ABSTRACT

Cutaneous overexposure to solar radiation, especially its ultraviolet (UVR) component can cause several skin related disorders, some of these include sunburns, immunosuppression and skin cancer. Changes in lifestyle together with depletion in the atmospheric ozone layer during the last decades have led to an increase in the skin cancer (both melanoma and non-melanoma). In Mexico, skin cancer ranks second in frequency among all malignancies, which constitutes 13.6%. Recently, considerable attention has been focused on identify natural products capable of inhibiting, retarding or reversing the multi-stage photocarcinogenesis. A wide array of phenolics substances have been reported to possess substantial antioxidant and anticarcinogenic properties. The photochemopreventive agents can act on molecular targets that modulate cellular processes such as inflammation, immunity, cell cycle progression, and apoptosis. This revision presents the main molecular mechanisms of various phytochemicals in the chemoprevention of skin cancer.

Key words: skin cancer, ultraviolet radiation, photochemoprevention, polyphenols, oxidative stress, signal transduction, immunosuppression

Abbreviations: ↓: down-regulation; ⊥: inhibition; ∼: modulation; ↑: up-regulation; (6-4): pyrimidin-pirimdona photo aducts; 6-Gin: 6-gingerol; 8-OHdG: 8-hydroxy 2´-deoxyguanosine; AP-1: activator protein-1; APC: antigen presenting cells; Apig: apigenin; BCC: basal cell cancer; Caff: caffeine; CHS: contact hypersensitivity; COX: cyclooxygenase; CPD: cyclobutane pyrimidine dimmer; Cur: curcumin; Cyt c: cytochrome c; Delp: delphinidin; DFC-DA: 2’,7’-dichlorofluorescin diacetate; ECGC: epigallocatechin gallate; Gen: genistein; GGR: global genomic repair; GSH: glutathione; GSP: grape seed proanthocyanidins; GTP: Green tea polyphenols; HEC: human epidermoid carcinoma; IL: interleukin; iNOS: inducible nitric oxide synthase; LPO: lipid peroxidation; MAPK: mitogen activated protein kinase; NER: nucleotide excision repair; NF-κB: nuclear factor kappa; NHEK: Normal human epidermal keratinocytes; ODC: ornithine decarboxylase; ON: nitric oxide; PCNA: proliferating cell nuclear antigen; PFE: Pomegranate fruit extract; Pro: proanthocyanidins; Resv: resveratrol; RHN: Registro histopatológico de neoplasias malignas; ROS: reactive oxygen species; Sang: sanguinarine; SCC: squamous cell cancer; Sil: sylimarin/silibinin; SPF: sun protection factor; TCR: transcription-coupled repair;
TPA: 12-O-tetradecanoylphorbol-13-acetate; UCA: urocanic acid; UVA: Ultraviolet radiation (320-400 nm); UVB: Ultraviolet radiation (280-320 nm); UVC: Ultraviolet radiation (200-280 nm); UVR: Ultraviolet radiation.

RESUMEN

La exposición excesiva a la radiación solar, especialmente la luz ultravioleta puede causar diversos padecimientos cutáneos, algunos de ellos incluyen las quemaduras solares, la inmunosupresión y el cáncer de piel. Durante las últimas décadas, cambios en el estilo de vida y la disminución en la capa de ozono han provocado el incremento en la incidencia del cáncer de piel melanómico y no melanómico. En México, el cáncer de piel ocupa el segundo lugar en incidencia con una prevalencia del 13.6% de acuerdo al Registro Histopatológico de Neoplasias Malignas. Recientemente, una gran cantidad de investigaciones se han enfocado en los productos naturales capaces de inhibir, retardar o revertir la fotocarcinogénesis. Se han propuesto una amplia variedad de compuestos fenólicos con propiedades antioxidantes y anti-mutagénicas como agentes quimioprotectores, los cuales pueden actuar en blancos moleculares que modulen diversos procesos celulares implicados en la inflamación, la inmunidad, el ciclo celular y la apoptosis. Esta revisión presenta los principales mecanismos moleculares de productos naturales en la quimioprevención del cáncer de piel.

Palabras clave: Cáncer de piel, radiación ultravioleta, fotoquimioprevención, polifenoles, estrés oxidativo, transducción de señales, inmunosupresión

Skin and UVR

In the last decades, several human activities such as the release to the environment of chlorofluorocarbon compounds have led to the diminution of the ozone layer. This has resulted in an increase in the amount of solar radiation reaching the earth’s surface. Ultraviolet radiation (UVR) from the sun is divided into UVC (200-290 nm), UVB (290-320 nm) and UVA (320-400 nm). The amount of UVR which crosses the atmosphere and reaching the earth is approximately 6% of the sun’s radiation. The radiation intensity depends on several factors like altitude, latitude and seasons of the year, hour of the day, cloudiness and light dispersion (Goettsch et al., 1998; De Grujl, 1999).

The skin is the body organ that is more exposed to UVR. Several studies in vitro and in vivo have demonstrated that UV-B causes damage to various molecules and cellular components, and produces alterations in cell functions. The DNA is the main target; its damage can lead to the skin cancer development (Marrot and Meunier, 2008). The harmful effects of UVR in the skin can be divided into acute (sunburn or erythema, phototoxic reactions, photoalergic, and photosensitivity) and chronic (photoaging, skin cancer and immunosuppression) (Matsumura and Ananthaswamy, 2004; Adhami et al., 2008).

