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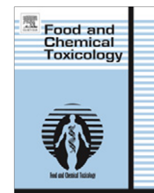

Caffeic acid attenuates 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced NF-κB and COX-2 expression in mouse skin: Abrogation of oxidative stress, inflammatory responses and proinflammatory cytokine production

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ABSTRACT

Polyphenols are the abundant micronutrient in our diet and attention has been given to them for the prevention of degenerative diseases. Since over production of ROS and proinflammatory cytokine are often act as the triggers for the promotion stage of carcinogenesis by transcriptional up-regulation of nuclear factor-κB (NF-κB) and cycloxygenage-2 (COX-2). We investigated the protective effects of caffeic acid (CA) on 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induced oxidative and inflammatory responses, expression of NF-κB and COX-2 in mouse skin. Animals were given pre-treatment of CA at two different doses 10 μmol (D1) and 20 μmol (D2)/0.2 ml of acetone 30 min prior to each TPA (10 nmol/0.2 ml of acetone) application. Our results show that CA significantly inhibit the TPA induced lipid peroxidation (LPO), inflammatory responses, tumor necrosis factor alpha (TNF-α) release and also found to up regulate GSH content and the activity of different antioxidant enzymes. Further, CA was found to inhibit the TPA induced expression of NF-κB and COX-2. Thus, our results suggest that CA attenuates TPA induced tumor promotional triggers possibly by inhibition of oxidative and inflammatory responses thereby diminishing the expression of NF-κB and COX-2.

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1. Introduction

In the present era, attention has been given to the use of naturally occurring compounds and their formulations for the treatment of different degenerative diseases. These effects are attributed to their antioxidant as well as anti-inflammatory potential. Moreover most of the plant based products are considered to be safe.

Epidemiological findings also revealed that use of natural compounds is a well promising approach for the chemoprevention and management of human cancers (Nakachi et al., 1996). Chemoprevention is a strategy of cancer control by administration of one or more naturally occurring and/or synthetic compounds to block initiation or suppress or delay promotion/progression of carcinogenesis (Surh, 2003).

Polyphenols represent one of the most diverse and widely distributed plant secondary metabolites including acids, flavonoids, lignins etc. Caffeic acid (CA) (3,4-dihydroxycinnamic acid), is one of the important phenolic acid present in medicinal plants, vegetables, beverages like wine, tea, coffee and apple juice (Shahidi and Naczk, 1995). It is also an active constituent of bee propolis (Grunberger et al., 1988). CA has been known to exhibit wide spectrum of positive biological effects such as antioxidant, anti-inflammatory, immunomodulatory, anti-HIV (Johnson et al., 2004), antitumor and anti-metastatic effects (Chung et al., 2004; Okutan et al., 2005; Nardini et al., 1995; Yamada et al., 2006).

Now, it is well established that oxidative stress and inflammation are the two concurrent conditions critically associated with etiology and progression of a number of human diseases. Reactive oxygen species (ROS) production is part of the inflammatory processes as activated inflammatory cells produce these kinds of free radicals to kill the pathogens in general. ROS production by activated neutrophil and macrophages leads to cancer promotion in

Abbreviations: CDNB, 1-chloro 2,4-dinitrobenzene; DTNB, 5,5′-dithio-bis [2-nitrobenzoic acid]; EDTA, ethylenediamine tetra acetic acid; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; CA, caffeic acid; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; D1, dose one (10 μmol); D2, dose two (20 μmol).

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epithelial cells is one of the modes by which inflammation triggers the way for tumor development (Pollard, 2004).

Oxidative stress results due to the imbalance between production of ROS and the antioxidant defense mechanism of the target cell or tissue (Sies, 1985). There is a growing awareness that oxidative stress or increased production of ROS has been associated with various clinically diagnosed conditions like malignancies, inflammation, diabetes and arthritis etc. (Apel and Hirt, 2004; Behrend et al., 2003; Witz, 1991).

