The Inhibitory Effects of Vanillin on the Growth of Melanoma by Reducing Nuclear Factor-κB Activation

Abstract

Background: Melanoma is skin cancer, and the treatments are not efficient enough. Therefore, finding new drugs seems to be an essential need. Vanillin, which is extracted from vanilla seed, has anti-cancer effects by reducing nuclear factor-κB (NF). We explored the anti-tumor effects of vanillin in the melanoma model and its possible mechanism. Materials and Methods: In the MTT assay, mice melanoma cells (B16F10) were treated with vanillin (1, 2, 3, 4, 5 μg/mL) for 24 and 48 h. In an animal model, B16F10 was subcutaneously injected into C57BL/6 mice. After the development of tumors, the mice were treated with 50 and 100 mg/kg/day of vanillin for 10 days. The tumor size and expression level of NF-κB protein were measured. Results: In the MTT assay, vanillin in all concentrations significantly decreased B16F10 cell viability after 24 h incubation. The size of melanoma tumors was reduced in both doses 50 and 100 mg/kg/day in mice. NF-κB protein expression was decreased in the 100 mg/kg/day group in comparison with the control group. Conclusion: We found that vanillin by reducing NF-κB expression may have anti-tumor effects and reduced melanoma tumor size and cell viability.

Keywords: B16F10, melanoma, nuclear factor-κB, vanillin

Introduction

Melanoma is a type of skin cancer which is one of the most common and aggressive skin cancer with a poor prognosis and high mortality rate.[1,2] Conventional treatments, such as chemotherapy, are associated with high side effects and drug resistance,[3] and advanced therapies, such as immunotherapy, are too expensive for all people.[4] Therefore, finding new drugs can be an essential step in melanoma treatment.[5] Natural products are important sources for the design and discovery of new anti-cancer drugs.[6] Today many of the anti-cancer drugs are derived from plant products.[7]

Vanillin is one of the main compounds extracted from the vanilla bean.[8] It is commonly used in the food and pharmaceutical industry as a flavoring ingredient.[9] Vanillin has antioxidant properties and prevents DNA mutagenesis by DNA repairing. This compound has anti-cancer, antimetastatic, antiangiogenic, and cytotoxic activity[10] with low side effects. Up to relatively high concentration, no significant side effects have been observed from this natural compound.[11] A study showed that vanillin up to 300 mg/kg orally or subcutaneously did not show significant toxic effects.[12]

Probably one of the mechanisms of the anti-cancer effects of vanillin is reducing nuclear factor-κB (NF).[13] NF-κB is a translation factor that plays a role in regulating and cell proliferation in the immune system.[14] There is a relationship between cancer and NF-κB. Researchers have shown that NF-κB expression in cancer cells results in the production of anti-apoptotic proteins which leads to the survival and growth of cancer cells.[15] NF-κB dysregulation can lead to melanocyte transformation to cancer cells.[16] In human melanoma, the NF-κB expression was increased especially in metastatic melanoma.[17,18] Vanillin can reduce the expression of NF-κB,[19] so we suggest that vanillin can be used as a new drug in the treatment of melanoma. In this study, we look into the effect of vanillin on melanoma and its possible anti-cancer mechanism.

How to cite this article: Pourhadi M, Ghasemi A, Abediny R, Haghjooy Javanmard S, Vaseghi G. The inhibitory effects of vanillin on the growth of melanoma by reducing nuclear factor-κB activation. Adv Biomed Res 2022;11:68.

Address for correspondence: Dr. Golnaz Vaseghi, Applied Physiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: golnazvaseghi@yahoo.com

Received: 06 September 2021 Revised: 29 September 2021 Accepted: 12 October 2021 Published: 26 August 2022

Marjan Pourhadi¹, Ahmad Ghasemi¹, Reza Abediny¹, Shaghyayegh Haghjooy Javanmard², Golnaz Vaseghi¹,³

¹Applied Physiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, ²Department of Physiology, Applied Physiology Research Center, School of Medicine, Cardiovascular Research Institute, Isfahan University of Medical Sciences, ³Isfahan Cardiovascular Research Institute, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran

Original Article

© 2022 Advanced Biomedical Research | Published by Wolters Kluwer - Medknow
Materials and Methods

Cell and materials
Vanillin was taken from Sigma (San Diego, California, USA) and dissolved in normal saline and 5% dimethyl sulfoxide (DMSO). B16F10 cell line was purchased from the Pasteur Institute (Tehran, Iran). Dulbecco’s Modified Eagle’s Medium (DMEM), antibiotics of penicillin and streptomycin, and the fetal bovine serum (FBS) were obtained from Gibco BRL (Carlsbad, CA, USA). Antibody against NF-κB was taken from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell treatment
The B16F10 cells were cultured in DMEM containing 10% (v/v) FBS and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin). The culture was maintained at 37°C in a 5% CO₂ incubator.

