

A NATURAL PRODUCT DECURSIN ENHANCES THE RADIOSENSITIZATION OF IONIZING RADIATION AGAINST DMBA-INDUCED TUMOR

ADEEB SHEHZAD^{1*}, HIRA ZAHID¹, WAZIR MUHAMMAD², RIZWAN AHMAD³, EBTESAM A. AL-SUHAIMI^{4*}

¹Department of Biomedical Engineering and Sciences, SMME, National University of Sciences and Technology, H-12, Islamabad, Pakistan.

²Department of Therapeutic Radiology, Yale School of Medicine, Yale University, New Haven, CT, USA. ³Department of Biology, Natural Products and Alternative Medicines, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, Saudi Arabia. ⁴Department of Biology, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Saudi Arabia.

Email: adeeb.shehzad@smme.nust.edu.pk

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ABSTRACT

Objective: Radiation therapy has gained significant attention for the treatment and prevention of solid and malignant human tumors. However, after periodical exposures, radiation therapy loses its efficacy against cancer cells displaying radio-resistant phenotypes. Therefore, decursin might improve the efficiency of radiotherapy against a variety of human cancers.

Methods: The chemopreventive efficacy of decursin was evaluated against B16F10 cancer cell lines and DMBA/croton oil-induced skin carcinogenesis in BALB/c mice. Decursin was administered intraperitoneal at the dose of 20 mg/kg/day for 8 weeks following exposure to 5 Gy of ionizing radiation (IR) after 1 month of DMBA application. Western blot was performed for underlying mechanism of radioresistance.

Results: Decursin suppressed the proliferation and viability of melanoma cancer cell lines in a concentration- and time-dependent manner. The *in vivo* data collected from mice model revealed that decursin reduced the precancerous skin lesions and the incidence of tumor bearing in radiation-exposed mice. Decursin also enhanced the effect of IR by downregulation of Akt/NFκB pathway through activation of IκBα.

Conclusion: Our results suggest that the activation of Akt/NFκB establishes a pro-survival response to radiation that may account for the development of radioresistance. Decursin blocks the abnormal expression of these proteins and potentiates the radiotherapeutic effect.

Keywords: Decursin, Ionizing radiation, DMBA, Inflammation, Squamous carcinoma.

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INTRODUCTION

Ionizing radiation (IR) is an established beneficial treatment modality for the treatment of pre- and post-operative cancer, which selectively target and destroy cancer cells [1]. Radioresistance is a biological phenomenon which limits and decreases the maximal therapeutic efficacy of radiotherapy for the treatment of tumors [2]. Among various human cancers, melanoma has been believed to be a radio-resistant tumor type as compared to other types of cancers [3]. Studies have reported various plant isolated compounds for radiosensitization as well as radioprotection [4]. Therefore, low cost and less toxic radiosensitizers are urgently required, which can reduce the radiation dose-response threshold for cancer cells and provide radioprotection to normal cells [4].

PI3k/Akt pathway has been involved in tumor cell proliferation and intrinsic radioresistance [5]. In melanoma, aberrant activity of the PI3k/Akt pathway has been demonstrated to promote melanogenesis [5]. A number of studies have shown that specific inhibition of PI3k/Akt/mTOR pathway has enhanced the radiosensitivity of various cancer cell lines both *in vitro* and *in vivo* [6]. On activation, PI3k/Akt pathway also regulates the expression of downstream targets such as NFκB, which further potentiates the process of radioresistance. NFκB activation constitutively leads to radioresistance. Inhibition of NFκB activation can lead to an increase in the efficacy of radiation therapy [7]. Therefore, downregulation of NFκB is a promising target to enhance radiosensitivity [7].

Decursin is a naturally occurring coumarin compound isolated from the roots of Korean Angelica also known as Angelica gigas Nakai [8].

Decursin exerts its anticancer effects by inducing apoptosis through activation of caspase 8, 9, and 3, downregulation of antiapoptotic proteins such as Bcl-2 and Bcl-xL, by inhibiting vascular endothelial growth factor (VEGF)-induced angiogenesis, by inducing cell cycle arrest through the downregulation of cyclin D1, and by modulating various signaling pathways including NFκB and PI3K pathways [9,10].

