 10 animals each, and received an application of 200 µL acetone either 1 h before or 1 h after UVB exposure. The experiment was carried out for 27 weeks.

For the dose-response experiment, four groups of animals were used. Group 1 served as control and received 200 µL of acetone, group 2, group 3, and group 4 received topical applications of 30, 45, and 60 µg of honokiol in 200 µL of acetone respectively. Treatments were administered one hour before UVB exposure (30 mJ/cm^2, Monday-Friday). The experiment was carried out for 25 weeks. Earlier reports and our previous studies have indicated that 200 µL acetone (topical) does not have effects on skin cancer development (*24,25*).

2.5. *Evaluation of tumor development*

Over the course of the experiments, the tumors' incidence, multiplicity, and volume were recorded once weekly. Mice's weights and external signs of toxicity also were closely monitored. Vernier caliper was used to determine the length, width, and height of the tumors, these values were then used to determine tumor volume by using the formula: Volume = $4\pi r^3/3$ where r is the radius, the diameter is the average of the three dimensional size of each mass (height, length, and width). Tumor areas were quantified as described elsewhere (*25*) by using images from the mice's backs which were taken at the end of 25 weeks. Tumor boundaries were determined and areas were measured by using by using Photoshop CS5 (Adobe systems, San Jose, CA, USA). Tumor counts, volume, and body weights were recorded on weekly basis for 25-27 weeks. Results were analyzed for tumor incidence, multiplicity, volume, and area.

2.6. *Histopathological analysis of mice tumors*

Mice were euthanized by cervical dislocation at the end of the above mentioned protocols. Skin samples randomly collected from five animals per group were fixed by immersion in 10% neutral buffered formalin for 24 h at room temperature. Fixed tissues were processed into paraffin-wax blocks, sectioned, stained with hematoxylin-eosin (HE), and evaluated under a light microscope.

2.7. *Statistical analysis*

INSTAT software (Graph Pad, San Diego, CA, USA) was used to analyze data. Chi square analysis was used for the data on tumor incidence. Analyses of variance followed by Tukey's test and Krushal-Wallis test (Nonparametric ANOVA) were used for tumor multiplicity and volume. Significance in all experiments was considered at $p < 0.05$. All values were expressed as mean ± standard error (SE).

3. Results

3.1. *Effects of honokiol on weight gain*

Treatment of animals with honokiol at all doses and different times did not have any effects on weight gain of mice (data not shown) suggesting safety in the application of honokiol at these doses and times.

3.2. *Effects of honokiol on tumor incidence*

Tumor incidence reflects the number of animals bearing at least one tumor. In the time response experiment, by the end of the 27th week, tumor incidence was 100% in the control group meaning that all control animals had at least one tumor. Tumor incidence ranged from 90-100% for all the honokiol (30 µg) pre-treated and post-treated groups. Tumor incidence was not significantly different between control and honokiol (30 µg) applied either before or after UVB exposure. The results for tumor incidence in the time response experiment are presented in Figure 2.

The effects of honokiol pretreatment at different doses on the tumor incidence in SKH-1 mice are shown in Figure 3. By the end of the 25th week, tumor

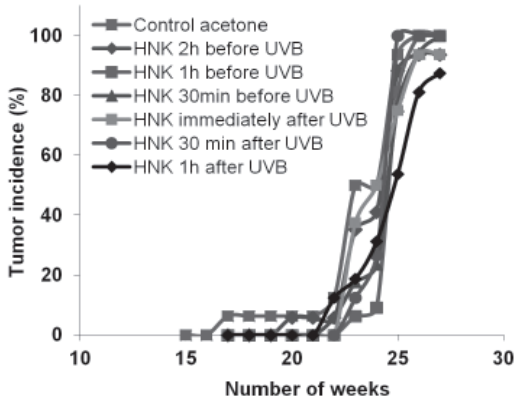

Figure 2. Effects of the topical application of honokiol (HNK) before and after UVB exposure on tumor incidence in SKH-1 hairless mice. Honokiol was applied topically on the mice's skin, either before (30 min-2 h) or after UVB exposure (immediately-1 h). The experiment was carried out for 27 weeks, and tumor counts were monitored weekly. Data represents the percentage of mice with at least one tumor (n = 20 per group). Honokiol did not reduce the tumor incidence when applied at 30 µg/dose either before or after UVB exposure.