There are experimental and epidemiologic evidences which have show the direct correlation between exposure to UVB with squamous cell cancer (SCC) basal cell cancer (BCC) and melanoma (Black et al., 1997, De Grujl, 1999, Armastrong and Kricker, 2001, Matsumura and Ananthaswamy, 2004). The skin cancer incidence has in-
increased so fast in the last decades that it has been considered as the silent epidemic of the XX century (Stratton et al., 2000). Cutaneous overexposure to solar radiation, especially its ultraviolet (UVR) component can cause several skin related disorders. In México, skin cancer ranks second in frequency among all malignancies, which constitutes 13.6% in the last report of the “Registro histopatológico de neoplasias malignas” (RHNM, 2001). The non-melanoma skin cancer comprising BCC and SCC, are the most frequently diagnosed cutaneous malignancies in the world now and its incidence is increasing. It is well know that they are associated with a low mortality rate, but they have a high cost in the health system. In addition, they produce a high range of disfigurement if the lesions are located in the head or neck. Melanoma is related to the frequency of severe sunburn in childhood. Melanoma can be surgery if it is detected in time, however, when it presents metastasis there is no cure (Stratton et al., 2000).

**UVR and DNA damage**

The genotoxic effect of UVR is mainly mediated by the absorption of photons by the DNA. The cyclic pyrimidine dimers (CPDs) and pyrimidin-piromidona (6-4) are the main DNA adducts induced by UVR (Hemminki et al., 2000; Marrot and Meunier, 2008). It also produces breaks in DNA, and DNA cross-linking of cross-linking DNA-protein (Pinnell, 2003; Afaq et al., 2005a; Melnikova and Ananthaswamy, 2005). The CPDs and 6-4 occur frequently in sequence with pyrimidines in tandem. Both injuries are potentially mutagenic if not repaired by the system of nucleotide excision repair (NER) (Black et al., 1997; Matsumura and Ananthaswamy, 2004). The mutations are in the form of transitions from C to T and CC to TT, which are characteristic of DNA damage induced by UVR (De Grujil, 1996; Marrot and Meunier, 2008). NER is a complex system involving more than 30 proteins (Ichihashi, et al., 2003). The mechanism of NER has great versatility in the ability to recognize and repair the alterations in DNA caused by UVR and other carcinogenic chemicals. 6-4 adducts are processed in about one hour after its formation, while the DCPs takes about fifteen hours to be repaired (Black, et al., 1997). It was established that also vary the speed of repairs. The genes active in the transcription process are repaired quickly through the system called the transcription-coupled repair (TCR), while other genes are processed by the system of global genomic repair (GGR). These mechanisms differ only in the process of recognition of the damage to the DNA (Matsumura and Ananthaswamy, 2004).

The p53 tumor suppressor gene plays a decisive role in protecting cell from DNA damage. The increased level of p53 protein after DNA damage is also associated with enhanced apoptosis, presumably in those cells that are too damage for adequate DNA repair. High doses of UVR have been show to be associated with the formation of sunburn cells, initiated by p53. On the other hand, p53 is also involved directly and indirectly in NER (Afaq et al., 2005a).

**UVR and oxidative stress**

The UVR generates ROS and induced oxidative stress in skin cells. Oxidative stress may cause damage at the cellular level, as well as at the molecular level, and this can result in cutaneous inflammation, lipid and protein oxidation, DNA damage, and activation or inactivation of certain enzymes, all of which could potentially contribute to UVB-induced photodamage of the skin. It has been estimated that there are approximately 35 DNA adducts in the presence of ROS, one of the key markers of oxidative DNA damage is 8-hydroxy-2-deoxy-guano-
sine (8-OHdG), which induce the change of guanine to thymine (G T) (Ichihashi et al., 2003). ROS are intracellular mediators that are implicated in signal transduction. ROS generation leads to the expression of specific genes involved in the development of pathological conditions such as immunossuppression and all stages of photocarcinogenesis in skin. Nuclear factor kappa (NF-kB) is a ubiquitously expressed transcription factor, regulates gene involved in inflammation, immunity, cell cycle progression, and apoptosis. NF-kB activation is subject to redox regulation. Activator protein-1 (AP-1) transcription factor is a protein dimer composed of proteins from the Fos and Jun families. AP-1 activation, which is also redox regulated, principally leads to cell proliferation and transformation. In addition, NF-κB and AP-1 are activated by UVR, either independently or coordinately regulate the expression of several target genes whose protein products are molecular markers of processes such as inflammation, immunossupression, and tumor transformation, such as cyclooxygenase (COX), nitric oxide synthase (NOS) and ornithine decarboxylase (ODC) (Afaq et al., 2005a).

UVR and immunossuppression

Epidermis keratinocytes with DNA damage UV induced, secrete interleukin 10 (IL-10), which produces an immunossuppressive microenvironment that alters the local and systemic immune response. Locally, on the site exposed to UVR, the contact hypersensitivity reaction (CHS) is reduced. Hypersensitivity to a large variety of antigens has been observed in systemic immune response. On the other hand, immunossupression may be a risk factor in the infections caused by microorganisms (Black et al., 1997; Ichihashi et al., 2003). Keratinocytes produced trans-urocanic acid (trans UCA), which participates in the differentiation of the stratum corneum and contributes to the homeostasis of the upper layers of skin maintaining the pH. In the skin, UVR induce isomerization of trans-UCA to the cis-UCA (Adhami et al., 2008). The cis-UCA has an immunossupressive effect by altering the activity of the antigen presenting cells (APC), possibly through the secretion of IL-10. In the skin exposed to UVR have seen a decrease in the number of APC, therefore does not start the immune response. Interleukin 12 (IL-12) can reverse the suppressive effect of IL-10 (Matsumura and Ananthasawamy, 2004). There is ample experimental and clinical evidence to suggest that immune factors contribute to photocarcinogenesis. Chronically immunosuppressed patients living in regions of intense solar radiation have an exceptionally high risk of skin cancer (Adhami et al., 2008).

