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Inhibitory effect of garcinol against 12-O-tetradecanoylphorbol 13-acetate-induced skin inflammation and tumorigenesis in mice

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ABSTRACT

Garcinol, a polyisoprenylated benzophenone derivative, is one of the major constituents isolated from the *Garcinia indica* fruit rind. Previous studies have reported that garcinol exhibits many biological benefits, including anti-oxidative, anti-inflammatory, and anti-tumour activities both *in vitro* and *in vivo*. However, little is known about the garcinol-mediated protection from inflammation-associated skin tumorigenesis. The aim of this study is to evaluate the inhibitory effects of garcinol against 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced mouse skin inflammation and tumour promotion. Topical pre-treatment of mouse skin with garcinol significantly reduced TPA-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Pre-treatment with garcinol on mouse skin also suppressed the TPA-induced nuclear translocation of nuclear factor-κB (NF-κB) and its subsequent DNA binding by blocking phosphorylation of inhibitor κB α (IκBα) and the p65 subunit leading to the degradation of IκBα. Moreover, garcinol markedly reduced TPA-induced activation of extracellular signal-regulated kinases (ERK), c-Jun-N-terminal kinases (JNK), p38 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt, which are upstream of NF-κB. Finally, topical application of garcinol significantly attenuated 7, 12-dimethylbenz[α]anthracene (DMBA)/TPA-induced mouse skin tumour promotion by reducing tumour incidence and papilloma tumour size at 18 weeks following treatment. Based on these findings, our data suggest that garcinol may serve as a potent chemopreventive agent capable of inhibiting inflammation-associated skin tumorigenesis.

1. Introduction

Carcinogenesis is a complex and multistep process that comprises three mechanistic phases, including initiation, promotion and progression. Many studies have indicated that inflammation is a critical component of tumour progression as numerous malignancies initiate from sites of infection, irritation and inflammation. It is estimated that 15% of the global cancer burden, approximately 1.2 million cases per year, is attributed to chronic

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viral or bacterial infections (Pisani, Parkin, Munoz, & Ferlay, 1997). In response to tissue damage, inflammatory cells such as neutrophils, monocytes, macrophages, dendritic cells, mast cells and lymphocytes are recruited to the inflamed site and secrete growth and angiogenic factors, cytokines and matrix-degrading proteases (Mantovani, Allavena, Sica, & Balkwill, 2008; Pan, Lai, Dushenkov, & Ho, 2009). These proteins significantly facilitate tumour survival, growth, proliferation, and invasion. Various studies have indicated that neoplasms can be initiated from chronic inflammation such as asbestosis, bladder inflammation, inflammatory bowel disease, chronic pancreatitis and skin inflammation (Coussens & Werb, 2002).

According to the cellular origin, skin cancer is typically classified as melanoma skin cancer or non-melanoma skin cancer (basal cell carcinoma and squamous cell carcinoma); non-melanoma skin cancer is the most commonly occurring malignancy in the Caucasian population each year (Diepgen & Mahler, 2002). Epidemiologic data and clinical observations strongly suggest that non-melanoma skin cancer is mainly caused by cumulative exposure to solar radiation (Armstrong & Kricker, 2001; Leffell, 2000). Many animal studies mimicking human skin diseases, including rodent and non-rodent models, have been employed to investigate this issue. Experimental mouse skin carcinogenesis is one of the most well-established models to study multistage tumorigenesis. Chemically-induced mouse skin carcinogenesis comprises three tumour development stages, including initiation, promotion and progression (Schwarz, Munzel, & Braeuning, 2013). In the two-stage mouse skin tumour model, tumours are generally initiated by a single application of a carcinogen, most commonly 7, 12-dimethylbenz[a]anthracene (DMBA), followed by repetitive treatment of tumour promoters, typically phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Furstenberger, Berry, Sorg, & Marks, 1981). During the initial stage of skin carcinogenesis, application of DMBA activates mutations in Ras proto-oncogenes, the first known event of mouse skin carcinogenesis (Balmain & Brown, 1988; Brown, Buchmann, & Balmain, 1990). TPA is a tumour promoter that activates a series of protein kinase C iso-enzymes and induces chronic inflammatory responses by up-regulating expression of numerous genes such as inducible *nitric oxide synthase (iNOS)*, *cyclooxygenase-2 (COX-2)* and *ornithine decarboxylase (ODC)* (Cianchi et al., 2003; Einspahr et al., 2003; Verma, Shapas, Rice, & Boutwell, 1979). Activation of nuclear factor-κB (NF-κB) is a crucial mediator in cell transformation and tumour promotion, as well as an important modulator of tumour surveillance and rejection. In the two-stage mouse skin tumour model, the activation of NF-κB by TPA induces inflammatory gene expression (Chun et al., 2004). In addition, previous studies have indicated that the mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathways are involved in the TPA-mediated induction of iNOS and COX-2 by activation of transcription factors, including NF-κB and activator protein 1 (AP-1) (Cichocki et al., 2008; Kuo et al., 2012).

