Review

Grape seed proanthocyanidines and skin cancer prevention: Inhibition of oxidative stress and protection of immune system

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Overexposure of the skin to UV radiation has a variety of adverse effects on human health, including the development of skin cancers. There is a need to develop nutrition-based efficient chemopreventive strategies. The proanthocyanidins present in grape seeds (*Vitis vinifera*) have been shown to have some biological effects, including prevention of photocarcinogenesis. The present communication discusses the *in vitro* and *in vivo* studies of the possible protective effect of grape seed proanthocyanidins (GSPs) and the molecular mechanism for these effects. In SKH-1 hairless mice, dietary supplementation with GSPs 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. It is suggested that the chemopreventive effects of dietary GSPs are mediated through the attenuation of UV-induced: (i) oxidative stress; (ii) activation of mitogen-activated protein kinases and nuclear factor-kappa B (NF-κB) signaling pathways; and (iii) immunosuppression through alterations in immunoregulatory cytokines. Collectively, these studies indicate protective potential of GSPs against experimental photocarcinogenesis in SKH-1 hairless mice, and the possible mechanisms of action of GSPs, and suggest that dietary GSPs could be useful in the attenuation of the adverse UV-induced health effects in human skin.

Keywords: Grape seed proanthocyanidins / Immunosuppression / Oxidative stress / Photocarcinogenesis / Ultraviolet radiation

Received: June 4, 2007; revised: September 14, 2007; accepted: September 17, 2007

1 Introduction

Proanthocyanidins are naturally occurring compounds that are widely available in fruits, vegetables, nuts, seeds, flowers, and bark. They are a class of phenolic compounds that take the form of oligomers or polymers of polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin [1]. The seeds of the grape (*Vitis vinifera*) are particularly rich source of proanthocyanidins, and the proanthocyanidins represent the major type of polyphenols in red wine. These grape seed proanthocyanidins (GSPs) are mainly dimers, trimers, and highly polymerized oligomers of monomeric catechins [2, 3]. GSPs have been shown to be potent antioxidants and free radical scavengers, being more effective than either ascorbic acid or vitamin E [4, 5]. Furthermore, GSPs have been shown to have anticarcinogenic activity in different cancer models [6, 7]. Feeding proanthocyanidins-rich extracts to rabbit significantly reduced severe atherosclerosis in the aorta [8], retarded the development of aortic atherosclerosis in male Watanabe heritable hyperlipidemic rabbits as demonstrated by significantly lower cholesterol content in the abdominal part [9]. Protective effects of grape seed extract against atherosclerotic lesions were also observed in hamsters [10], and cardioprotection in mice and hamster [11]. GSPs were subjected to a limited toxicity testing which included acute and subchronic toxicity in rats, and genotoxicity testing (comprising test for induction of gene mutation in bacteria, test for induction of chromosomal aberrations in mammalian cell *in vitro*, and mouse micronucleus test *in vivo*), the results of which indicate that these compounds are of a low toxicity

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Abbreviations: CHS, contact hypersensitivity; **GSH,** glutathione; **GSPs,** grape seed proanthocyanidins; **MAPK,** mitogen-activated protein kinase; **NF-κB,** nuclear factor-kappa B; **ROS,** reactive oxygen species

and have no genotoxic potential [1]. As there has been considerable interest in the use of botanicals for the prevention of various diseases, phytochemical compounds/phytochemical dietary supplements might be of interest as protective compounds for skin cancer.

2 Solar UV-spectrum, its effect on immune system and photocarcinogenesis

Skin represents the first defense barrier against external physical, chemical, and environmental pollutants, including solar UV radiation [12]. The solar UV-spectrum can be divided into UVC (<280 nm), UVB (280–320 nm), and UVA (320–400 nm) bands [13]. UVC component is almost completely absorbed by the atmospheric ozone layer and does not reach the earth's surface. The UVC wavelengths are mutagenic in nature. UVB spectrum constitutes approximately 5% of the total solar UV radiation and possesses suppressive effects on the immune system [14]. It can act as a tumor initiator [15], tumor promoter [16], and cocarcinogen [17, 18]. Exposure of the skin to UVB radiation induces a variety of biologic effects, including inflammation, sunburn cell formation, hyperpigmentation, immunologic alterations, and induction of oxidative stress, that altogether contribute to the development of the skin cancers [19–21]. UVA comprises the largest spectrum of solar UV radiation (90–95%). UVA can penetrate deeper into the dermis than UVB radiation. Exposure to UVA radiation induces the generation of singlet oxygen and hydroxyl free radicals, which can cause damage to cellular macromolecules, such as proteins, lipids, and DNA [22]. It can also suppress some immunologic functions [23].

