Research Article

CAFFEIC ACID PREVENTS LIPID PEROXIDATION AND ANTIOXIDANT STRESS IN CHRONIC UVB EXPOSED MICE SKIN

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Abstract

In this study, we analyzed antioxidants status of caffeic acid on chronic UVB exposed skin in Swiss albino mice. We found that the completion of chronic UVB exposure (210 days) mice skin shows increased lipid peroxidation and depletion of enzymatic and non-enzymatic antioxidants. Treatment of caffeic acid prevents lipid peroxidation and depletion of antioxidants in chronic UVB exposed mouse skin. Further caffeic inhibits the expressions of COX-2 and ODC in UVB exposed mice. Thus, prevention of chronic UVB induced COX-2 and ODC expressions counteracted by the free radical scavenging potential of caffeic acid.

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1. Introduction

Extensive exposure of human skin to solar ultraviolet (UV) radiation particularly, UVB (285-320) is a major environmental factor that induces serious of adverse effects on the structure and function of the skin (Chilampalli et al., 2011). Acute skin reactions induced by UVB exposure are erythema (skin reddening) or sunburn and long time severe exposure of UVB is the main cause of skin cancer, including cutaneous malignant melanoma (CM), nonmelanoma skin cancer (NMSC) (IARC, 2012). The incidence of both CM and NMSC has increased precipitously in fair-skinned populations over the past 50 years. Worldwide the highest incidence rates are by far those observed in Australia and New Zealand, where fair-skinned populations are exposed to intensive UV radiation (Erdmann et al., 2013; Lomas et al., 2012).

Skin possesses a variety of enzymatic or non-enzymatic as well as small molecular antioxidants that can inhibit oxidative damage from the exposure of UVB radiation (Nichols and Katiyar, 2010). Excessive ROS generation from UVB exposure can overwhelm the antioxidant defense capacity of the skin, implicating in oxidative stress consequently in photo damaging effects of the skin (Yuanqin et al., 2013). UVB-induced lipid peroxidation and depletion of antioxidant enzymes are important biomarker of oxidative stress in the skin. UVB induced lipid peroxidation have been linked to injurious effects such as inactivation of membrane enzymes, loss of fluidity, increased permeability to ions and eventually rupture of the cell membrane leading to release of cell organelles (Prasad et al., 2009).

Ornithine decarboxylase (ODC) is an important enzyme that catalyzes the cellular polyamine biosynthesis, i.e., the formation of putrescine from ornithine. It has been associated with cellular transformation as well as cell cycle
regulation (Afaq et al., 2003). Cyclooxygenase-2 (COX-2) is another rate limiting enzyme which is involved in synthesis of prostaglandin E2 (PGE2) from arachatic acid in the skin. The enhanced expression of ODC and COX-2 during the course of UVB exposure plays a key role in carcinogenesis by contributing to uncontrolled proliferation of damaged cells that ultimately form tumors (Burns et al., 2013).

Dietary supplementation with vitamins, minerals or essential fatty acids results in improved skin condition in animals; it has been reported that nutrients such as vitamin A, E, C, and herbal extracts which having polyphenols and flavanoids, protect skin against photodamage (Mallikarjuna et al., 2005). Polyphenol compounds are very promising molecules against UVB damaging effects, as their absorption spectrum can filter UV radiation and reduce penetration of radiations into skin, and subsequently decrease adverse effects (Nichols and Katiyar, 2010).

Caffeic acid (3,4-dihydroxycinnamic acid), a naturally occurring major hydroxycinnamic acid derivative, is an active phenolic component of propolis extract and is also found in a wide variety of vegetables and fruits (Chen et al., 1997; Chang et al., 2010 and Rajendra Prasad et al., 2011). It has biological and pharmacological properties that include antiviral, antioxidant, anti-inflammatory, anticarcinogenic, and immunomodulatory activity (Prasad et al., 2009). Recently, we reported that the caffeic acid inhibits short-term UVB induced oxidative damage and inflammatory signaling (Balupillai et al., 2015). In this present investigation we studied the effect of caffeic acid on chronic UVB radiation induced oxidative damage, COX-2 and ODC expressions in the skin of Swiss albino mice.