Chemoprevention is the use of natural agents or synthetic chemicals that inhibit, reverse or retard tumorigenesis (Hong & Sporn, 1997). Many naturally occurring dietary compounds have been demonstrated to exhibit chemopreventive effects via several mechanisms such as antioxidative activity, anti-inflammatory activity, induction of phase II enzymes, apoptosis, and cell cycle arrest (Pan & Ho, 2008; Surh, 2003). Garcinol is a polyisoprenylated benzophenone derivative mainly derived from the fruit rind of *Garcinia indica*. The extract from fruit of *G. indica*, known as Kokum, has traditionally been used as a garnish in Indian curries or steeped in syrup for refreshing drink preparation. The major chemical constituents of fruit extract of *G. indica* include garcinol, citric acid, hydroxycitric acid and oxalic acid. Previously we have demonstrated that oral administration of garcinol could suppress dimethylnitrosamine-induced liver fibrosis in rats and inhibit inflammation-associated colon carcinogenesis in mice (Hung et al., 2014; Tsai, Chiou, Chiou, Ho, & Pan, 2014). Many studies have indicated that garcinol has many biological health benefits, including antioxidative, anti-inflammatory and anti-cancer activities. Garcinol exhibited potent antioxidative activities by scavenging free radicals such as superoxide anions and hydroxyl, methyl and hydroxyl radicals (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000). Similar to a well-known antioxidant, curcumin, the structural features that confer garcinol with profound antioxidant capabilities are the phenolic hydroxyl group and β-ketone moieties. The anti-cancer activities of garcinol have been investigated in both *in vitro* and *in vivo* model systems. For example, garcinol induced apoptosis human leukaemia HL-60 cells to a greater extent than did curcumin through the release of cytochrome *c* and the activation of caspases (Pan, Chang, Lin-Shiau, Ho, & Lin, 2001). A recent study indicated that garcinol effectively induced G1 arrest in human lung cancer p53-null H1299 cells by up-regulation of $p21^{Waf/Cip1}$ triggered by p38-MAPK signalling inactivation (Yu et al., 2014). Ahmad et al. also found that garcinol greatly suppressed cell invasion in breast, prostate and pancreatic cancer cells by reducing signal transducer and activator of transcription-3 (STAT-3) expression (Yu et al., 2014). Administration of garcinol significantly inhibited breast and liver cancer cell growth through suppression of STAT-3 expression in mouse xenograft models (Sethi et al., 2014; Yu et al., 2014). Most importantly, a recent study documented that topical application of garcinol effectively suppressed 7, 12-dimethylbenz[*a*]anthracene (DMBA)-induced hamster cheek pouch carcinogenesis (Chen et al., 2012). However, the inhibitory effects of garcinol during skin carcinogenesis are not clear. The present study is designed to use two-stage skin carcinogenesis as an animal model to investigate the anti-inflammatory and chemopreventive effects of garcinol against skin tumorigenesis. The incidence and volume of tumours and the inflammation-related signalling pathways are also discussed.

2. Methods and materials

2.1. *Reagents and antibodies*

Garcinol was isolated from *G. indica* fruit rind. TPA and DMBA were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used were available commercially. Antibodies against iNOS, IκB-α, phospho-IκB-α (p-IκB-α), p50, p65, vascular endothelial growth factor (VEGF), MMP-9, phospho-PI3K (Tyr508) and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against cyclooxygenase-2 (COX-2) were purchased from BD Transduction Laboratories (Lexington, KY, USA). Phospho-p65 (Ser536),