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BOTANICAL ANTIOXIDANTS FOR CHEMOPREVENTION OF PHOTOCARCINOGENESIS

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1. ABSTRACT

The incidence of non-melanoma skin cancer, consisting of basal- and squamous- cell carcinoma, continues to increase in the United States and elsewhere. Solar ultraviolet (UV) B radiation has been implicated as its main cause. This adverse effect of UVB has become a major human health concern. Therefore, development of novel strategies to reduce the occurrence of skin cancer is a highly desirable goal. Because UV radiation is known to cause excessive generation of reactive oxygen species (ROS) thereby resulting in an oxidative stress condition, the approaches aimed at counteracting ROS production may be useful for the prevention of skin cancer. One approach to reduce its occurrence is through 'Photochemoprotection', which we define as 'the use of agents capable of ameliorating the adverse effects of UVB on the skin'. Among many photochemoprotective agents, botanical antioxidants are showing promise. We propose that the use of botanical antioxidants, in combination with the use of sunscreens and educational efforts to avoid excessive sun exposure, may be an effective strategy for reduction of incidence of skin cancer and other UV-mediated damage in humans.

2. INTRODUCTION

Ultraviolet (UV) radiation in sunlight is the most prominent and ubiquitous physical carcinogen in our natural environment and is divided into three categories dependent on wavelength, short wave UVC (200-280 nm), mid wave UVB (280-320 nm) and long wave UVA (320-400 nm). UVC in solar radiation is effectively blocked by ozone layer of the Earth's atmosphere and therefore its role in human pathogenesis is minimal. UVB and, to a much lesser extent, UVA radiation are responsible for inducting various skin disorders including skin cancer (1-3). Because greater than 90% of the solar radiation at the earth's surface is UVA (320-400 nm), in recent years the role of UVA in skin carcinogenesis has begun to be appreciated. It has

become clear that UVA accounts for at least 10% of the carcinogenic dose of sunlight. UV irradiation to skin results in erythema, edema, hyperplasia, pigmentation, sunburn cell formation, immunosuppression, photaging and photocarcinogenesis (1, 4-7). UVB irradiation to skin has direct effects on biomolecules, for example the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photodimers (8), photoisomerization of trans- to cis-urocanic acid (9) and generation of reactive oxygen species (ROS) (7, 10-12). These effects of UVB may result in a variety of skin disorders including skin cancer. UVB damage may also be mediated through the production of molecular mediators of inflammation that may contribute to the development of immunosuppression (13,14). CPDs are located in keratinocytes and Langerhans cells following UVB exposure, and also in dendritic cells in lymph nodes draining the irradiated sites (15). The macrophages are $CD1a^{-}CD11b^{+}$ while Langerhans cells are $CD1a^{+}CD11b^{-}$ (16). They produce high levels of IL-10 and low levels of IL-12, and express different co-stimulatory molecules from Langerhans cells, thereby promoting suppressed immune responses in the skin.

The skin serves as a protective defense barrier from external environmental pollutants, including solar UV radiation. The major role of skin is to provide a protective covering at this crucial interface. Mammalian skin shows an impressive variety of passive and active protective features. The coordinated functions of multiple epidermal and dermal cell population allows the skin immune system to respond rapidly to a wide variety of insults occurring at the interface of the organism and its environment. These environmental factors may jeopardize the integrity of the skin's oxidizable structures that are critical for cellular homeostasis. UV exposure to the skin results in the generation of ROS, such as singlet oxygen, superoxide anion, peroxy radicals, and hydroxyl radicals that damage DNA, proteins, and lipids (17-21). UV-induced generation of ROS in the skin causes oxidative stress, when their formation exceeds the antioxidant defense ability of the cellular target system. The induction of oxidative stress, and subsequent imbalance of the antioxidant defense system, has been associated with the onset of several disease states including inflammation, premature skin aging, immunosuppression, and skin cancer. Although the skin possesses a complex and interlinked antioxidant defense system to protect itself from damage by UV-induced ROS, the capacity of this antioxidant defense system is not unlimited and it can be overwhelmed by excessive exposure to solar UV radiation (22).