Photocarcinogenesis

The development of skin cancer is a complex multistage phenomenon. Photocarcinogenesis involves several simultaneous and sequential events UVR induced that result in the development of skin cancer. The development of carcinoma depends on the time, the intensity and the wavelength at which the skin is exposed (Black et al., 1997). UVR is a complete carcinoen because it induces the three stages of carcinoenesis: initiation, promotion and progression The initiation is the first event in the process of photocarcinogenesis, is an indispensable and irreversible step in which the DNA damage altered gene expression of epidermal cells (Afaq et al., 2005a). Tumor promotion is the process that involves clonal expansion of initiated cells that produce the pre-malignat and malignat lesions, essentially by alterations in signal transduction pathways. The promotion of damaged cells to a state preneoplastic lasts about 10 years. Finally, the
progression of carcinoma in situ and the conversion of the lesion into an invasive and potentially metastatic malignant tumor occur in one year (Surh, 1999; Stratton et al., 2000; Matsumura and Ananthaswamy, 2004; Afaq et al., 2005a).

**Photoprotection**

Exposure to UVR has several adverse effects on the skin. Actions recommended to prevent overexposure to UVR include: avoiding exposure to the sun, wear clothing that protects the neck, arms and legs, using hats and sunglasses; the use of sunscreens and blockers with a sun protection factor (SPF) of 15 or higher; to teach children to protect themselves from the sun; deliberately avoid tanning in tanning beds and examine the skin regularly to detect the presence of early cancers (Agarwal and Mukhtar, 1996; Kullavanijaya and Lim, 2005). Sunscreens are chemicals that can absorb UV light efficiently, are applied topically and protected from the adverse effects of sunlight, mainly erythema. Most of the commercial formulations contain several active ingredients for a broad SPF of 280 to 400 nm (UVB and UVA). The application of sunscreen before exposure to UV radiation prevents sunburn, DNA damage and skin cancer (Pinnell, 2003). However, sunscreens can induce side effects such as irritation, allergy, phototoxic reactions, affect the synthesis of vitamin D, generate ROS and act as photosensitizers. These primary prevention approaches have had limited success. Therefore, additional efforts are needed to prevent skin cancer (Kullavanijaya and Lim, 2005).

**Photochemoprevention**

Several studies have evaluated the protective effect of natural products against damage induced by UVR in cells, tissues, animals and humans. Photochemoprevention is the use of synthetic or natural substances that prevent, delay or reverse the damage caused by UVR (Agarwal and Mukhtar, 1996; Surh, 1999; Stratton et al., 2000; Afaq et al., 2005a). Within this concept we can find a variety of polyphenolic compounds with antioxidant, anti-inflammatory, immunomodulatory and antimutagenic properties. In general, photochemopreventive agents act on two levels: a) prevention of the damage caused by UVR and b) modulation of different cellular responses to UVR that can prevent, stop or correct tumor promotion and progression (Afaq et al., 2005a; Adhami et al., 2008).

The photochemoprevention involves substances capable of absorbing the UV and act as filters, prevent DNA damage and immunosuppresion. In addition, polyphenolic compounds with antioxidant activities protect against photooxidative damage on DNA, lipids and proteins. On the other hand, exposure to UVR initiates a cascade of events that altered gene expression, which means the signal transduction involved in inflammation, apoptosis, immunosuppression, and cell proliferation. Photochemopreventive agents can act on molecular targets that modulate cellular processes such as the activation of the transcription factors as NF-κB, AP-1, and several kinases like mitogen-activated protein kinase (MAPK) (Afaq et al., 2005a).