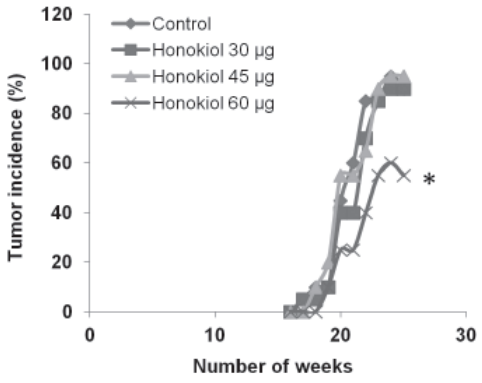

Figure 3. Dose-response effects of honokiol pretreatment on tumor incidence in UVB-induced skin carcinogenesis in SKH-1 hairless mice. From the 20th week to the end of the experiment honokiol 60 µg reduced significantly tumor incidence. At the end of the 25th week the honokiol 60 µg group had a tumor incidence 40% lower than the control group. The groups treated with honokiol 30 µg and 45 µg did not differ significantly from the control group. Each point represents the percentage of animals bearing at least one tumor, values derived from 20 mice per group. * Significant difference ($p < 0.05$).

incidence was 90% in both the control group and honokiol treated groups (30 and 45 µg). The honokiol (60 µg) pretreated group showed delayed appearance of tumors as compared to control, 60 µg application of honokiol resulted in a decrease in tumor incidence by 40% ($p < 0.05$) at the end of the experiment.

3.3. *Effects of honokiol on tumor multiplicity*

Results of the multiplicity of the time-response experiment are presented in Figure 4. Tumor multiplicity is the total number of tumors on back per mouse, for comparison purposes the number of tumors in the control group was considered as 100%. Topical application of 30 µg of honokiol before or after UVB treatments showed protection against skin tumor development in SKH-1 mice. At the end of the experiment (27th week), we found that Honokiol (30 µg) 30 min, 1 h, and 2 h before UVB treatments resulted in 57.5%, 54%, and 48% decrease in tumor multiplicity, respectively. Honokiol (30 µg) immediately, 30 min, and 1 h after UVB exposure resulted in 55%, 39%, and 48% reduction in tumor multiplicity, respectively. Tumor multiplicity was significantly ($p < 0.05$) lower in the honokiol pretreated groups (30 min and 1 h before UVB exposure) and in the post treated groups (immediately and 1 h after UVB exposure) when compared to the combined control group. Interestingly, honokiol applied 2 h before UVB exposure and 30 min after UVB exposure did reduce the tumor multiplicity but this difference was not statistically significant.

The effects of honokiol dose-response pretreatment on tumor multiplicity are shown in Figure 5. Topical application of 30, 45, and 60 µg of honokiol 1 h before UVB exposure showed protection against skin tumor development in SKH-1 hairless mice. Average tumor numbers were found to be lower in the honokiol pretreated groups from the 20th week until the end of the experiment (25th week), when compared to the control group treated with acetone ($p < 0.05$). At the end of the experiment, honokiol pretreatment resulted in a 36-78% decrease in tumor multiplicity with 30, 45, and 60 µg of honokiol application, respectively.

3.4. *Effects of honokiol on tumor volume*

The effects of honokiol (30 µg) before and after UVB treatments on the tumor volume are shown in Figure 6. In the control group, the mean tumor volume per animal was 35.3 mm^3, in the groups 30 min, 1 h, and 2 h before UVB exposure the average tumor volumes per animal were 3.77, 5.83, and 10.3 mm^3, respectively. In the post UVB exposure treated groups, the average tumor volumes per animal were 7.69, 5.66, and 6.28 mm^3 for the honokiol (30 µg) immediately, 30 min, and 1 h after UVB exposure, respectively. The control group had a high standard deviation among the animals' tumor volumes. As a consequence, only one group (honokiol treatment 30 min before UVB exposure) had a statistically significant reduction in tumor volume. Results for the tumor volume for the time response experiment are presented in Figure 6.