2. Materials and Methods

Chemicals

Caffeic acid, primary monoclonal antibodies for cyclooxygenase-2 (COX-2), ornithine decarboxylase (ODC), β-actin and secondary antibodies were obtained from Sigma, St. Louis, MO, USA. 1-chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitrophenylhydrazine, 5,50-dithiobis (2-nitro-benzoic-acid) (DTNB), glutathione reductase (GR), hydrogen peroxide (H2O2), nicotinamide adenine dinucleotide reduced (NADH), nicotinamide adenine dinucleotide phosphate reduced (NADPH), nitroblue tetrazolium (NBT), reduced glutathione (GSH), 2-thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Mumbai, India. All other reagents used were of analytical grade.

Experimental animals and drug preparation

Male Swiss albino mice aged six weeks weighing about 18 – 20 g were maintained at Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India. The mice were housed in well ventilated rooms (temperature 23 ± 2°C, humidity 65-70 % and 12 h light/dark cycle). All studies were carried out in accordance with the protocol (Reg No./160/1999/CPCEA) approved by the Institutional Ethical Committee, Annamalai University.

CA (15 mg per mouse) was dissolved in 0.1% DMSO and 200 µl volume applied for topical studies; for intraperitoneal administration, caffeic acid was prepared by dissolving in 0.1% DMSO and made up with sterile molecular grade water to obtain the final required concentration. Treatments of the entire six experimental groups were terminated at end of 30th week.

Experimental design

For this experiment, mice were divided into six experimental groups of ten mice each. Group I mouse received only vehicle (0.1% DMSO) and served as vehicle control. Group II, III mouse received topical application of caffeic acid (CAT), 15 mg/kg.b.wt, and intraperitoneal administration of 15 mg/kg.b.wt of caffeic acid (CAIP). Group IV mouse received 180 mJ/cm² of UVB irradiation and Group V, VI mouse received topical and intraperitoneal administration of caffeic acid followed by UVB irradiation. UVB irradiation was performed at the dose rate of 180 mJ/cm² exposure for weekly thrice. 1 hour before each UVB exposure mice were pretreated with caffeic acid (15 mg/kg b.wt.).

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UVB-irradiation

The dorsal portions of the mice hair were shaved using an electric shaver (Philips, India) followed by the application of hair removing cream (Veet, Bombay, India) at least two days before treatment. Mice that showed no symptoms of hair regrowth were used for photocarcinogenesis experiments. The dorsal portion of skin was exposed to UVB-radiation, Philips TL40W/12 RS lamp, emitting 312 nm. The lamp is exactly mounted 10 cm above the table where the mice placed on. Mice were then UVB-irradiated (180 mJ/cm²) and sacrificed as per the protocol described earlier (Ambothi et al., 2015). The full thickness of the dorsal skin was removed for further experiments.

Estimation of lipid peroxidation and antioxidant status

The levels of lipid peroxide markers of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide (LPH) in the mouse skin lysates were estimated by the methods of Niehaus and Samuelsson 1968, Jiang et al., 1992 respectively. The activities of enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxides (Gpx) were assayed in mouse skin lysates by the method of (Kakkar et al., 1984, Sinha, 1972 and Rotruck, 1973). The levels of non-enzymatic antioxidants such as reduced glutathione and vitamin C and vitamin E were estimated on the mouse skin by the method of Ellman (1959), Roe and Kuether (1943) and Baker et al. (1980) respectively.

Western blot analysis

Immunoblot analysis was carried out for COX-2 and ODC protein expressions in CA plus UVB-irradiated mice skin lysates. The results were normalized to β-actin expression. Following the protein estimation, the samples were separated using SDS-PAGE gel electrophoresis and the separated molecules were blotted in PVDF membrane as per the method described previously (Towbin et al.,1979)

Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) on SPSS (Statistical Package for Social Sciences) and the group means were compared to Duncan’s Multiple Range Test (DMRT). The results were considered statistically significant if the $P$ value < 0.05 levels.

3. Results

Effect of CA on chronic UVB radiation induced lipid peroxidation markers in the mouse skin

Lipid components in the membranes are highly susceptible to radiation damage. Lipid peroxidation in biological membranes is a free radical-mediated event. The level of lipid peroxidation in UVB-exposed and/or CA treated mouse skin was determined by analyzing TBARS and LPH (Fig. 1). We found that chronic UVB-exposure showed increased levels of TBARS and LHP in the mouse skin. Treatment of CAT and CAIP before chronic UVB-exposure resulted in significantly reduced lipid peroxidative markers in mouse skin.