Previously, we have summarized and reported the anti-inflammatory and synergistic role of decursin in various cancers [11]. In the current study, we have investigated the role of decursin in modulating the radioresponse of melanoma cancer *in vitro* and *in vivo*. Decursin enhanced radiosensitization through downregulation of NFκB and PI3K.

METHODS

Cell culture and treatment

B16F10 cells of Mus musculus skin melanoma were obtained from ATCC, CRL-6475™. Cells were cultured at a density of 5×10⁵ in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum, L-glutamine and supplemented with 1% (v/v) antibiotic-penicillin streptomycin (Gibco, Invitrogen Corporation) at 37°C in a 5% CO₂-humidified atmosphere. 70% confluent growing cells were seeded at 1×10⁶ cells per dish followed by exposure to various concentrations (2, 5, 10, 20, 40, 80, and 100 μM) of decursin (≥97% Sigma-Aldrich, USA). For 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, cells were exposed to various concentrations of decursin for next 24 h. After this, medium was removed and 50 μl of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well. After 3 h, the MTT solution was removed and 200 μl of

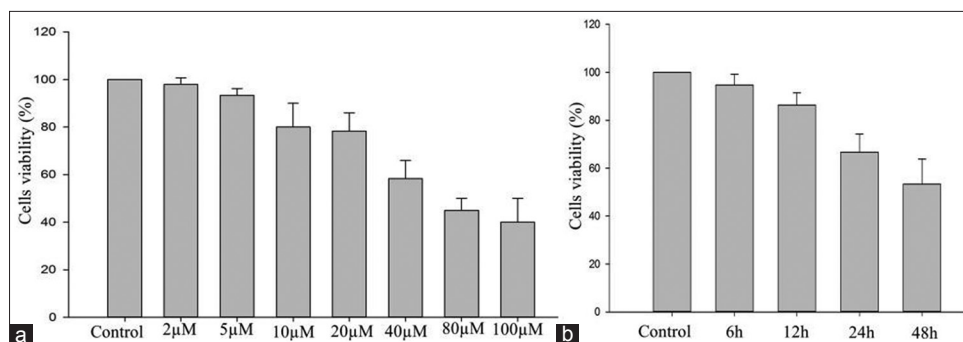


Fig. 1: (a) Concentration-dependent effect of decursin against B16F10 cell lines. (b) Time-dependent effect of 40 µL of decursin against B16F10 cell lines. $p \leq 0.05$

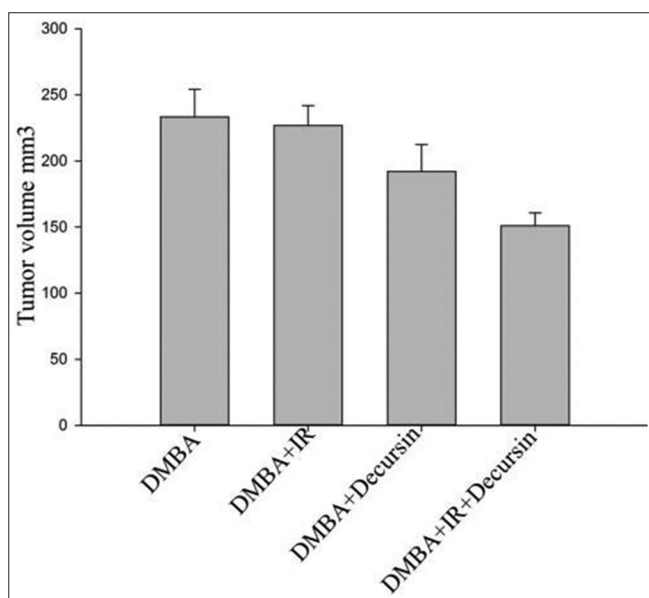


Fig. 2: Decursin sensitizes DMBA-induced tumors to radiation in BALB/C mice. Decursin and IR together reduced tumor size as compared to their individual treatments. Tumor volumes were measured using Vernier calipers, calculated as described in materials and methods. $p \leq 0.05$ (n=6)

dimethyl sulfoxide was added and analyzed by microplate reader (BioTek, USA) at 570 nm in a triplicate.