UVB radiation has multiple effects on the immune system [24, 25] and there is ample clinical and experimental evidence to suggest that suppression of the immune system plays a role in solar UV-induced skin cancer [26, 27]. It has been noted that chronically immunosuppressed patients living in regions of intense sun exposure experience an exceptionally high rate of skin cancer [28]. The incidence of skin cancers, especially squamous cell carcinoma, is also increased among organ transplant recipients [29–31]. This increased frequency of squamous cell carcinoma in transplant patients is presumably attributable to long-term immunosuppressive therapy [32], however, nonimmune mechanisms may also have a role [33].

Photocarcinogenesis is a complex, multistep process. UV-induced tumor development generally is considered to consist of three distinct stages: (i) tumor initiation, which is associated with the genotoxic effects of UV light on normal cells; (ii) tumor promotion, which consists of clonal expansion of initiated cells and is considered to be reversible; and (iii) tumor progression, which consists of malignant transformation of papillomas to carcinomas through a process that appears to require additional genotoxic stimuli (Fig. 1).

3 Effects of GSPs on experimental photocarcinogenesis and possible molecular mechanisms of action

3.1 Experimental photocarcinogenesis

The chemical composition of the GSPs used in the author's laboratory (Gravinol, Kikkoman, Noda, Japan) is presented in Table 1. To evaluate the efficacy of GSPs in the prevention of photocarcinogenesis, GSPs were used to supplement the diet (AIN76A) of SKH-1 hairless mice ($n = 20$) at the levels of 0.2 and 0.5% w/w. Administration of the GSPs at dietary concentration of 0.2 and 0.5% w/w following exposure of the mice to a complete photocarcinogenesis protocol (initiation + promotion stages) resulted in a dose-dependent reduction in photocarcinogenesis when expressed in terms of percent of mice with tumors, tumor multiplicity (0.2%, $p < 0.05$; 0.5%, $p < 0.005$), or tumor size ($p < 0.001$) as compared to those in the control group (fed unsupplemented diet) [34].

The tumor promotion stage of carcinogenesis is reversible; therefore, it is most suitable for targeting to prevent, reverse, or slow the process of carcinogenesis. In the same study, the control group (not fed GSPs) exhibited 100% tumor incidence at week 15th of tumor promotion whereas the tumor incidence in mice fed as GSPs-supplemented diet (0.5% w/w) exhibited only a 60% tumor incidence at this time point.

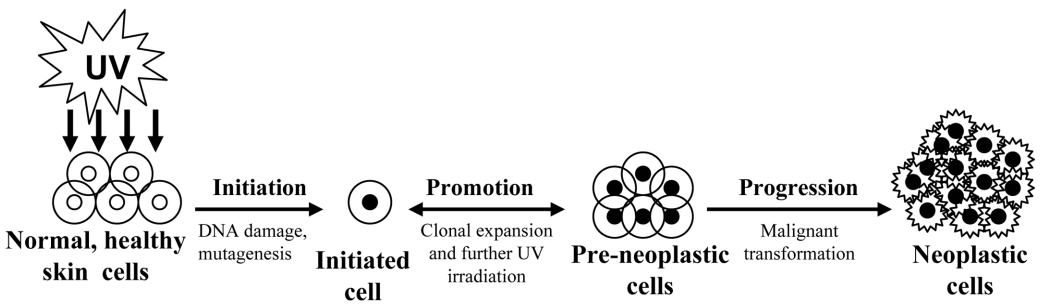

Figure 1. Schematic representation of UV radiation-induced multistage skin carcinogenesis. UV radiation acts as a tumor initiator, promoter, and complete carcinogen. Multiple UV exposures are required for tumor growth and progression.