Cell viability assay
The effects of vanillin on B16F10 viability were measured by MTT assay. Briefly, 2 × 10⁴ cells were placed in each well of the 96-well plate in DMEM medium for 1 day. The cells were incubated with 20 μL of vanillin in different concentrations (1, 2, 3, 4, 5 μg/mL). For each concentration, three wells were used. After 24 or 48 h treatment, 20 μL of MTT solutions was added to each well and incubate the plate at 37°C for 4 h. The crystals were dissolved in 150 μL DMSO for each well (150 μL of DMSO alone as blank), and absorbance was measured by ELISA plate reader (ELX 800-BioTek-USA) at 570 nm. The formula (1) was used to determine the viability of cells:

\[
\text{Percent cell viability} = \frac{\text{Mean absorption of sample wells} - \text{Mean absorption of blank wells}}{\text{Mean absorption of control wells} - \text{Mean absorption of blank wells}} \times 100
\]

Experimental animals
Male C57BL6 mice aged 6–8 weeks, weighing 20–28 g, were purchased from Pasteur Institute (Tehran, Iran). They were kept in the Animal Laboratory with a standard 22°C–23°C temperature. Water and foods were freely available. The animal experiments were done according to the Ethical Committee of Isfahan University of Medical Sciences Isfahan, Iran (approval ID: IR.MUI.MED.REC.1398.651 on February 23, 2020).

In vivo experimental
The B16F10 was suspended in PBS till a concentration of 1 × 10⁶ cells/100 μL was achieved. The cell suspensions (100 μL/mouse) were subcutaneously injected into the back of mice. On day 7 after injection mice randomly were divided into three groups (6–8 mice in each group). Two groups received 50, 100 mg/kg/day vanillin respectively through intraperitoneal injections. The control group received normal saline and 5% DMSO as the vehicle. The mice were sacrificed after 10 days of treatment with vanillin, and the tumors were excluded for further studies.

Tumor volume
The anti-tumor effect was investigated by measuring the weight and volume of the tumor. The Vernier calipers were used to determine the length and width of the tumor, and finally, the tumor volumes were calculated by the formula (2) as known the modified ellipsoidal formula:\[^{[20]}\]

\[
\text{Tumor volume} = \frac{\text{Length} \times \text{width}^2}{2}
\]

Immunohistochemical staining
Immunohistochemical (IHC) was used to determine the expression of NF-κB. Tumor tissues were fixed in 10% formalin solution for 24 h. The tissue block is embedded in paraffin, then affixed onto the slide. Slides were deparaffinized in xylene and rehydrated in ethanol gradient (100%–70%). The antigen retrieval step has been done according to the antibody datasheet. The slides were incubated with 3% H₂O₂ for 30 min. For IHC staining, the slides were washed with water and treated with the NF-κB antibody (mouse monoclonal, sc-293072 hP) overnight at 4°C. The next day, the slides were incubated by 3, 3′-diaminobenzidine, and hematoxylin staining, respectively. The slides were then photographed using a Leica camera (DFC450 C). FIJI (ImageJ) software was used to analyze the NF-κB expression.

Statistical analysis
Statistical analysis was performed one-way analysis of variance followed by post hoc Tukey using SPSS version 20 statistical software (SPSS Inc, IL, USA). All values have been expressed as mean ± standard error of the mean. P < 0.05 was considered to be statistically significant when compared to control.

Results

The effect of vanillin on B16F10 cell viability
In the MTT assay, after 24 h treatment of B16F10 cells with vanillin, cell viability significantly decreased in all concentrations (P < 0.001). After 48 h incubation of cell, the significant decrease of cell viability was only observed with concentrations of 2 (P = 0.003) and 5 μg/ml (P < 0.001) of vanillin [Figure 1].