3.5. *Effects of honokiol on tumor area*

The effects of honokiol pretreatment on the ratio of total tumor area to total back area are shown in Figure 7. The mean ratio of tumor area to total back area in the control group was 4.0%, in the honokiol pretreated groups (30, 45, and 60 µg) was 0.32%, 0.95%, and

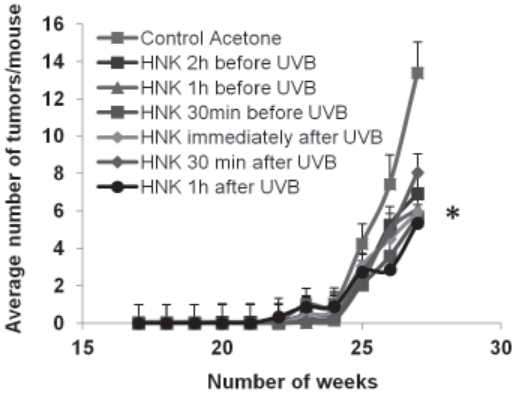

Figure 4. Effects of the topical application of honokiol (HNK) before and after UVB exposure on the tumor multiplicity in SKH-1 hairless mice. Skin carcinogenesis was performed as described in the methods section. Honokiol 30 µg was applied either before or after the UVB radiation. At the end of the experiment (27 weeks) the groups 30 min and 1 h before UVB as well as the groups immediately and 1 h after UVB showed a significant reduction in tumor multiplicity. Each point represents the average number of tumors per animal, $n = 20$, * $p < 0.05$.

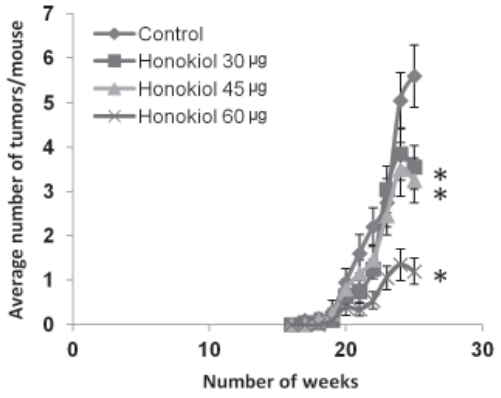

Figure 5. Dose-response effects of honokiol pretreatment on tumor multiplicity. Honokiol 30, 45, and 60 µg pretreatment decreased tumor multiplicities from the 20th to 25th week of UVB induced carcinogenesis. At the end of the experiment, honokiol significantly reduced the tumor multiplicity in 36-78% with 30, 45, and 60 µg applications, respectively. Each point represents mean number of tumors per mouse ± SE derived from 20 mice. * Significant difference ($p < 0.05$).

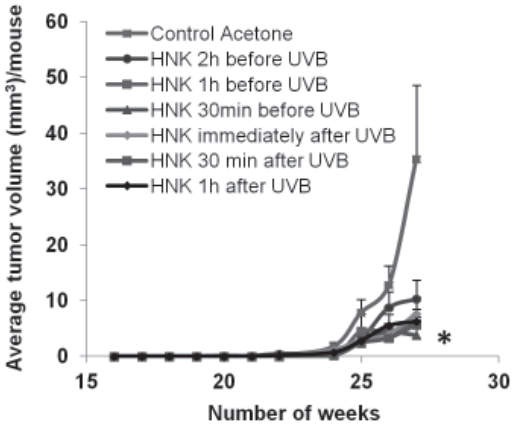

Figure 6. Effects of honokiol (HNK) on tumor volume in SKH-1 hairless mice. Skin carcinogenesis protocol was followed as described in the methods section. Tumors were measured weekly. It was observed an average reduction in tumor volumes in all honokiol treated groups pre and post UVB exposure. Each point represents the average tumor volume per animal, $n = 20$, * $p < 0.05$.

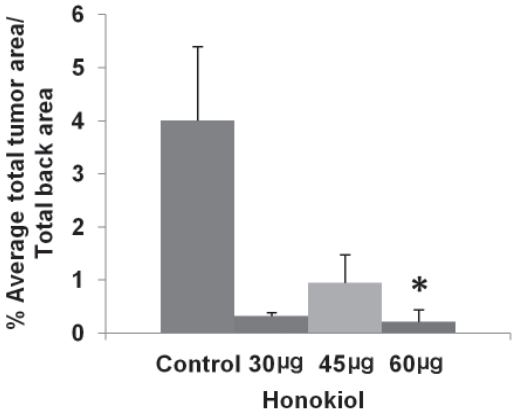

Figure 7. Dose-response effects of honokiol treatment on tumor area in SKH-1 mice. Graph bar represents the average ratio of total tumor area to total back area of the SKH-1 mice. Pictures of the backs of the animals were taken at the end of the 25th week. * Significant difference ($p < 0.05$).

