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Geraniol attenuates 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced oxidative stress and inflammation in mouse skin: Possible role of p38 MAP Kinase and NF-κB

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ABSTRACT

Abnormal production of reactive oxygen species (ROS) and proinflammatory cytokines often act as trigger for development of most of the chronic human diseases including cancer via up-regulation of transcription factors and activation of MAP kinases. We investigated the protective effects of geraniol (GOH) against 12-O-tetradecanoyl phorbol-13-acetate (TPA) induced oxidative and inflammatory responses, expression of p38MAPK, NF-κB and COX-2 in mouse skin. Animals were divided into four groups I-IV (n = 6). Group II and III received topical application of TPA at the dose of 10 nmol/0.2 ml of acetone/animal/day, for two days. Group III was pre-treated with GOH (250 μg) topically 30 min prior to each TPA administration. While group I and IV were given acetone (0.2 ml) and GOH respectively. Our results show that GOH significantly inhibited TPA induced lipid peroxidation (LPO), inflammatory responses, proinflammatory cytokine release, up regulates reduced glutathione (GSH) content and the activity of different antioxidant enzymes. Interestingly, GOH also inhibited TPA induced altered activity of p38MAPK. Further, TPA induced altered expression of NF-κB (p65) and COX-2 was also attenuated by GOH. Thus, our results suggest that GOH attenuates early tumor promotional changes, and it may serve as one of the various ways to prevent carcinogenesis.

Introduction

Dietary natural products showed protection against various degenerative diseases including cancer and therapeutic importance has been given to them because of the virtue of their anti-oxidant and anti-inflammatory potential. Epidemiological findings implicate that substantial intake of fruits and vegetables have shown protective effects against different types of cancer (Singletery, 2000). Dietary isoprenoids, originate from the mevalonate pathway, are one of the important categories of natural products that comprised of thousands of constituents distributed widely in fruits, vegetables and have been found to have promising chemopreventive potential (He et al., 1997; Mo and Elsonm, 2004). Geraniol is one of the important isoprenoids and main ingredient of oil of rose and palmosora also present as an active constituent in essential oil of lemon, ginger, rose, orange etc.

GOH has been shown to possess antioxidant (Tiwari and Kakkar, 2009) antinflammatory, anti-apoptotic and anticarcinogenic potential (Shoff et al., 1991). Previous findings reflect that GOH has potent anti-tumour activity against different types of malignancies in animal models (Cardozo et al., 2011; Yu et al., 1995).

Inflammatory responses play important role in multistage carcinogenesis, including initiation, promotion, malignant conversion, invasion and metastasis (Balkwill and Mantovani, 2002; Coussens and Werb, 2002). During inflammation, leucocytes and mast cell are recruited first to produce ROS and various mediators like chemokines and cytokines which further recruit inflammatory cells to produce ROS (Henricks and Nijkamp, 2001; Lin and Karin, 2007). Uncontrolled production of ROS or change in intracellular antioxidants level causes damage or modification of cellular macromolecules (Droge, 2002; Waris and Ahsan, 2006).

Aberrant production of inflammatory mediators like pro-inflammatrory cytokines TNF-α, IL-6, IL-1β and nitric oxide have critical role in roles in multistage cancer development, including initiation, promotion, malignant conversion, invasion and metastasis (Barker et al., 1991; Hong et al., 2000; Hussain and Harris, 2007).

Most of the tumour promoting agent can induce activation of NF-κB (Bowie and O'Neill, 2000). NF-κB, one of the most important ubiquitous redox sensitive transcription factor known to regulate the expression of genes involved in inflammation, cell proliferation and survival (Karin and Delhase, 2000). Although, various signalling cascade component have been found to involve in promotional

Abbreviations: BSA, bovine serum albumin; COX-2, cyclooxygenase-2; DTNB, 5, 5 -dithio bis-[2-nitrobenzoic acid]; FAD, flavin adenine dinucleotide; GSH, reduced glutathione; MDA, malondialdehyde; NF-kB, nuclear factor-kappaB; NO, nitric oxide; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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stage of cancer development but those that congregate with NF-κB has prominent intimacy with tumour promotion (Balkwill and Mantovani, 2002). One of the downstream targets of NF-κB is COX-2. Uncontrolled expression of COX-2 has been observed in different premalignant as well as in malignant stage (Williams et al., 1999).

TPA is the most widely used distinguished promoting agent to understand the cellular and molecular alterations associated with skin carcinogenesis (Nakadate, 1989) and a well-known model to understand the role of ROS, inflammation and hyperplasia in the promotion stage of carcinogenesis (Ha et al., 2006).

Taking this into account we postulated that GOH a natural compound having combined antioxidant and anti-inflammatory effects could be effective in preventing damage caused by topically applied TPA-induced early tumor promotional events in mouse skin. To test this possibility, we have studied the effects of GOH on the TPA-induced cutaneous oxidative stress and inflammation.