In recent years, epidemiological and experimental studies have focused on a wide variety of natural products that provide protection to the development of skin cancer because it can alter or correct a variety of cellular functions induced by the UVR. The use of natural products as photochemopreventive agents can contribute in reducing the risk of skin cancer in combination with changes in lifestyle, diet and products for skin care. This revision presents the main molecular mechanisms of various phytochemicals in the chemoprevention of skin cancer (Fig 1 and Table 1).
Fig 1. Schematic representation of the fotocarcinogenesis in skin and molecular mechanisms of the photochemopreventive agents. ↑: up regulation; ↓: down-regulation; ⊥: inhibition; CPDs: cyclobutane pyrimidine dimmer; 8-OHdG: 8-hydroxy 2’-deoxyguanosine; ROS: reactive oxygen species; Cyt c: cytochrome c; IL: interleukin; CHS: contact hypersensitivity; NF-κB: nuclear factor kappa; AP-1: activator protein-1; MAPK: mitogen activated protein kinase; COX: cyclooxygenase; ODC: ornithine decarboxylase; PCNA: proliferating cell nuclear antigen; ECGC: epigallocatechin gallate; Resv: resveratrol; Cur: curcumin; 6-Gin: 6-gingerol; Sil: sylimarina/silibinin; Gen: genistein; Apig: apigenin; Delp: delphinidin; Caff: caffeine; Sang: sanguinarine; Pro: proanthocyanidins.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Structure</th>
<th>Model</th>
<th>Target/Mechanism</th>
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<td>EGCG</td>
<td>Green tea</td>
<td><img src="image" alt="Structure" /></td>
<td>Balb/c</td>
<td>↓ incidence, multiplicity, volume of tumors</td>
<td>Ahmad and Mukhtar, 1999</td>
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<td></td>
<td>Camellia sinensis</td>
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<td>In vitro</td>
<td>↓ LPO, ↓ Protein oxidation</td>
<td>Pinnell, 2003</td>
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<td>C3H/HeN</td>
<td>Restoration GSH level, ↓ ROS</td>
<td>Katiyar and Mukhtar, 2001</td>
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<td></td>
<td>↓ Infiltration CD11b+ cells</td>
<td>Afaq et al., 2005a</td>
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<td>of cell signaling pathways; ↓ NF-κB, ↓ AP-1</td>
<td>Matsumura and Anastawamy, 2004</td>
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<td>~ cytokine IL 10/1IL 12, ~ infiltration of cell signaling pathways;</td>
<td>Katiyar et al., 2007</td>
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<td>SKH-1</td>
<td>↓ Immunosuppression, ↓ Inflammation</td>
<td>Lu et al., 2000</td>
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<td>↑ p53, ↑ NER</td>
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<td>~ Cell cycle</td>
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<td>Resveratrol</td>
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<td><img src="image" alt="Structure" /></td>
<td>In vitro</td>
<td>Antioxidant, anti-inflammatory, anti-proliferative properties</td>
<td>Dong, 2003</td>
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<td>SKH-1</td>
<td>↓ Edema, ↓ H₂O₂, ↓ infiltration of leukocytes, ↓ ODC, ↓ COX</td>
<td>Afaq et al., 2003</td>
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<td>↓ NF-κB, ↓ AP-1, ↓ Caspases, ↓ Cytochrome c, ~ Apoptosis</td>
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<td>NHEK</td>
<td>↓ NF-κB</td>
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<td>Curcumin</td>
<td>Curcuma longa</td>
<td><img src="image" alt="Structure" /></td>
<td>CD-1 mice/TPA</td>
<td>↓ COX, ↓ ODC</td>
<td>Ishizaki et al., 1996</td>
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<td>HEC cell</td>
<td>↓ Caspases, ↓ Cytochrome c, ↓ oxidative stress, ~ Apoptosis</td>
<td>Oguro and Yoshida, 2001</td>
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<td>A431</td>
<td>↓ COX, ↓ AP-1, ↑ Caspases, ↓ Cytochrome c, ~ Apoptosis</td>
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<td>HaCat cell</td>
<td>↓ COX, ↓ AP-1, ↑ Caspases, ↓ Cytochrome c, ~ Apoptosis</td>
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<td>Gingerol</td>
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<td>HaCat cell</td>
<td>↓ ROS, ↓ Caspases, ↓ COX-2, ↓ NF-κB</td>
<td>Kim et al., 2007</td>
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<td>SENECAR</td>
<td>↓ COX-2</td>
<td>Agarwal et al., 1994</td>
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<td>SKH-1</td>
<td>↓ Number of tumors</td>
<td>Katiyar et al., 1997</td>
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<td>Compound/Plant</td>
<td>Cells/Tissues</td>
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<td>C3H/HeN</td>
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<td>Meeran et al., 2006</td>
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<td>Mohan et al., 2004</td>
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<td>A431</td>
<td>↓Apoptosis (post-treatment)</td>
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<td>Genistei Soy isoflavones</td>
<td>Tumor cells</td>
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<td>↓c-fos, ↓c-jun</td>
<td>Wang et al., 1998</td>
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<td>SENECAR Reconstituted skin</td>
<td>↓c-fos, ↓c-jun</td>
<td>Moore et al., 2006</td>
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<td>Abu-Yousit et al., 2008</td>
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<td>↑Cytochrome c</td>
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<td>Pomegranate fruit extract</td>
<td>NHEK</td>
<td>~MAPK, ~NF-κB</td>
<td>Afaq et al., 2005b</td>
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<td>Delphinidin Punica granatum</td>
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<td>Apoptosis (Bax, Bcl 2), ↓CPDs, ↓8-OHdG</td>
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Table 1. Molecular mechanisms of photochemopreventive agents. ↓: down-regulation; ↑: up-regulation; ~: modulation; ⊥: inhibition; NHEK: Normal human epidermal keratinocytes; HEC: Human epidermoid carcinoma; LPO: lipid peroxidation; NF-κB: nuclear factor kappa; AP-1: activator protein-1; IL: interleukin; NER: nucleotide excision repair; ODC: ornithine decarboxylase; COX: cyclooxygenase; ROS: reactive oxygen species; CPDs: cyclobutane pyrimidine dimer; iNOS: inducible nitric oxide synthasae; PCNA: proliferating cell nuclear antigen; 8-OHdG: 8-hydroxy 2’-deoxyguanosine; MAPK: mitogen activated protein kinase.
Epigallocatechin gallate (EGCG)

Epidemiological studies have shown that high consumption of green tea decreases the frequency of various types of malignancies, including skin cancer (Katiyar and Mukhtar, 1996). Green tea is a rich source of polyphenols (GTP), mainly of EGCG, as they constitute 30 to 35% of the dry weight of the leaf (Table 1). The GTP are mainly derived from catechin (Ahmad and Mukhtar, 1999). In studies with mice Balb/c it has been determined that both topical and oral administration of EGCG decrease the incidence, multiplicity and the volume of tumors induced by UV (Ahmad and Mukhtar, 1999; Katiyar et al., 2001). The application of EGCG induces partial regression of papillomas in mice. Furthermore, chronic administration showed no visible signs of toxicity. Several studies in experimental models in vitro and in vivo indicated that the GTP prevents photocarcinogenesis through several molecular mechanisms (Katiyar et al., 2007). EGCG acts as potent antioxidant and can scavenge ROS, as superoxide radicals, hydroxyl radicals, hydrogen peroxide and singlet oxygen (Pinnell, 2003; Adhami et al., 2008).