Effect of vanillin on tumor volume and tumor weight
Tumor developing in the untreated control mice was higher than two groups receiving vanillin. After 10 days of treatment with vanillin, the average tumor...
Pourhadi, et al.: Vanillin suppresses melanoma by inhibiting NF-κB

The NF-κB expression was significantly decreased in the 100 mg/kg/day group compared with the control group \(P = 0.019\); Figure 4. Vanillin reduced NF-κB expression in the 50 mg/kg/day group, but it was not significant when compared with the control group \(P = 0.086\); Figure 4. There was no significant reduction of NF-κB expression between 50 and 100 (mg/kg/day) groups \(P = 0.619\).

**Discussion**

In this study, we show that vanillin had anti-tumor activity both in vivo and in vitro and reduced melanoma tumor size by suppressing the NF-κB expression.

Vanillin is a natural compound with anti-neoplasm effects\(^{[21]}\) and low adverse effects.\(^{[22]}\) Hitherto, several studies have been done to find the anti-neoplastic effects of vanillin.\(^{[21]}\) For example, one study has been shown vanillin could prevent breast cancer metastasis by inhibiting the enzyme activity of matrix metalloproteinases (MMPs).\(^{[11]}\) It had synergistic effects with doxorubicin in breast cancer. Vanillin without doxorubicin also was able to reduce the tumor volume significantly compared to the control group \(P < 0.01\).\(^{[23]}\) The anti-cancer effects of vanillin on gastrointestinal malignancies have also been studied. It could cause death in colon cancer cells (HT-29) by inducing apoptosis, and cell cycle arrest both low (200 mg/ml) and high concentrations (1000 mg/ml). G0/G1 arrest was achieved in low concentrations and G2/M arrest in high concentrations of vanillin.\(^{[24]}\) The reduction of cell proliferation by vanillin in melanoma has also been reported. This natural compound inhibited the proliferation of cancer cells by reducing the expression of HIF-1 in melanoma cancer cell lines (A2058 and A375).\(^{[25]}\) Our MTT and animal test results show that vanillin could inhibit the growth of melanoma cells. However, in the MTT assay, the reduction of cell proliferation was significant in all concentrations of vanillin in 24 h but 48 h just in two concentrations. Also, in animal study, vanillin reduce the tumor volume and weight by 50 mg/kg/day more than 100 mg/kg/day. Probably that this paradox could be due to the effect of vanillin in DNA damage repair, which increased after 48 h and by increasing the dose (10).

The result suggested that vanillin suppressed melanoma tumor volume and weight. However, the precise
mechanism whereby vanillin decreases tumor size is not well known.[25] In this research work, we investigated the hypothesis that vanillin could reduce melanoma tumor size by inhibiting NF-κB expression. Increased NF-κB expression has been seen in many cancers such as melanoma.[26] NF-κB activation can lead to drug and radiation resistance.[27,28]

Canonical and noncanonical are generally two main pathways for NF-κB activation. In the canonical pathway, one of the factors that activate NF-κB is the phosphorylation of p65. Therefore, any factors that prevent p65 phosphorylation can inhibit NF-κB and may have anti-cancer.[29] Various studies have been performed on the reduction of NF-κB expression by vanillin. One study has been shown vanillin inhibited p65 phosphorylation. This led to a decrease in NF-κB expression and finally increased the induction of apoptosis in cancer cells mediated by TRAIL (an anti-cancer agent).[30] These results were repeated in another study and vanillin reduced

Figure 3: Mean melanoma tumors weigh in different groups. Tumor-bearing animals were treated with different dose of vanillin for 10 days. *P < 0.05, **P < 0.01 and values are expressed as mean ± standard error of the mean (n = 6–8)

Figure 4: Immunohistochemical (IHC) evaluation of nuclear factor-κB. (a) IHC staining of different doses of vanillin, (b) IHC quantifications relative to respective treatment control in melanoma tumors. *P < 0.05 and Values are expressed as mean ± standard error of the mean (n = 6–8)
NF-κB expression and translocation of p65. Vanillin by inhibiting NF-κB could reduce the expression of MMP-9, which is one of the factors required for cancer cell metastasis. NF-κB could increase the expression of MMP-9, and vanillin inhibited MMP-9 in liver cancer cells (HepG2) by reducing NF-κB.