Free radicals play an important role in the inflammation induced cancer development (Ballaz and Mulshine, 2003; Farrow and Evers, 2002; Halliwell, 2007; Seril et al., 2003). Cytokines, chemokines and eicosanoids are the inflammatory mediators known to have important role at promotion phase of carcinogenesis (Balkwill and Mantovani, 2002). Proinflammatory cytokine, TNF-α is the most important mediator of inflammation and a well known endogenous tumor promoter as previous findings show that mouse deficient in TNF-α have fewer skin tumors after dimethylbenz anthracene (DMBA) and TPA application (Moore et al., 1999; Scott et al., 2003).

Most of the tumor promoting agents and other stimuli like oxidative stress, TNF-α and lipopolysaccharides (LPS) 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 different cellular activities like inflammation, cell proliferation and survival (Karin and Delhase, 2000). Although different signalling cascade components have been found to involve in promotional stage of cancer development but those that congregate with NF-κB known to mediate inflammatory responses and has prominent intimacy with tumor promotion (Balkwill and Mantovani, 2002; Greten et al., 2004; Pikarsky et al., 2004).

One of the downstream targets of NF-κB is COX-2 which plays central role in the inflammation. Uncontrolled expression of COX-2 has been observed in different premalignant as well as in malignant stage (Williams et al., 1999; Mohan and Epstein, 2003). Cells or tissues exposed with inflammatory stimuli such as tumor promoters, cytokines, endotoxins and reactive oxygen shows transcriptional up-regulation of COX-2 (Prescott and Fitzpatrick, 2000; Aggarwal and Gehlot, 2009).

TPA is the most widely used distinguished promoting agent to understand the cellular and molecular alterations associated with promotion stage (Nakadate, 1989; Sharma and Sultana, 2004), and also a well-known model to understand the role of inflammation, generation of reactive oxygen species (ROS) and hyperplasia in cancer promotion (Ha et al., 2006).

Taking this into account, the present study was designed to investigate the preventive effects of CA on TPA induced promotional changes in mouse skin and to explore the underlying molecular mechanism. To test this possibility, we have studied the preventive effects of CA on the TPA induced activation of NF-κB and its downstream target COX-2 via cutaneous oxidative stress, inflammation and TNF-α production.

2. Experimental procedures

2.1. 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), horseradish peroxidase (HRP), xanthine, xanthine oxidase (XO), caffeic acid (CA) and 12-*O*-tetradecanoyl-phorbal-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.

2.2. 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 light–dark cycle in polypropylene cages and had free access to standard laboratory feed (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. Present study protocols were approved by the Animal Ethics Committee (IAEC) of the university that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India, and animals were undergone to experiment with the approved ethical guidelines.

2.3. Treatment schedule

To observe the effect of CA on TPA induced cutaneous cellular and molecular alterations, animals were divided into five groups (I–V) of six animal ($n = 6$) each. Dorsal skin of all the animals was shaved using surgical clipper (Oster Professional Products, McMinnville, TN, and USA) 2 days prior to the start of the experiment. Animals of group I received topical application of acetone only (0.2 ml/mouse) and served as negative control. Group II animals treated with the topical application of TPA (10 nmol/200 μl acetone) and served as positive control. Animals of the groups III and IV were given topical pretreatment of CA at the dose of 10 (D1) and 20 (D2) μmol/200 μl of acetone 30 min before TPA application respectively. Group V animals were given topical application of higher dose (D2) of CA. Same dose of TPA or acetone or test compound was given after 24 h.

2.4. Tissue processing

Animals of the entire group were sacrificed 1 h after the second TPA application by cervical dislocation and skin tissue was processed for sub cellular fractionation. A piece of skin was preserved in 10% neutral buffered formalin for histological observation. Ten percentage 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).

2.4.1. Edema measurement

Effect of CA on TPA induced skin edema was assessed by using 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.

2.4.2. Assay for myeloperoxidase activity

MPO activity in the skin was assayed by the method of Bradley et al. (1982). Skin tissue homogenate was prepared in 50 mM K_2HPO_4 buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide. Samples were centrifuged at 2500×*g* for 30 min at 4 °C immediately after three cycles of sonication and freezing–thawing. In 0.1 ml of supernatant MPO activity was measured, using 0.3 ml phosphate buffer (50 mM, pH 6.0) containing 0.167 mg/ml *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide as substrates, at 460 nm over 5 min. MPO activity was ex-

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