The topical application of EGCG decreases the production of H$_2$O$_2$ and nitric oxide (NO) as well as the expression of NOS in epidermis and dermis in mice C3H/HeN. EGCG also protect human skin from oxidative stress induced by UV and to restore the level of glutathione (GSH) and antioxidant enzymes in the skin. In the same way, the EGCG pretreatment inhibits UVR-induced infiltration of inflammatory leukocytes, particularly CD11b$^+$ cells (a surface marker of monocytes/macrophages and neutrophils), these cells are the main producers of ROS in the skin (Katiyar and Mukhtar, 2001). It has been demonstrated that oxidative stress caused by exposure to UVR, active transcription factors such as NF-κB and members of the complex AP-1, c-Fos and c-Jun. The GTP regulate signal transduction in human keratinocytes, inhibit the expression of c-fos, the activity of AP-1 and modulate MAPK signaling and NF-κB induced by UV (Afaq et al., 2005a; Matsumura and Ananthaswamy, 2004). EGCG produces a balance in the relationship between cytokines IL-10/IL-12. This can be mediated by antigen presenting cells in the skin and lymph nodes or to block the infiltration of CD11b$^+$ cells that secrete IL-10 (Afaq et al., 2005a; Katiyar et al., 2007). This suggests that the application of EGCG on the skin reduces inflammation and inhibits the signals involved in cellular processes such as inflammation, proliferation and cell transformation that play an important role in the development of skin cancer.

Cutaneous overexposure to UVR produces direct damage to DNA (CPDs). CPDs have been implicated in UVR induced immunosuppression and initiation of photocarcinogenesis. The p53 tumor suppressor gene plays a decisive role in protecting cell form DNA damage. The increased level of p53 protein after DNA damage is also associated with enhanced apoptosis. The oral administration of GTP in hairless mice SKH-1 increases the number of cells positive for p53 (Lu et al., 2000) and induces the DNA repair process (Katiyar et al. 2007). EGCG regulate cell cycle progression and induce apoptosis in p53-dependent transformed cells (Ichihashi et al., 2003).

Resveratrol (trans-3,4´,5-trihydroxystilbene)

Resveratrol is present in at least 72 species of plants and also in the red wine (Table 1). This compound possesses antiinflammatory, antiproliferative and antioxidant properties. It inhibits platelet aggregation and modulates the metabolism of lipids (Dong, 2003). Resveratrol is considered as a potential chemopreventive agent in various types of cancer, due to the impact of this compound in several cellular events related to the initiation, promotion and progres-
Vishion of tumors (Surh, 1999; Dong, 2003). Single topical application of resveratrol to SKH-1 hairless mice was found to result in significant inhibition of UVB mediated skin edema and generation of \( H_2O_2 \) as well as leukocyte infiltration in epidermis. The resveratrol treatment to mouse skin was also found to result in significant inhibition of UVB-mediated induction of COX enzyme and ODC activities and protein expression of ODC, which are well-established markers for tumor promotion. Resveratrol inhibits UVB-mediated increased level of lipid peroxidation, a marker of oxidative stress (Afaq et al., 2003). Pre-treatment of normal human epidermal keratinocytes (NHEK) with resveratrol inhibited UVR-mediated activation of NF-κB in dose and time dependent manner (Adhami et al., 2003).

Curcurmin

Curcurmin (diferuloylmethane) is a yellow odorless pigment isolates from the rhizome of turmeric (Curcuma longa L.) (Table 1). Curcurmin possesses anti-inflammatory, anti-tumoral and antioxidant properties (Ammon and Wahl, 1991). The molecular basis of anticarcinogenic and chemopreventive effects of curcurmin is attributed to its effect on several targets, including transcription factors, growth regulators, adhesion molecules, apoptotic genes, angiogenesis regulators and cellular signaling molecules (Aggarwal et al., 2003). It has been found that topical application of curcurmin in 12-O-tetradecanoylphorbol-13-acetate (TPA)-pretreated epidermis of CD-1 mice significantly inhibited UV-A induced ODC activity and ODC gene expression. The inhibitory effects of curcurmin were attributed to its ability to scavenge ROS (Ishizaki et al., 1996; Oguro and Yoshida, 2001). Curcurmin can prevent UV irradiation-induced apoptotic changes in human epidermoid carcinoma A431 cells, including mitochondrial release of cytochrome c, caspase-3 activation. Flow cytometric analysis using the cell permeable dye 2',7'-dichlorofluorescin diacetate (DCF-DA) as an indicator of ROS generation, revealed that the increase in intracellular oxidative stress caused by UV irradiation could be abolished by curcurmin (Chan et al., 2003). Treatment of curcurmin and UVR synergistically induced apoptotic cell death in HaCaT cells through activation of caspase 8, 3 and 9 followed by released of cytochrome c. Treatment with curcurmin strongly inhibited COX-2 mRNA and protein expression in UV-B irradiated HaCaT cell. Curcurmin inhibited UVB-induced AP-1 transcriptional activation in HaCaT cells (Cho et al., 2005).