0.22%, respectively. Honokiol pretreatments caused a 76-94.5% reduction in tumor area as compared to control. As in the section 3.4 (tumor volume), the control group had high standard deviation among animal's tumor areas, as consequence only one treatment (honokiol 60 µg) had a statistically significant reduction in tumor area.

3.6. *UVB induced squamous cell carcinoma in controls and honokiol treated mice*

The histopathological examination of the tumors after 25-27 weeks of treatments indicated that controls and honokiol treated mice in both protocols developed squamous cell carcinoma in the skin (Pictures not shown).

4. Discussion

Honokiol is a small-molecule, hydroxylated biphenolic compound isolated from *Magnolia* genus plants. It has been used in traditional Chinese medicine for thousands of years, and in recent years has been investigated for its effects on cancer and skin carcinogenesis. Previous findings from our laboratory indicated the chemopreventive effects of honokiol when applied topically in doses as low as 30 µg/dose (*21*). In this study, we investigated the effects of honokiol in a UVB-induced skin carcinogenesis model with a UVB radiation dose of 30 mJ/cm^2/day which is more translational and relevant to human skin cancer (*23*) as compared to previous studies that used honokiol as chemopreventive agent and higher doses of UVB radiation (180 mJ/cm^2) (*20*). We evaluated the effects of a low dose of honokiol (30 µg) applied topically either before or after the UVB exposure. Similar effects in tumor incidence were observed in all groups. Tumor multiplicity was decreased by the honokiol treatment, the average number of tumors per mouse in the control group was 13.38, while in the honokiol treated groups was 5.69, 6.13, and 6.94 for the honokiol pre-treated groups (30 min, 1 h, 2 h); and 5.94, 8.06, and 6.88 for the honokiol post-treated groups (immediately, 30 min, 1 h) respectively. Tumor volume was also decreased by the honokiol treatment, we observed a reduction in tumor volume of 89%, 83.5%, and 70.8% for the honokiol pre-treated groups (30 min, 1 h, 2 h); and 78%, 84%, and 82% for the honokiol post-treated groups (immediately, 30 min, 1 h) respectively. However, because of the high standard deviation in the tumor volumes in the control group, only the 30 min pre-treated group resulted in a statistically significant reduction in tumor volume. Previous studies showed chemopreventive effects when honokiol is applied topically within 30 min before or immediately after UVB exposure (*20*). In that study, they proved that topical honokiol (1 mg, 3 mg) either before or after UVB exposure (180 mJ/cm^2) prevented skin carcinogenesis. The novelty of our study is that we used very low doses of honokiol (30 µg) and low-chronic UVB exposure (30 mJ/cm^2). With this model we aim to show that honokiol at very low doses prevents skin carcinogenesis by mechanisms that are retained even after the UVB exposure, so it could be included in lotions applied prior to sun exposure (sunscreens) or in products used after sun exposure such as humectants, still retaining preventive effects. This model of low and chronic UVB exposure simulates human behavior of exposing skin to sunlight every day.

In the dose response experiment, we found that honokiol 60 µg markedly reduced the tumor incidence and multiplicity as compared to the control treated with acetone. The results demonstrated that honokiol 60 µg reduced tumor incidence by 40%. Honokiol 30, 45, and 60 µg in 200 µL of acetone showed a protective effect in a dose dependent manner when applied topically. Tumor

multiplicity was reduced by 36-78% while tumor area was reduced by 76-94.5% with treatments of 30, 45, and 60 µg of honokiol respectively as compared to the control.