Effect of CA on chronic UVB radiation induced enzymatic antioxidants in the mouse skin

To protect against oxidative damage, skin cells have evolved a complex of cellular antioxidant defense system which includes enzymatic antioxidants, such as SOD, Catalase and GPx. UVB-exposure due to its free radical generation capacity depletes the antioxidant defense capability of the skin. Figure. 2 demonstrated that chronic UVB-irradiation resulted in significant depletion of the SOD, Catalase and GPx activities in chronic UVB-exposed mouse skin. Treatment of CAT and CAIP prevents chronic UVB-induced depletion of enzymatic activities (SOD, CAT and GPx) in the mouse skin.

Effect of CA on chronic UVB radiation induced non-enzymatic antioxidant levels in the mouse skin

Intracellular GSH is considered to be a free radical-scavenger or a cofactor for protective enzymes, which plays a pivotal role in the cellular defense system against oxidative damage. Vit-C and Vit-E are well-known free radical scavengers and could prevent free radical mediated cellular changes.
Figure – 1: CA inhibits chronic UVB radiation induced lipid peroxidation in mouse skin. A) Thiobarbituric acid reactive substances (TBARS) B) Lipid hydroperoxide (LPH). Values are expressed as mmol per mg protein. Values are given as means ± SD of six experiments in each group. Values not sharing a common marking (*, ** and ***) differ significantly at P < 0.05 (Duncan’s multiple range test [DMRT]).

Figure – 2: Effect of CA on chronic UVB radiation induced activities of SOD, catalase and Gpx in mouse skin. The activities of SOD, catalase and Gpx were expressed as units per mg protein. Values are given as means ± SD of six experiments in each group. Values not sharing a common marking (*, ** and ***) differ significantly at P < 0.05 (Duncan’s multiple range test [DMRT]).
Figure – 3: Effect of CA on chronic UVB radiation induced activities of non enzymatic antioxidants in mouse skin. The activity of GSH was expressed as mmol per mg protein and Vit-C,E were expressed as units per mg protein. Values are given as means ± SD of six experiments in each group. Values not sharing a common marking (*, ** and ***) differ significantly at P < 0.05 (Duncan’s multiple range test [DMRT]).

Figure – 4: CA inhibits the expression of ODC and COX-2 in chronic UVB exposed mouse skin. Mice were euthanized at 24 h after completing 30 weeks of UVB irradiation, and skin lysates were prepared. (A) Immunoblotting analysis of ODC and COX-2 expression in the mouse skin lysates. The signal of β-Actin confirms equal loading of protein samples. (B) The quantification of expression intensity was performed by densitometric analysis using Image-studio software (LI COR, USA.). The densitometry data represent means ± SD from 3 immunoblots and are shown as relative density of protein bands normalized to β-Actin. Values not sharing a common marking (* and **) differ significantly at P < 0.05 (Duncan’s multiple range test [DMRT]).
Non-enzymatic antioxidants are important endogenous antioxidants, whose nucleophilic and reducing properties play a central role in metabolic pathways. Chronic UVB-irradiation (30 weeks) resulted in the reduction of intracellular GSH, Vit-C and Vit-E levels in mice skin (Fig. 3). Conversely, treatment of CAT and CAIP completely prevented UVB-irradiation-induced depletion of GSH, Vit-C and Vit-E levels in mouse skin.

Effect of CA on UVB induced COX-2 and ODC expressions in mouse skin

COX-2 is an important enzyme that is regarded to play critical role in inflammation and cancer development. ODC plays an important role in normal cellular proliferation, growth and development of tumors. Therefore, we studied the effect of CA on UVB-mediated modulation in COX-2 and ODC expressions (Fig. 4). Western blotting analysis shows that mouse exposed with UVB showed a significant increase in COX-2 and ODC expressions. Treatment with CAT and CAIP inhibits the expressions of COX-2 and ODC in chronic UVB exposed mouse skin.