[6]-Gingerol

Ginger (Zingiber officinale Roscoe) one of the most heavily consumed dietary substances in the world; have been shown to inhibit tumor promotion in mouse skin (Katiyar et al., 1996) (Table 1). The oleoresin from the root of ginger contains [6]-gingerol, the major pharmacologically active component. This compound has antibacterial, anti-inflammatory, analgesic and anti-tumor activity (Shukla and Singh, 2007). It has been found to possess potent antioxidant activity as determined by inhibition of phospholipids peroxidation induced by \( FeCl_3 \)-ascorbate system (Aeschbach et al., 1994). [6]-gingerol block EGF-induced cell transformation, inhibited EGF-induced AP-1 DNA binding activity in a concentration-dependent manner in JB6 cells (Bode et al., 2001). In vitro, pre-treatment with gingerol reduced UVB-induced intracellular reactive oxygen species levels, activation of caspase 3, 8,-9, and Fas expression. It also reduced UVR-induced expression and transactivation of COX-2. Translocation of NF-κB from cytosol to nucleus in HaCaT cells was inhibited by [6]-gingerol via suppression of IkBα phosphorylation. Topical
application gingerol prior to UVB irradiation of hairless mice SKH-1, also inhibited the induction of COX-2 mRNA and protein, as well as NF-κB translocation (Kim et al., 2007). These results suggest that 6-gingerol could be an effective therapeutic agent providing protection against UVB-induced skin disorders.

**Silymarin and Silibinin**

Silymarin is an extract of the milk thistle plant, *Silybum marianum* L. Silymarin consists of a mixture of 3 flavonoids found in the fruit, seeds, and leaves of the milk thistle plant: silibinin, silydianin, and silychristine (Table 1). Silibinin is the main component (70%-90%) and is thought to have the most biologic activity (Pinnell, 2003). Topical silymarin could almost completely inhibit the effect of TPA, a tumor promoter, from inducing ODC activity in SENCAR mice (Agarwal et al., 1994). Topical silymarin was demonstrated to have a remarkable antitumor effect, the number of tumors induced in the skin of hairless mice SKH-1 by UVB irradiation was reduced by 92% (Katiyar et al., 1997). Silymarin has strong antioxidant activity and prevented lipid peroxidation and scavenged ROS. The antioxidant and anticarcinogenic effects of silymarin in mouse models has been established, and silibinin has been shown to be the main constituent responsible for these effects. Treatment of irradiated HaCaT cells with silymarin resulted in concentration-dependent diminution of oxidative stress induced by UV-A. Silymarin application reduced GSH depletion, ROS production and lipid peroxidation in irradiated cells (Svobodová et al., 2007). In addition, silymarin inhibited UVB-induced sunburn cell formation and apoptosis. Topical silymarin prevented the formation of pyrimidine dimers after UVB exposure to hairless mouse skin. Silymarin reduced the UV-B induced enhancement of the levels of the immuno-supresor cytokine IL-10 in the skin, and increase de levels of IL-12 (Meeran et al., 2006). C3H/HeN mice were irradiated with or without topical treatment of silymarin. Treatment with the mix of flavonoides inhibited UV-induced oxidative stress through inhibition of infiltrating CD11b+ cells (Katiyar et al., 2008).

Topical or dietary silibinin treatment caused protection against photocarcinogenesis in terms of delay in tumor appearance, multiplicity, and volume. Silibinin decreased inducible iNOS and (COX-2) levels. Simultaneously, silibinin also decreased UVB-caused increase in cell proliferation and microvessel density. This compound inhibited UVB-caused phosphorylation and nuclear translocation of NF-κB DNA binding activity (Gu et al., 2007). Dietary feeding of silibinin to hairless mice SKH-1 for 2 weeks before irradiation with UV-B resulted in a strong decrease in UV-B-induced CPDs positive cells and proliferating cell nuclear antigen (PCNA). Silibinin enhances UVB-induced p53 as a possible mechanism to protect skin epidermal cells from DNA damage and inhibit proliferation and apoptosis or sunburn cell formation (Dhanalakshmi et al., 2004; Mallikarjuna et al., 2004). Irradiation of human epidermoid carcinoma A431 cells with UVB resulted in a dose- and time-dependent increase in apoptosis. Silibinin pre-treatment, resulted in an increase in UVB-induced apoptosis, but its post-treatment caused a decrease in UVB-induced apoptosis. A similar pattern in the activation of caspases 9, 3, and 7 was observed with silibinin treatment. Further, silibinin treatment prior to or immediately after UVB exposure altered Bcl-2, Bax, Bak, and cytochrome c levels in mitochondria and cytosol in favor of or against apoptosis, respectively. Silibinin treatment prior to UVB also increased the activation of mitogen/stress activated protein kinases Erk1/2, JNK, and p38 kinase as compared to its post-treatment (Mohan et al., 2004). Together, these results sug-
suggest that silibinin has multiple targets in the cell to prevent UVB-induced skin carcinogenesis.