Material and methods

Chemicals and reagents

Ethylenediamine tetra acetic acid (EDTA), reduced glutathione (GSH), glutathione reductase (GR), oxidized glutathione (GSSG), 1-chloro 2,4-dinitrobenzene (CDNB), 5, 5 -Dithio-bis-[2-nitrobenzoic acid] (DTNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), geraniol (GOH), 12-*O*-tetradecanolyphorbal-13-acetate (TPA) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All the other reagents used were of highest purity and commercially available.

Animals

Eight week old female Swiss albino mice (25–30 g), free from infection, were obtained from the Central Animal House facility of Hamdard University, New Delhi, India. Animals were housed in a well-ventilated room at 25 °C under a 12 h light–dark cycle in polypropylene cages and have free access to standard laboratory feed (Hindustan Lever Ltd., Bombay, India) and water. Present study protocols were approved by the Institutional Animal Ethics Committee (IAEC) of the university and animals were undergone to experiment with the approved ethical guidelines.

Treatment schedule

To observe the effect of GOH on TPA induced cutaneous oxidative and inflammatory responses, animals were divided into four groups (I–IV) of six animal (n = 6) each. Dorsal skin of all the animals was shaved with the electric clipper two days prior to the start of the experiment. Only mice that showed no signs of hair re-growth were included in the experiments. All the treatments were done topically onto the shaved area of dorsal skin.

Group I — Animals of this group were given topical application of vehicle (0.2 ml acetone) only and served as control group.
Group II — Animals of this group were given topical application of TPA (10 nmol) in 0.2 ml acetone.
Group III — Animals of the groups III were given topical pretreatment of GOH at the dose of 250 μg in 0.2 ml acetone 30 min before TPA [(10 nmol) in 0.2 ml acetone)] application.
Group IV — Animals were given topical application of GOH (250 μg) in 0.2 ml acetone.

The treatments were carried out for 2 days at the interval of 24 h. We have performed the experiment once. Animals of the entire group were sacrificed by cervical dislocation 1 h after the last TPA treatment and skin tissue was processed for the evaluation of different parameters.

Tissue processing

Animals of all the groups were sacrificed by cervical dislocation 1 h after the last application and dorsal skin tissue was processed for sub-cellular fractionation. A piece of skin was preserved in 10% neutral buffered formalin for histological observation. 10 % homogenates were prepared in chilled phosphate buffer (0.1 M, pH 7.4) using Polytron homogenizer (Kinematica, Inc., Switzerland). The homogenized tissue was centrifuged at 10,500 *g* for 30 min at 4 °C to obtain the post mitochondrial supernatant (PMS).

Edema measurement

Effect of GOH on TPA induced Skin edema was assessed by the method of Afaq et al., 2005. Weight of skin punch (1 cm diameter, free from extraneous materials) was measured. After drying for 24 h at 50 °C, the skin punch was reweighed and loss of water content was determined. Increase in the mass of skin punch is directly proportional to the degree of inflammation. The extent of skin edema was determined by using difference in the water gain between control and treated groups.

Measurement of nitric oxide (NO)

Production of NO, after GOH and TPA application in the skin tissue, was evaluated by measuring the level of nitrite (an indicator of NO) in the tissue supernatant with the help of Griess reagent (Green et al., 1982). Briefly, 150 μl of tissue supernatant was mixed with 150 μl Griess reagent [0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% H_3PO_4]. After incubation at room temperature in the dark for 10 min, absorbance was measured with a microplate ELISA reader (Bio RAD, U.S.A.) at 540 nm.

Measurement of lipid peroxidation (LPO)

The assay for membrane lipid peroxidation was done following method of Wright et al., 1981, with slight modifications. Reaction mixture in a total of 3 ml containing 1 ml of (10% homogenate), 1.0 ml of TCA (10%), and 1.0 ml TBA (0.67%). Reaction mixture was incubated in boiling water for 45 min. After cooling the tubes were centrifuged at 2500 ×*g* for 10 min. Absorbance of the supernatant was taken at 532 nm. Rate of LPO was assessed by measuring malondialdehyde (MDA) content as nmol MDA formed/h/g tissue at 37 °C by using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Measurement of reduced glutathione (GSH)

GSH content of the skin was determined by the method of Jollow et al., 1974. Briefly 1 ml of PMS (10%) was mixed with 1.0 ml of sulphosalicylic acid (4%). The samples were incubated at 4 °C for 1 h and then centrifuge at 1200 ×*g* for 15 min at 4 °C. The assay mixture (3 ml) consists of 0.4 ml supernant, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml DTNB (4 mg/ml). The yellow colour developed was read immediately at 412 nm. The GSH concentration was calculated as nmol DTNB conjugate formed/g tissue.

Measurement of quinone reductase (QR) activity

The QR activity was determined by the method of Benson et al., 1980. The 3 ml reaction mixture consisted of 2.13 ml Tris–HCl buffer (25 mM, pH 7.4), 0.7 ml BSA, 0.1 ml FAD, 0.02 ml NADPH (0.1 mM), and 50 μl PMS (10%). The reduction of dichlorophenolindophenol (DCPIP) was recorded calorimetrically at 600 nm and the enzyme