UV radiation is the major cause for the vast majority of cutaneous malignancies diagnosed in the human population, more so in Caucasian individuals (23). UV radiation, particularly UVB (280-320 nm), is responsible for more than 1,000,000 cutaneous malignancies diagnosed each year in the USA alone, making it the most hazardous environmental carcinogen (23, 24). Thus, chronic exposure of UV to the skin is the leading cause of skin cancer the incidence of which is roughly equivalent to the annual incidence of all other malignancies combined (23, 24). Cutaneous malignancies are comprised mainly of non-melanoma basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) derived from keratinocytes of the epidermis. SCC is chronically more aggressive accounting for most of the non-melanoma skin cancer deaths (25). Melanoma, the malignant form of skin cancer, accounts for 1-3% of new cancer cases diagnosed in the USA, totaling ~47,700 new diagnosed cases and ~7,700 deaths in the year 2000 (24, 26). Recently, Godar et al. (27) have shown that the average erythemal UV doses of Americans are about 25,000 J/m^2/year, with 22,000 for females and 28,000 for males. This average may increase to about 33,000 J/m^2/year when a conservative, continental US vacation (about 8000 J/m^2/year) is included.

Previous experimental studies have shown that ROS can act as both initiator and promoter of tumors by damaging critical cellular macromolecules such as DNA, proteins, and lipids, and by acting as stimulator or inducer of cell-signaling molecules (28,29). Skin is easily accessible and constantly exposed to ROS-generating agents such as solar radiation, ozone and other environmental pollutants. It is well known that ROS are associated with skin cancers, premature skin aging, immunosuppression, and many other cutaneous inflammatory disorders, although causes and effects are not well known (30).

3. PHOTOCHEMOPREVENTION OF SKIN CANCER

For prevention of photodamage and skin cancer, education about the harmful effects of solar UV light, the need to avoid its excessive exposure, wearing protective clothing and the use of sunscreen has been emphasized. Sadly these effects are only partially effective. Therefore, additional efforts are needed to protect skin against the deleterious effect of UVB exposure. Because of the mortality and morbidity associated with skin cancer and other UV radiation-mediated cutaneous damage, concerted efforts are needed to design novel approaches for the prevention of UV responses. One such approach to ameliorate the occurrence of skin cancer is through chemoprevention, which by definition is a means of cancer control in which the occurrence of the disease can be entirely prevented, slowed, or reversed by topical or oral administration of naturally occurring agents (31). An expanded definition of cancer chemoprevention also includes the chemotherapy of precancerous lesions (31). For chemoprevention of photodamage, including photocarcinogenesis, we have coined the term 'photochemoprotection' (32).

4. PHOTOCHEMOPREVENTION BY BOTANICAL ANTIOXIDANTS

In recent years, naturally occurring compounds, especially the botanical antioxidants, present in the common diet and beverages consumed by the human population have gained considerable attention as chemopreventive agents against many cancers including skin cancers (32-35). Studies from this laboratory and

elsewhere have shown that many naturally occurring botanicals present in the human diet and beverages afford protection against the development of cutaneous malignancies (11, 36-38). Thus, a chemopreventive approach appears to have practical implications in reducing skin cancer risk as, unlike the carcinogenic environmental factors that are difficult to control, individuals can modify their dietary habits and lifestyle in combination with careful use of skin care products. Many studies from this laboratory and elsewhere have shown the efficacy of naturally occurring botanical antioxidants such as green tea polyphenol, silymarin, curcumin, apigenin and resveratrol against UV radiation-induced inflammation and cancer (11,39-44). In this review, we will discuss the photochemoprotective potential of some of the naturally occurring botanical antioxidants present in diet and beverages against photocarcinogenesis.

4.1. Green tea

Tea is obtained from the leaves and buds of the plant *Camellia sinensis* and is the most popular beverage consumed by more than two thirds of the world's population. Tealeaves are processed differentially and are available as black, green, and oolong tea. Of the total tea production, 78% is commercially available as black tea and is consumed in many Asian and Western countries (41). Green tea accounts for about 20% of the total tea production and is consumed in some Asian countries like Japan, China, Korea and India and in Middle East countries. The remaining 2% is manufactured as oolong tea and is consumed primarily in Southern china (41). Green tea polyphenols gained considerable attention in recent years due to their antimutagenic and anticarcinogenic potential (45-46). Green tea contains four major types of polyphenols: (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG). All of these polyphenols act as a potent antioxidant and can scavenge ROS, such as lipid free radicals, superoxide radical, hydroxyl radicals, hydrogen peroxide and singlet oxygen. However, their scavenging effect differs due to differences in their structure. EGCG is the major polyphenol in tea that is responsible for these effects (31, 47).