The inhibitory effect of vanillin and nine other aromatic aldehydes on doxorubicin NF-κB activation was evaluated in melanoma. The in vitro study showed that some of these aromatic aldehydes could reduce the expression of NF-κB in A375 cells. Finally, only two compounds (not include vanillin) that had a better effect on suppression of A375 NF-κB activity were selected to treat the mice melanoma tumor (13); while in this study, we studied the effect of vanillin on reducing NF-κB expression in mouse tissue. The treatment of C57BL/6 mice with vanillin showed that vanillin could reduce NF-κB expression.

Conclusion

The present study provided evidence that vanillin could inhibit melanoma cell viability in vitro and reduce melanoma tumor size through inhibiting NF-κB signaling in an animal model. We observed that Vanillin in both doses (50 and 100 mg/kg) reduced melanoma tumor size compared to the controls. The results showed a decrease in NF-κB expression in vanillin groups, but only at the high dose was significantly different compared to the control group. Due to the effectiveness of vanillin in reducing tumor size and its low side effects, probably it can be used as a new drug for melanoma treatment. However, more studies are needed to find an effective dose of vanillin for melanoma treatment.

Financial support and sponsorship

This work was supported by the Isfahan University of Medical Science [grant number 298208].

Conflicts of interest

There are no conflicts of interest.

References

1. Rastrelli M, Tropea S, Rossi CR, Alaibac M. Melanoma: Epidemiology, risk factors, pathogenesis, diagnosis and classification. In Vivo 2014;28:1005-11.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
3. Singh S, Zafar A, Khan S, Naseem I. Towards therapeutic advances in melanoma management: An overview. Life Sci 2017;174:50-8.
4. Lin J, Lin Y, Huang Z, Li X. Identification of prognostic biomarkers of cutaneous melanoma based on analysis of tumor mutation burden. Comput Math Methods Med 2020;2020:8836493.
5. Yang Y, Guo W, Ma J, Xu P, Zhang W, Guo S, et al. Downregulated TRPV1 expression contributes to melanoma growth via the calcineurin-AIF3-p53 pathway. J Invest Dermatol 2018;138:2205-15.
6. Pereira GJ, Tavares MT, Azevedo RA, Martins BB, Cunha MR, Bhardwaj R, et al. Capsaicin-like analogue induced selective apoptosis in A2058 melanoma cells: Design, synthesis and molecular modeling. Bioorg Med Chem 2019;27:2893-904.
7. Wu YJ, Hsu WJ, Wu LH, Liou HP, Pangilinan CR, Tyan YC, et al. Hinokitiol reduces tumor metastasis by inhibiting heparanase via extracellular signal-regulated kinase and protein kinase B pathway. Int J Med Sci 2020;17:403-13.
8. Zamzuri NA, Abd-Aziz S. Biovanillin from agro wastes as an alternative food flavour. J Sci Food Agric 2013:93:429-38.
9. Guo W, Liu B, Hu G, Kan X, Li Y, Gong Q, et al. Vanillin protects the blood-milk barrier and inhibits the inflammatory response in LPS-induced mastitis in mice. Toxicol Appl Pharmacol 2019;365:9-18.
10. Bezerra DP, Soares AK, de Sousa DP. Overview of the role of vanillin on redox status and cancer development. Oxid Med Cell Longev 2016;2016:9734816.
11. Lindprapamongkol K, Sakurai H, Kawasaki N, Choo MK, Saitoh Y, Aozuka Y, et al. Vanillin suppresses in vitro invasion and in vivo metastasis of mouse breast cancer cells. Eur J Pharm Sci 2005;25:57-65.
12. Ho K, Yazan LS, Ismail N, Ismail M. Toxicology study of vanillin on rats via oral and intra-peritoneal administration. Food Chem Toxicol 2011;49:25-30.
13. Marton A, Kúsz E, Kolozsi C, Tubak V, Zagotto G, Buzás K, et al. Vanillin analogues o-vanillin and 2,4,6-trihydroxybenzaldehyde inhibit NFκB activation and suppress growth of A375 human melanoma. Anticancer Res 2016;36:5743-50.
14. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023.
15. Zubair A, Friere M. Role of nuclear factor-kb in breast and colorectal cancer. Curr Allergy Asthma Rep 2013;13:44-49.
16. Dana N, Vaseghi G, Haghjooy Javanmard S. Activation of PARP inhibits TLR4 signal transduction pathway in melanoma cancer in vitro. Adv Pharm Bull 2020;10:458-63.
17. Huang S, DeGuzman A, Bucana CD, Fielder JI. Nuclear factor-kappaB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res 2000;6:2573-81.
18. Ueda Y, Richardson A. NF-kappaB activation in melanoma. Pigment Cell Res 2006;19:112-24.
19. Yan X, Liu DF, Zhang XY, Liu D, Xu SY, Chen GX, et al. Vanillin protects dopaminergic neurons against inflammation-mediated cell death by inhibiting ERK1/2, P38 and the NF-κB signaling pathway. Int J Mol Sci 2017;18:389.
20. Jensen MM, Jørgensen JT, Binderup T, Kjaer A. Tumor volume in subcutaneous mouse xenografts measured by microCT is more accurate and reproducible than determined by 18F-FDG-microPET or external caliper. BMC Med Imaging 2008;8:16.
21. Srimul S, Chanvorachote P, Pongrakhananon V. Suppression of cancer stem-like phenotypes in NCI-H460 lung cancer cells by vanillin through an Akt-dependent pathway. Int J Oncol 2017;50:1341-51.
22. Naz H, Tarique M, Khan P, Luqman S, Ahamad S, Islam A, et al. Effect of vanillin binding to CAMKIV explains the anti-cancer mechanism in human hepatic carcinoma and neuroblastoma cells. Mol Cell Biochem 2018;438:35-45.
23. Elsherbiny NM, Younis NN, Shaheen MA, Elsewedy MM. The synergistic effect between vanillin and doxorubicin in ehrlich ascites carcinoma solid tumor and MCF-7 human breast cancer cell line. Pathol Res Pract 2016;212:767-77.
24. Ho K, Yazan LS, Ismail N, Ismail M. Apoptosis and cell cycle
Pourhadi, et al.: Vanillin suppresses melanoma by inhibiting NF-κB