4. Discussion

It is a well-known fact that oxidants are believed to have a crucial role in all the stages of cancer. A number of experiments reported that many modulators, which have the ability to prevent oxidative stress are also capable of inhibiting tumor promotion-related biochemical alterations (Rahman 2007). UVB irradiation is a consequence of the generation of ROS resulting in imbalance between oxidants and antioxidants, in favor to the former, shifts the cellular redox-sensitive cellular signal transduction pathways and gene expression (Yin et al., 2013). It has been reported that excessive generation of ROS resulting in oxidative stress and consequently depletion of antioxidant defense enzymes. The strategies targeted at counteracting ROS generation and antioxidant defense enzymes could be helpful for prevention of skin cancer development (Afaq et al., 2002). Lipid peroxidation of polyunsaturated fatty acids is a well-known marker of oxidative stress. Exposure of skin cells to UVB induces an immediate release of labile iron, which can catalyze production of the highly toxic •OH via the Fenton reaction (Kruszewski, 2003). •OH is the primary reactive oxygen species responsible for the formation of lipid radicals in the epidermis following exposure to UV light (Ogura et al., 1991). This lipid radicals damage the membrane integrity, membrane fluidity and membrane structure in the skin cells. In this study, caffeic prevents lipid peroxidative markers i.e TBARS and LPH in chronic UVB exposed tumor bearing mice skin (Fig. 1). The antioxidative potential of caffeic acid has been well documented in the literature (Prasad et al., 2010) and this might be the reason for the prevention of lipid peroxidation during chronic UVB exposure. Previously, caffeic acid inhibits lipid peroxidation during liver toxicity and diabetes mellitus (Jayanthi and Subash, 2010). This may be attributed to the antiradical activity of caffeic acid which inhibits lipid peroxidation and enhances the antioxidant defense against UVB induced oxidative damage in skin.

Enzymatic antioxidants (SOD, catalase and Gpx) and non-enzymatic antioxidants (GSH, Vit-C and Vit-E) are contributed to the homeostasis of oxygen radicals in the skin (Bouayed and Bohn, 2010). Superoxide dismutase belongs to major antioxidant enzymes; dismutation of superoxide by SOD results in the production of hydrogen peroxide, which is subsequently converted to H2O and O2 through a reaction that is catalyzed by Catalase (Flip et al., 2011). GPx is a selenoprotein that catalyses the conversion of UV-induced hydrogen peroxide into water and molecular oxygen using GSH as unique hydrogen donor (Black et al., 2008). The activities of SOD, catalase, Gpx were decreased following UVB exposure (Fig. 2). In contrast, caffeic acid treatment restores the activities of these enzymes in chronic UVB irradiated mice skin. Quercetin, a phytochemical has been documented that protects UVB induced oxidative damages in mouse skin (Yin et al., 2013). Recently, we reported ferulic acid, one of the derivatives of caffeic acid, prevents UVB induced depletion of enzymatic and non enzymatic antioxidants thereby prevented photocarcinogenesis (Ambothi et al., 2015)
GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body’s antioxidant defense process. GSH directly scavenges radicals by hydrogen transferring and acts as a cofactor for the enzyme glutathione peroxidase, this enzyme in turn scavenges peroxides and regenerates vitamins E and C (Carini et al., 2000). In this study, caffeic acid inhibits chronic UVB induced depletion of GSH, Vit-C and Vit-E in mouse skin. Previously, we reported that phytochemicals such as sesamol and ferulic acid has been effectively restores the activities of non-enzymatic antioxidants in chronic UVB radiation exposed mice skin (Ramachandran 2012; Ambothi et al., 2015).

Induction of ODC activity is considered to be an important biochemical marker of tumor promotion as it is associated with cell proliferation. COX-2 is a heme-containing enzyme that plays an important role in inflammation and cancer progression (Afaq et al., 2003). Experimental reports demonstrated that in human keratinocytes, COX-2 and ODC expression is up-regulated by UVB radiation and that the mechanism of activation probably involves the oxidative component of UVB radiation (Soriani et al., 1999). Our results also confirmed the chronic UVB exposure enhance expression of ODC and COX-2 in mouse skin. On the other hand caffeic acid inhibits the expressions of ODC and COX-2. Previously, ODC and COX-2 expression levels were down regulated by caffeic acid and its derivatives in different experimental models (Kang et al., 2009; Rossi et al., 2002).

5. Conclusion

Thus, in this study caffeic acid ameliorates chronic UVB radiation induced lipid peroxidation, antioxidants depletion, ODC and COX-2 expressions in mouse skin. This protection may be due to its antioxidative nature and sunscreen property. Hence, caffeic acid may be considered as potential photoprotective and sunscreen agent.

6. References

10) Casagrande R, Georgetti SR, Verri WA, Dorta DJ, dos Santos AC, Fonseca MJ. Protective effect of topical formulations containing...


31) Rajendra Prasad N, Karthikeyan A, Karthikeyan S and Reddy BV. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080
42) Zhao J, Wang J, Chen Y, Agarwal R. Anti tumor promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two stage-initiation promotion protocol and identification of procyanidin B 5-3_galactose as the most effective antioxidant constituent. Carcinogenesis. 1999, 20, 1737–1745.