Experimental design

BALB/C male mice (27–30 g, 5–6 weeks) were purchased from the National Institute of Health (NIH, Islamabad, and were divided into four groups, each containing six mice. Our experimental protocol was approved by the Institutional Animal Care and Ethics Committee, in line with international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH publication No. 85–23, 1985). These groups were assigned to different treatments as follows: Group A (DMBA/Croton oil): The animals in this group received topical applications of DMBA in acetone (100 µL) at a dose of 400 nmol per application at an interval of 72 h per mouse. Starting from the 8 days after the first DMBA application, croton oil (1% w/v) in acetone (100 µL) was applied twice in a week for a total of 8 weeks. Group B mice were exposed to 5 Gy/month of IR after induction of tumor with DMBA/croton oil. Group C (decursin 20 mg/kg ip injection): The animals in this group were applied with DMBA/croton oil following concomitant treatment with ip injection of decursin. Group D received decursin and IR after treatment with DMBA/croton oil. During the 8 weeks of experimental period, the animals were observed daily for the appearance of skin papillomas and the tumor volume was recorded. At the start of 9 weeks, all the mice were sacrificed and the skin samples were collected.

Western blotting

In brief, proteins were extracted from the developed tumor of different treatment groups through tissue lysis buffer. Proteins were transferred onto nitrocellulose membranes (0.2 µM, Schleicher and Schuell). Membranes were blocked using 5% non-fat dry milk and probed with primary antibody in TBS containing 3% non-fat dry milk and 0.1% Tween 20. Antibodies in the ratio of 1:1000 v/v were used and detected using an enhanced chemiluminescence kit (Amersham Corp).

RESULTS

Decursin inhibited B16F10 cells proliferation

Cell viability assay determines the inhibitory concentration of the drug, which has therapeutic effect. B16F10 cell lines were treated with various concentrations (0–100 µM) of decursin. As shown in Fig. 1a, decursin reduced the cells viability of B16F10 cells in a concentration-dependent manner. Furthermore, we checked the time-dependent effects of 40 µM of decursin various time periods. Decursin significantly reduced the cells viability after 12 h (Fig. 1b). These results demonstrate the therapeutic potential of decursin *in vitro* against melanoma cell lines.

Decursin reduced DMBA-induced tumor in BALB/C

We evaluated the combined effects of decursin and IR against the growth of DMBA-induced tumor model. As shown in Fig. 2, combined administration of decursin and IR reduced the tumor growth as compared to their individual effect. Tumor volume was measured according to the formula: $V = 4/3\pi W^2L$ (short size² × long size/2 (mm³)). The mice were sacrificed for the isolation of tumor after the 8th week.

Decursin inhibited the DMBA-induced expression of NFκB and Akt

In our study, DMBA treatment activates Akt and NFκB, whereas degraded IκB (Fig. 3). However, decursin treatment reversed the carcinogenic and tumorigenic effect of DMBA. Decursin potentiated the effects of IR in DMBA-induced tumor (Fig. 3). One of the known activators of IKK signaling pathway is Akt, which also mediates radioresistance through induction of pro-survival signaling. Decursin significantly inhibited DMBA-induced Akt and NFκB (Fig. 3).

DISCUSSION

Skin cancers are counted among curable cancers, but the development of resistant phenotype makes it hard to treat with standard chemo and radiotherapy [12,13]. The study was conducted to investigate the radiosensitizing effect decursin in DMBA-induced carcinoma. For this purpose, B16F10 cells and carcinogen model were exposed to decursin and IR. Decursin reduced the cells viability in a concentration- and time-dependent manner. Decursin reduced the cancer lesions in squamous cell carcinoma and potentiated the effect of IR in DMBA-induced tumor model. The previous studies have shown that activation of Akt and NFκB provides a pro-survival response to radiation that may account for the development of radioresistance [14,15]. Decursin activates IκBα and inhibits Akt/NFκB pathway and boosts the efficacy of radiotherapy.