arrest of human colorectal cancer cell line HT-29 induced by vanillin. Cancer Epidemiol 2009;33:155-60.

25. Park EJ, Lee YM, Oh TI, Kim BM, Lim BO, Lim JH. Vanillin suppresses cell motility by inhibiting STAT3-mediated HIF-1α mRNA expression in malignant melanoma cells. Int J Mol Sci 2017;18:532.

26. Bassères DS, Baldwin AS. Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. Oncogene 2006;25:6817-30.

27. Labbozzetta M, Notarbartolo M, Poma P. Can NF-κB be considered a valid drug target in neoplastic diseases? Our point of view. Int J Mol Sci 2020;21:3070.

28. Russo SM, Tepper JE, Baldwin AS Jr., Liu R, Adams J, Elliott P, et al. Enhancement of radiosensitivity by proteasome inhibition: Implications for a role of NF-kappaB. Int J Radiat Oncol Biol Phys 2001;50:183-93.

29. Gupta SC, Sundaram C, Reuter S, Aggarwal BB. Inhibiting NF-κB activation by small molecules as a therapeutic strategy. Biochim Biophys Acta 2010;1799:775-87.

30. Lirdprapamongkol K, Sakurai H, Suzuki S, Koizumi K, Prangsaengtong O, Viriyaroj A, et al. Vanillin enhances TRAIL-induced apoptosis in cancer cells through inhibition of NF-kappaB activation. In Vivo 2010;24:501-6.

31. Li JM, Lee YC, Li CC, Lo HY, Chen FY, Chen YS, et al. Vanillin-ameliorated development of azoxymethane/dextran sodium sulfate-induced murine colorectal cancer: The involvement of proteasome/nuclear factor-kB/mitogen-activated protein kinase pathways. J Agric Food Chem 2018;66:5563-73.

32. Liang JA, Wu SL, Lo HY, Hsiang CY, Ho TY. Vanillin inhibits matrix metalloproteinase-9 expression through down-regulation of nuclear factor-kappaB signaling pathway in human hepatocellular carcinoma cells. Mol Pharmacol 2009;75:151-7.