In several publications from this laboratory, it has been demonstrated that topical application or oral feeding of green tea polyphenols (GTP) protects immune functions and prevents photocarcinogenesis (48-54). Long-term oral feeding of GTP in mice exposed chronically to UV radiation resulted in lower tumor burden in these animals compared to their non-GTP-fed control (50). Oral feeding of GTP to SKH-1 hairless mice, followed by irradiation with UVB, resulted in significant protection against UVB radiation-mediated cutaneous edema; depletion of the antioxidant-defense system in the epidermis; induction of epidermal ornithine decarboxylase (ODC) and cycloxygenase (COX) enzymes activities that play an important role in cutaneous inflammation and tumor promotion (50, 55). Oral administration of green tea, black tea, and decaffeinated green and black teas inhibited the formation and size of malignant and nonmalignant tumors (56). Wang et al. (57) have shown that oral consumption of green tea to mice with established tumors resulted in a significant regression of these tumors. Oral feeding of GTP to SKH-1 hairless mice enhanced UVB-induced increases in epidermal wild type *p53*, *p21* and apoptotic sunburn cells in the epidermis (58). In a recent study, conducted by Lu et al. (38), it was shown that orally administered green tea, black tea, and caffeine decreased the size of the parametrial fat pad, and the thickness of the dermal fat layer both distant and directly under tumors. We have demonstrated that topical application of EGCG (3 mg/mouse/3m^2 of skin area) to C3H/HeN mice, before a single dose of UVB (90 mJ/cm^2) irradiation, decreases hydrogen peroxide and nitric oxide synthase-expressing cells and inhibits hydrogen peroxide and nitric oxide production both in the dermis and epidermis (53). Additionally, EGCG inhibits the migration, depletion, or death of antigen presenting cells when detected as class II MHC^+Ia^+ cells (53). Polyphenols extracted from both black and green teas were found to possess an anti-inflammatory effect in the mouse skin and inhibited epidermal lipid peroxidation (59). Recently, Kim et al (60) demonstrated that EGCG treatment to guinea pig inhibits lipid peroxidation and skin damage. These studies also suggested anti-photoaging properties of EGCG. Peus et al. (61) have shown that physiologic doses of UVB irradiation is involved in the activation and regulation of mitogen activated protein kinase (MAPK) signal transduction pathways. EGCG also inhibits UVB-induced release of intracellular hydrogen peroxide from normal human epidermal keratinocytes with concomitant inhibition in the phosphorylation of MAPK (40). Pretreatment of JB6 Cl 41 cells with tea polyphenol EGCG or theaflavins inhibits UVB-induced phosphatidylinositol 3-kinase activation (62).

The relevance of the extensive *in vitro* and *in vivo* laboratory data showing the protective effect of GTP against UV radiation-mediated damage to human skin has been considered. It was demonstrated that a topical application of GTP or EGCG protects against 4 MED (minimal erythema dose) of UVB radiation. This dose normally induces: (i) erythema (sunburn), (ii) an influx of inflammatory cells, (iii) prostaglandin synthesis, (iv) myeloperoxidase activity, (v) production of hydrogen peroxide and nitric oxide, both in the epidermis and dermis, (vi) IL-10 positive cells, (vii) depletion of Langerhans cells, (iii) alteration in endogenous antioxidant levels, and (ix) induction of lipid peroxidation (11,42, 63, 64). GTP has been shown to scavenge the ROS generated by UV irradiation and prevent single stand breaks in DNA (47,65). Topical application of GTP 30 min prior to UVB irradiation resulted in reduced production of cyclobutyl pyrimidine dimers (CPD) in the epidermis and dermis of human volunteers (66). This reduction in CPD formation is probably due to protection of the DNA repair enzyme from inactivation of ROS and by the absorption of UV energy by the antioxidant EGCG (λ_{max} 270-273 nm). The importance of preventing DNA damage extends beyond preventing mutation since production of CPD initiates UV-induced immune suppression that plays a critical role in photocarcinogenesis (67-69).