We used very low doses (in micrograms) of honokiol compared to other chemopreventive agents which use milligrams per applications (*8,26,27*), thus indicating the higher potency and improved potential of honokiol over other agents. Previous mechanistic studies from our laboratory showed that topical application of honokiol (30 µg) on mice induced apoptosis through the intrinsic and extrinsic pathways, increasing the activation of caspase 8, caspase 9, caspase 3, and PARP. Honokiol 30 µg applied topically also increased the expression of p53 protein in mice skin (*21*). Additionally, we reported the effects of honokiol on A431 squamous carcinoma cell line. We used this *in vitro* model to gain insight and understanding of signaling mechanisms involved in the honokiol anti-carcinogenic effect. Honokiol overall inhibited cell growth in A431 cells at concentrations 50-75 µM starting at 12 h treatments. Honokiol induced G0/G1 cell cycle arrest and significant apoptosis in A431 cells, down regulated cyclins and cdks protein expressions and up-regulated the expression of cell cycle inhibitors p21 and p27 (*19*). These cell cycle modulator effects observed *in vitro* were confirmed *in vivo* by Vaid *et al*. (*20*). They used 1 mg and 3 mg of honokiol per application in SKH-1 mice. They found decreased expression of cyclin D1, D2, E, CDK2, CDK4, and CDK6, as well as increased expression of the cell cycle inhibitors p21 and p27 in the skin of honokiol treated animals. Furthermore, they found that the chemopreventive effects of honokiol at 1 mg and 3 mg involved modulation of PI3K/p-Akt pathway, decrease in inflammation and inflammatory mediators associated with tumorigenesis, and inhibition of UVB-induced cell survival signals in the tumors. Further mechanistic studies on the mice's skin are necessary to confirm if the same mechanisms of action found when honokiol was applied at 1 mg and 3 mg (100 times more) would be responsible for the low dose (30 µg) preventive effects observed in our study.

Our study provided evidence that honokiol pre or post UVB treatment at very low doses (micrograms per applications compared to most other agents which are used in milligrams per application) prevents UVB-induced skin cancer development in SKH-1 mice. Our studies also showed that honokiol exhibited potent chemopreventive effects at doses as low as 30-60 µg when applied topically; the preventive response was dose dependent, being the lowest with 30 µg and highest with 60 µg. Future studies on formulations to increase the retention of low doses of honokiol in skin are warranted, as well as their pharmacokinetic profile.

Honokiol has a great potential to be a safe and potent chemopreventive agent against skin cancer development in human.

Acknowledgements

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References

1. Rogers HW, Weinstock MA, Harris AR, Hinckley MR, Feldman SR, Fleischer AB, Coldiron BM. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. Arch Dermatol. 2010; 146:283-287.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012; 62:10-29.
3. Preston DS, Stern RS. Nonmelanoma cancers of the skin. N Engl J Med. 1992; 327:1649-1662.
4. Strickland PT. Photocarcinogenesis by near-ultraviolet (UVA) radiation in Sencar mice. J Invest Dermatol. 1986; 87:272-275.
5. Wright CY, Reeder AI. Youth solar ultraviolet radiation exposure, concurrent activities and sun-protective practices: A review. Photochem Photobiol. 2005; 81:1331-1342.
6. Alcalay J, Freeman SE, Goldberg LH, Wolf JE. Excision repair of pyrimidine dimers induced by simulated solar radiation in the skin of patients with basal cell carcinoma. J Invest Dermatol. 1990; 95:506-509.
7. Matsumura Y, Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. Toxicol Appl Pharmacol. 2004; 195:298-308.
8. Zhang X, Dwivedi C. Skin cancer chemoprevention by alpha-santalol. Front Biosci (Schol Ed). 2011; 3:777-787.
9. Nichols JA, Katiyar SK. Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. Arch Dermatol Res. 2010; 302:71-83.
10. Watanabe K, Watanabe H, Goto Y, Yamaguchi M, Yamamoto N, Hagino K. Pharmacological properties of magnolol and honokiol extracted from *Magnolia officinalis*: Central depressant effects. Planta Med. 1983; 49:103-108.
11. Dikalov S, Losik T, Arbiser JL. Honokiol is a potent scavenger of superoxide and peroxyl radicals. Biochem Pharmacol. 2008; 76:589-596.
12. Chao LK, Liao PC, Ho CL, Wang EI, Chuang CC, Chiu HW, Hung LB, Hua KF. Anti-inflammatory bioactivities of honokiol through inhibition of protein kinase C, mitogen-activated protein kinase, and the NF-kappaB pathway to reduce LPS-induced TNFalpha and NO expression. J Agric Food Chem. 2010; 58:3472-3478.
13. Kuribara H, Stavinoha WB, Maruyama Y. Behavioural pharmacological characteristics of honokiol, an anxiolytic agent present in extracts of *Magnolia* bark, evaluated by an elevated plus-maze test in mice. J Pharm Pharmacol. 1998; 50:819-826.
14. Konoshima T, Kozuka M, Tokuda H, Nishino H, Iwashima A, Haruna M, Ito K, Tanabe M. Studies on inhibitors of skin tumor promotion, IX. Neolignans from *Magnolia officinalis*. J Nat Prod. 1991; 54:816-822.
15. Mannal PW, Schneider J, Tangada A, McDonald D, McFadden DW. Honokiol produces anti-neoplastic effects on melanoma cells *in vitro*. J Surg Oncol. 2011; 104:260-264.