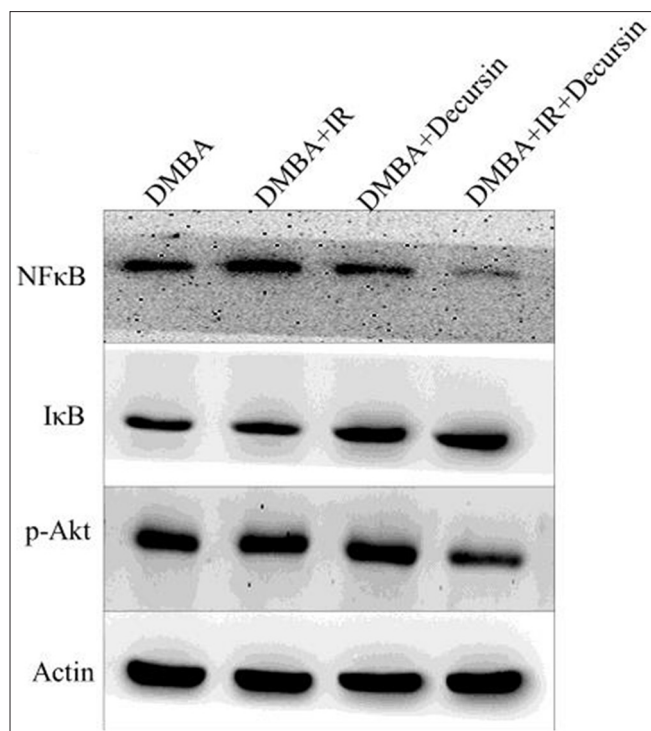


Fig. 3: Decursin suppressed the expression of NFκB and Akt in DMBA-induced tumor mice. Developed tumors were isolated, homogenized and total protein was isolated from cells and analyzed by western blotting. Actin was used as a loading control

There is accumulating evidence that PI3k/Akt and NFκB signaling pathways are involved in radioresistance [12,14]. Activation of Akt causes downregulation of tumor suppressor gene phosphatase and tensin homolog and inhibits the process of apoptosis [16,17]. Furthermore, Akt has been reported to phosphorylate the NFκB, increasing the transcription of NFκB downstream targets involved in cancer development and therapeutic resistance [18,19]. Therefore, inhibition of Akt will suppress the activation of NFκB in malignant melanoma cells [18]. Decursin treatment decreased the expression of p52 subunit of NFκB in a DMBA-treated group. The expression of IκB was also increased in the decursin-treated group. Our results indicate that decursin has the ability to block Akt and NFκB activation in DMBA-induced tumor model. Decursin can provide alternate strategy to potentiate the therapeutic effect of IR.

The underlying mechanism of tumor resistance is mediated through multiple interrelated signal transduction pathways. Deep understanding of these multiple molecular processes underlying resistance to cancer therapeutics has led to the demand for the less toxic and cost-effective novel agents [20,21].

CONCLUSION

Decursin has the ability to reduce the cells viability of B16F10 cells in a concentration- and time-dependent manner. Decursin also enhances the effect of IR by downregulating the expression of Akt/NFκB, which is involved in cancer cells survival and radioresistance. Therefore, decursin-induced radiosensitization may have potential therapeutic effect in cancer radiotherapy.

Further *in vitro* and *in vivo* studies are needed to investigate the molecular mechanism of decursin in various cancers to further advocate the radiosensitizer role. Furthermore, pharmacodynamics, pharmacokinetics, and safety studies of decursin are further needed to

confirm the detail mechanism of action with optimum dose and dosage form in cancer chemotherapy.

CONFLICTS OF INTEREST STATEMENT

All authors have declared no conflicts of interest.

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