**Soy isoflavones**

Soybeans and their associated food products are a rich source of flavonoids called isoflavones (Table 1). The most plentiful isoflavones in soy are genistein and daidzein. Genistein have potent anticarcinogenic effects that are largely independent of their estrogenic activities (Bingham et al., 1998). Genistein is a strong inhibitor of tyrosine protein kinases (TPK), which is necessary for regulation of cell division and topoisomerase II, and ribosomal S6 in the cell culture (Barnes and Peterson, 1995). Genistein is a potent antioxidant. Pretreatment of hairless mice with genistein prior to UV-B exposure inhibited UV-B induced H$_2$O$_2$, lipid peroxyl radicals and 8-OHdG in epidermis (Wei et al., 2002). Genistein inhibited in vitro UV-induced DNA oxidation (Wei et al., 1996). Genistein reduced erythema and histologic inflammation induced by PUVA in mouse skin. In addition, genistein inhibited UV-induced apoptotic changes, including caspase-3 and p21 activated kinase (AK) 2 activation in human epidermal carcinoma cells A431 (Chan and Yu, 2000). Topical administration of genistein before UV-B radiation reduced c-fos and c-jun expression in SENECAR mouse skin in dose dependent manner (Wang et al., 1998). In human reconstituted skin, genistein dose-dependently preserved cutaneous proliferation and repair mechanics as demonstrated by the preservation of proliferating cell populations with increasing genistein concentrations (Moore et al., 2006). In conclusion, genistein presents compelling photoprotective efficacy and potently minimizes the detrimental effects of UVB irradiation in skin. Its capacity for regulating these events is likely to be attributable to the scavenging of reactive oxygen species, the inhibition of oxidative and photodynamic damage to DNA.

**Apigenin (5,7,4’-trihydroxyflavone)**

Apigenin is a natural, plant flavonoid present in the leaves and stems of vascular plants, including fruits and vegetables (Table 1). It is relatively nontoxic and nonmutagenic in comparison with other flavonoids such as quercetin. Apigenin and other flavonoids are strong antioxidants which exert a number of effects on cell biology. Apigenin scavenge free radicals, which are involved in cell damage and tumor promotion. Apigenin treatment to murine skin resulted in inhibition of UV-B mediated induction of ODC activity as well as reduction in cancer incidence (Birt et al., 1997). Initial studies examined the effects of apigenin on the cell cycle. DNA flow cytometric analysis indicated that culturing cells for 24 h in medium containing apigenin induced a reversible G2/M arrest in two mouse skin derived cell lines, C50 and 308, as well as in human HL-60 cells (Lepley et al., 1996). Apigenin induced the level of p53 protein in mouse keratinocyte 308 cells. The half-life of p53 protein was found to be increased an average of 8-fold in apigenin-treated cells compared with vehicle-treated cells and increase the expression of p21/waf1. The mechanism of p53 protein stabilization and transactivational activity were by stimulating the p53-p21/waf1 response pathway (McVean et al., 2000).

Apigenin inhibits UV-induced COX-2 expression is through modulation of USF transcriptional activity in the 5’ upstream region of the COX-2 gene. Apigenin treatment also increased COX-2 mRNA stability. Two RNA-binding proteins, HuR and the T-cell-restricted intracellular antigen 1-related protein (TIAR) were associated with endogenous COX-2 mRNA in 308 keratinocytes, and apigenin treatment increased their localization to cell cytoplasm. Reduc-
tion of HuR levels by small interfering RNA inhibited apigenin-mediated stabilization of COX-2 mRNA. Cells expressing reduced TIAR showed marked resistance to apigenin’s ability to inhibit UVB-induced COX-2 expression. Taken together, these results indicate that in addition to transcriptional regulation, another mechanism by which apigenin prevents COX-2 expression is through mediating TIAR suppression of translation (Tong et al., 2007). Apigenin treatment enhanced UVB-induced apoptosis in HaCaT human keratinocyte cells, primary keratinocyte cultures isolated from human neonatal foreskin, and human organotypic keratinocyte cultures. When keratinocytes were exposed to UVB, apigenin treatment stimulated changes in Bax localization and increased the release of cytochrome c from the mitochondria compared with UVB exposure alone. Overexpression of the antiapoptotic protein Bcl-2 and expression of a dominant-negative form of Fas-associated death domain led to a reduction in the ability of apigenin to enhance UVB-induced apoptosis. These results suggest that enhancement of UVB-induced apoptosis by apigenin treatment involves both the intrinsic and extrinsic apoptotic pathways. The ability of apigenin to enhance UVB-induced apoptosis may explain, in part, the photochemopreventive effects (Abu-Yousif et al., 2008).

Delphinidin

Pomegranate fruit extract (PFE) derived from the tree Punica granatum L. possesses strong antioxidant and antiinflammatory properties (Table 1). PFE contains anthocyanins, ellagitannins and hydrolyzable tannins. PFE inhibits UVB-mediated phosphorylation of mitogen-activated protein kinase; activation of IKKα; degradation and phosphorylation of IκBα; and nuclear translocation and phosphorylation of NF-κB in normal human epidermal keratinocytes. Among the six anthocyanidins present in pomegranate fruit extract, delphinidin is the most abundant and is known to be present in many other pigmented fruits and vegetables like berries, dark grapes, egg plant, tomato, carrot, purple sweet potatoes, red cabbage, and red onion (Afaq et al., 2005b). The pretreatment of HaCaT cells with delphinidin protected against UV-B mediated decrease in cell viability and induction of apoptosis. Furthermore, pretreatment of HaCaT cells with delphinidin inhibited UV-B induced increase in lipid peroxidation; suppression of oxidative DNA damage (8-OHdG); decrease in PCNA expression; activation of caspases; increase in Bax; decrease in Bcl-2. Topical application of delphinidin to SKH-1 hairless mouse skin inhibited UVB-mediated apoptosis and markers of DNA damage such as CPDs and 8-OHdG. These results suggest that treatment of HaCaT cells and mouse skin with delphinidin inhibited UVB-mediated oxidative stress and reduced DNA damage, thereby protecting the cells from UVB-induced apoptosis (Afaq et al., 2007).