We have also examined the protective efficacy of GTP against UVB radiation-induced local, as well as

systemic, suppression of contact hypersensitivity and the edema response in C3H/HeN mice. Among the four major epicatechin derivatives known to be present in GTP, EGCG, a major constituent of green tea, was found to be most effective in affording protection against UVB-mediated contact hypersensitivity and inflammation (53). UV-induced suppression of cutaneous immunity is mediated in part through the cytokine IL-10, which is produced by macrophages infiltrating into U V-irradiated human (70,71) and murine (72) skin. The production of IL-10 by UV-irradiated skin or by highly immunogenic skin tumors is a major mechanism of escape from immune destruction. Thus, reduction of these cells and their products in UV-irradiated skin is expected to preserve cutaneous immunity. Topical application of EGCG to the skin of mice prior to UV irradiation, was found to preserve the contact hypersensitivity response of the animals and block IL-10 production in their skin and draining lymph nodes (52). The increased production of IL-12, which is regarded as mediator and adjuvant for induction of contact hypersensitivity, is increased in the draining lymph nodes, thus enhancing T helper cell type 1 functions. Alterations in the IL-10/IL-12 cytokine balance by EGCG may be mediated by the antigen presenting cells in the skin and draining lymph nodes or by blocking the infiltration of IL-10 secreting $CD11b^+$ macrophage into the UV-irradiated site. These data, in concert with other data, suggest that application of GTP to human skin reduces inflammation and inhibits formation of several mediators that are involved in immunosuppression that plays an important role in skin cancer development. In addition, these data suggest that certain green tea ingredients might be useful as photoprotectants in skin care products.

4.2. Silymarin

Silymarin, a flavonoid isolated from milk thistle plant *(Silybum marianu*m), is a mixture of different flavonolignans, which includes silybin, silidianin, silychristin and isosylibin (73,74). Silybin is the most biologically active component with regard to its antioxidant and anti-inflammatory properties (74). It is also known to be an antioxidant compound with skin cancer chemopreventive properties (75). In short-term experiments, the topical application of silymarin was found to result in significant inhibition against UVB-induced (i) skin edema, (ii) formation of sunburn and apoptotic cells, (iii) depletion of catalase activity, and (iv) induction of COX and ODC activities and ODC mRNA expression (30). This study suggested that silymarin might provide protection against different stages of UVB-induced carcinogenesis, possibly *via* its strong antioxidant properties. Indeed, it was shown that topical application of silymarin protects against UVB radiation-induced non-melanoma skin cancer in mice (30). In this study, SKH-1 hairless mice were subjected to (i) UVB-induced tumor initiation followed by TPA-mediated tumor promotion, (ii) DMBA-induced tumor initiation followed by UVB-mediated tumor promotion, and (iii) UVB-induced complete carcinogenesis. In all three protocols, topical application of silymarin prior to UVB irradiation/DMBA exposure, significantly reduced tumor incidence (% of mice with tumors), tumor multiplicity (number of tumors per mouse), and average tumor volume per mouse (30). With respect to the potential molecular basis of the photochemopreventive effects of silymarin, Chatterjee *et al*, employing ^{32}P post-labeling technique, demonstrated that topical application of silymarin or green tea polyphenols, as well as sunscreen containing ethylhexyl-p-methoxycinnamate, resulted in protection against UVB-mediated formation of CPD in mouse skin (76). Silymarin showed a protective effect against UV induced oxidative damage by modulating the activation of the transcription factors nuclear factor kappa B (NF-κB) in HaCaT keratinocytes (77). NF-κB, a redox sensitive transcriptional factor, plays an important role in regulating the expression of various genes that participate in many physiological processes such as inflammation, apoptosis, and cellular proliferation. Silymarin was found to inhibit NF-κB activation induced by UV radiation in a dose dependent manner in human keratinocytes (77).

4.3. Curcumin

Curcumin (diferuloylmethane), a yellow ingredient isolated from the rhizome of turmeric (*Curcuma longa*), has been extensively investigated for its cancer chemopreventive potential in many tumor model systems (44). Curcumin possesses anti-inflammatory and antioxidant properties (78-81). Studies have shown that it exhibits antimutagenic activity in the Ames Salmonella test and possesses anticarcinogenic activity. It inhibits chemically induced neoplastic lesions in many organs, including skin, probably *via* an antioxidant mechanism (82). Topical application of curcumin to mouse skin has been shown to enhance glutathione content and glutathione-S-transferase activity and inhibits lipid peroxidation and arachidonic acid metabolism in mouse skin (83,84). Further, topical application of curcumin has been shown to decrease the induction of ornithine decarboxylase in mouse skin (85). The antioxidant and anti-inflammatory properties of curcumin have been well documented (86,87).