16. Arora S, Bhardwaj A, Srivastava SK, Singh S, McClellan S, Wang B, Singh AP. Honokiol arrests cell cycle, induces apoptosis, and potentiates the cytotoxic effect of gemcitabine in human pancreatic cancer cells. PLoS One. 2011; 6:e21573.
17. Liu H, Zang C, Emde A, Planas-Silva MD, Rosche M, Kuhnl A, Schulz CO, Elstner E, Possinger K, Eucker J. Anti-tumor effect of honokiol alone and in combination with other anti-cancer agents in breast cancer. Eur J Pharmacol. 2008; 591:43-51.
18. Leeman-Neill RJ, Cai Q, Joyce SC, Thomas SM, Bhola NE, Neill DB, Arbiser JL, Grandis JR. Honokiol inhibits epidermal growth factor receptor signaling and enhances the antitumor effects of epidermal growth factor receptor inhibitors. Clin Cancer Res. 2010; 16:2571-2579.
19. Chilampalli C, Guillermo R, Kaushik RS, Young A, Chandrasekher G, Fahmy H, Dwivedi C. Honokiol, a chemopreventive agent against skin cancer, induces cell cycle arrest and apoptosis in human epidermoid A431 cells. Exp Biol Med (Maywood). 2011; 236:1351-1359.
20. Vaid M, Sharma SD, Katiyar SK. Honokiol, a phytochemical from the *Magnolia* plant, inhibits photocarcinogenesis by targeting UVB-induced inflammatory mediators and cell cycle regulators: Development of topical formulation. Carcinogenesis. 2010; 31:2004-2011.
21. Chilampalli S, Zhang X, Fahmy H, Kaushik RS, Zeman D, Hildreth MB, Dwivedi C. Chemopreventive effects of honokiol on UVB-induced skin cancer development. Anticancer Res. 2010; 30:777-783.
22. Chilampalli C, Guillermo R, Zhang X, Kaushik RS, Young A, Zeman D, Hildreth MB, Fahmy H, Dwivedi C. Effects of magnolol on UVB-induced skin cancer development in mice and its possible mechanism of action. BMC Cancer. 2011; 11:456.
23. Lu YP, Lou YR, Xie JG, Peng QY, Liao J, Yang CS, Huang MT, Conney AH. Topical applications of caffeine or (–)-epigallocatechin gallate (EGCG) inhibits carcinogenesis and selectively increase apoptosis in UV B-induced skin tumors in mice. Proc Natl Acad Sci U S A 2002; 99:12455-12460.
24. Lowe NJ, Connor MJ, Breeding J, Chalet M. Inhibition of ultraviolet-B epidermal ornithine decarboxylase induction and skin carcinogenesis in hairless mice by topical indomethacin and triamcinolone acetonide. Cancer Res. 1982; 42:3941-3943.
25. Zhang X, Bommareddy A, Chen W, Hildreth MB, Kaushik RS, Zeman D, Khalifa S, Fahmy H, Dwivedi C. Chemopreventive effects of sarcophine-diol on ultraviolet B-induced skin tumor development in SKH-1 hairless mice. Mar Drugs. 2009; 7:153-165.
26. Gu M, Singh RP, Dhanalakshmi S, Agarwal C, Agarwal R. Silibinin inhibits inflammatory and angiogenic attributes in photocarcinogenesis in SKH-1 hairless mice. Cancer Res. 2007; 67:3483-3491.
27. Katiyar SK, Korman NJ, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J Natl Cancer Inst. 1997; 89:556-566.

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