Miscellaneous

Caffeic acid and ferulic acid are two hydroxycinnamic acid largely present in plant and also in vegetable foods, such as olives and olive oil. Topical application of olive oil before and after UV-B exposure protects against UV-induced oxidative stress and the immunosuppressive effect (Ichihashi et al., 2003). Applied topically caffeic acid and ferulic acid protect against UV-B induced erythema in healthy human volunteers (Saija et al., 2000). Dihydrocaffeic acid, a dietary constituent and a microbial metabolite of flavonoids, is an antioxidant and reduces the cytotoxicity and pro-inflammatory cytokine production (IL-6 and IL-8) in HaCaT cells, following UV radiation (Poquet et al., 2008).

Studies have demonstrated that oral administration of caffeine to hairless mice
SKH-1 enhanced UV-induced p53-positive cells and apoptotic sunburn cells in epidermis (Lu et al., 2000). Caffeine decreases the size and thickness of the dermal fat layer under tumors and may decreases tumor multiplicity (Lu et al., 2001). Oral administration of a caffeine solution for 2 weeks enhanced UVB-induced increases in apoptosis in the epidermis, but these treatments had no effect in non-UVB treated normal epidermis. Topical applications of caffeine to mice previously treated with UVB for 20 weeks (high risk mice without tumors) inhibited the formation of tumors and stimulated apoptosis in the tumors but not in areas of the epidermis away from tumors. Caffeine has selective effect on apoptosis in DNA damaged tissues (Conney et al., 2007).

Sanguinarine isolated from Sanguinaria canadiensis, is a benzophenanthridine alkaloid (Table 1). It has been shown to possess antimicrobial, antioxidant, and antiproliferative properties. Recently, one study suggested that sanguinarine suppresses the growth and survival of human epidermoid carcinoma cells. In addition, this agent has been shown to inhibit the NF-κB (Chaturvedi et al., 1997) by induction of apoptosis pathway (Ahmad et al., 2000). Pretreatment of the HaCaT cells with sanguinarine caused a significant enhancement in the antiproliferative response of UVB. These responses on UVB and/or sanguinarine treatments were associated with decrease in Bcl-2 and Bcl-X and increase in Bax, Bid, and Bak protein levels. Furthermore, sanguinarine treatment was found to result in significant modulations in p53, and superoxide dismutase levels. The sanguinarine may protect skin cells from UVB-mediated damages via apoptotic elimination of damaged cells that escape programmed cell death (Reagan-Shaw et al., 2006). Topical application of sanguinarine in hairless mice SKH-1 resulted in a significant decrease in UVB-mediated increases in skin edema, skin hyperplasia and infiltration of leukocytes. Further, sanguinarine treatment also resulted in a significant decrease in UVB mediated generation of H₂O₂, and increases in the protein levels ODC and PCNA (Ahsan et al., 2007).

The seeds of the grape (Vitis vinifera) are particularly rich source of proanthocyanidins, and that compounds represent the major type of polyphenols in red wine. Grape seed proanthocyanidins (GSPs) have been shown to be potent antioxidants and free radical scavengers. Furthermore, GSPs have been shown to have anti-carcinogenic activity in different cancer models including skin cancer. Dietary supplementation with GSPs in SKH-1 hairless mice is associated with a decrease of UVB-induced skin tumor development in terms of tumor incidence, tumor multiplicity, and a decrease in the malignant transformation of papillomas to carcinomas (Mittal et al., 2003). The chemopreventive effects of dietary GSPs are mediated through the attenuation of UV-induced: oxidative stress; activation of MAPK and NF-κB signaling pathways (Mantena and Katiyar, 2006; Sharma et al., 2007) as well as immunosuppression through alterations in immunoregulatory cytokine (Sharma et al., 2006). Collectively, these studies indicate protective potential of GSPs against experimental photocarcinogenesis in SKH-1 hairless mice, and the possible mechanisms of action, suggest that dietary GSPs could be useful in the attenuation of the adverse UV-induced health effects in human skin (Katiyar, 2008).

CONCLUSION

The bulk of the research involving natural products and skin cancer has focused on the polyphenolics antioxidants. The general consensus was that antioxidants might play a role in modulating the initiation of the carcinogenesis process by protecting against DNA damage. Phytochemicals an-
Antioxidants have been proven to be remarkably efficient in protecting the skin against UVR-induced photooxidative damage, and this photoprotective effect has been associated with their ability to quench free radical formation. However, antioxidants have more complex mechanisms through which they may prevent or modulate the process of photocarcinogenesis. Emerging findings suggest a variety of potential mechanisms of action of polyphenols in cytoprotection against oxidative stress, which may be independent of conventional antioxidant-reducing activities. Such mechanisms might entail the interaction of polyphenols with cell signaling and influence gene expression, with the consequent modulation of specific enzymatic activities that drive the intracellular response against oxidative stress. The natural products constitute an important group of pharmacological agents capable of preventing the occurrence and reducing the severity of UVR induced skin diseases.

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