Because of these properties, curcumin was evaluated for its photochemopreventive effects. Ishizaki *et al* demonstrated that UVA irradiation significantly enhanced ODC induction after topical application of TPA in the epidermis of CD-1 mice and aggravated TPA-mediated dermatitis (85). A pretreatment of skin with curcumin was found to significantly inhibit these UVA-enhancing effects (85). In another study, Iersel *et al.* demonstrated that curcumin was the most potent inhibitor of glutathione S-transferase, the major pi-class GST subunit P1 activity, towards 1-chloro-2, 4-dinitrobenzene in intact human IGR-39 melanoma cells (88). Jee *et al.* have shown that curcumin induces apoptosis in human basal cell carcinoma cells in a dose- and time-dependent manner where p53-associated signaling pathway is critically involved in curcumin-mediated apoptotic cell death (89). These studies suggest that curcumin may impart beneficial effect against the responses of ultraviolet radiation in skin and in *in vitro* models of skin cancer. Curcumin supplemented cosmetics are sold in many parts of the world especially in India. More studies are needed to examine the effect of curcumin on photodamage, including photocarcinogenesis.

4.4. Apigenin

Apigenin (5,7,4'-trihydroxyflavone) is a natural flavonoid present in the leaves and stems of vascular plants, including fruits and vegetables (90). Foods rich in apigenin include apples, endive, beans, broccoli, celery, cherries, cloves, grapes, leeks, onions, barley, parsley and tomatoes, while plant-derived beverages containing apigenin include tea and wine (91). Apigenin treatment was found to be effective in the prevention of UV-induced skin carcinogenesis in SKH-1 mice. When apigenin was applied to mouse skin, it was found to inhibit UV-mediated induction of ODC activity and resulted in reduction in tumor incidence and an increase in tumor free survival (43). With respect to mechanism of action, apigenin was found to affect (i) G1 cell-cycle arrest by inhibiting cdk2 kinase activity in human diploid fibroblasts, (ii) accumulation of the hypophosphorylated form of retinoblastoma protein, (iii) induction of the cdk inhibitor p21/WAF1, and (iv) stabilization of tumor suppressor gene *p53* (92). These studies suggest that apigenin may exert photoprotective action by stimulating the p53-p21/waf1 response pathway.

4.5. Resveratrol

Resveratrol (trans-3,4',5-trihydroxystilbene) is a polyphenolic phytoalexin found largely in the skin and seeds of grapes, but in many other plant species including peanuts and mulberries. Resveratrol is a potent antioxidant with ant-inflammatory, antiproliferative and anti-cancer properties (93, 94). Recently, we demonstrated that topical application of resveratrol (25 μmole/0.2ml acetone/mouse) to SKH-1 hairless mice resulted in significant inhibition of UVB-induced skin edema. As evaluated by histochemistry, pre-application of resveratrol caused a significant decrease in UVB-mediated generation of hydrogen peroxide and infiltration of leukocytes. In addition, topical application of resveratrol resulted in significant inhibition of UVB-mediated induction of cyclooxygenase and ornithine decarboxylase activities and protein expression of ornithine decarboxylase (42). Ornithine decarboxylase is thought to represent a marker of tumor promotion.

5. CONCLUSIONS

Data are accruing that consistently support the notion that many botanical agents, with antioxidant properties, exert anti-inflammatory and anti-carcinogenic effects in skin. This suggests the possibility that specific botanicals might be used for the prevention and treatment of a variety of human skin disorders. The use of skin care products supplemented with botanicals, in conjunction with the use of sunscreens and educational efforts, may be an effective approach for reducing UV-generated ROS-mediated photodamage, inflammatory responses, and skin cancer in humans. Based on this information, many health care products such as toothpastes, shampoos, depilatory creams, cleansing lotions, scented sprays, body lotions, bath and shower gels, and moisturizing lotions have been supplemented with herbal extracts. Because of the role of UVA and UVB in cutaneous damage, the agent(s) that can protect against these radiations could be ideal photochemoprotective agent(s) for the skin.

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