Table 1. Chemical composition of GSPs used in author's laboratory

Components	% of total GSPs
Total proanthocyanidins	89.3
Dimers	6.6
Trimers	5.0
Tetramers	2.9
Oligomers	74.8
Total monomeric flavanols	6.6
(+)-Catechin	2.5
(−)-Epicatechin	2.2
(−)-Epigallocatechin	1.4
(−)-Epigallocatechin-3-gallate	0.5
Moisture	2.2
Protein	1.1
Ash	0.8

In that study the chemopreventive efficacy of GSPs also was determined in terms of their ability to prevent the malignant transformation of papillomas to carcinomas [34]. Histological observations indicated that papillomas had started to transform into carcinomas during the 21st week of the tumor promotion protocol. A total of 70% mice in the control group developed carcinomas, whereas only 25% of the mice in the GSPs-fed group (0.5%) developed carcinomas. A total of 18 papillomas had converted into carcinomas in the control group in comparison to only 7 in the group fed 0.5% GSPs in the diet. These data indicated that dietary GSPs may have the ability to inhibit UVB-induced transformation of benign papillomas to carcinomas.

3.2 UV-induced oxidative stress

Wavelengths in the UVA (320–400 nm) and UVB (280–320 nm) region of the solar spectrum are absorbed by the skin and produce oxidative stress that contributes to the development of skin cancer [35, 36]. Acute UVB (120 mJ/cm^2) exposure as well as repeated UVB exposure (1 month on alternate days) resulted in a reduction in the levels of reduced glutathione (GSH), GSH peroxidase (GPx), and catalase in the exposed skin as compared to the levels in the skin of control mice that were not exposed to UVB irradiation [37]. The depletion in the levels of GSH, GPx, and catalase in response to acute and repeated UV irradiation was reduced significantly on provision of 0.2 and 0.5% w/w GSPs in the diet. Catalase has a role in the catalytic conversion of H_2O_2 to oxygen and water and thus contributes to reductions in the levels of oxidative stress. It would be expected that the prevention of the UV-induced depletion of the antioxidant defense system would result in suppression of oxidative stress and the oxidative stress-mediated adverse effects in the skin. 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 [35, 38–40], all of which could potentially contribute to UVB-induced photodamage of the skin.

Under the above-mentioned experimental conditions, acute or repeated exposure of the mouse skin to UVB resulted in a three- to five-fold enhancement of the intracellular release of reactive oxygen species (ROS), including H_2O_2, in the skin compared to the non-UVB-exposed control mice. Administration of dietary GSPs (0.5% w/w) significantly decreased the UVB-induced intracellular release of ROS in both acutely ($p < 0.01$) or repeatedly ($p < 0.005$) UVB-exposed mouse skin [37]. Oxidation of some amino acid residues of proteins leads to the formation of carbonyl derivatives that affects the nature and function of the proteins [41]; thus, the analysis of carbonyl groups has been used as a measure of oxidative damage of proteins under conditions of oxidative stress [42]. It was observed that acute and repeated UV exposure of the mouse skin resulted in a multifold increase in the levels of protein carbonyls as compared to the levels observed in the skin of non-UV-exposed control mice. The enhancement of protein carbonyl formation or protein oxidation after UV irradiation of the skin was reduced on provision of GSPs in the diet. Similarly, GSPs significantly decreased UVB-induced production of nitric oxide ($p < 0.01–0.005$) in the skin under conditions of both acute and repeated UVB exposures [37].

Mitogen-activated protein kinases (MAPKs) are important upstream regulators of transcription factor activities that control cellular proliferation, differentiation, and apoptosis in response to external signals or stimuli [43]. UV-induced oxidative stress has been implicated in the activation of MAPK proteins. It was observed that UVB-induced phosphorylation of proteins of the MAPK family, such as ERK1/2, JNK, and p38, in mouse skin was decreased by the dietary GSPs. These *in vivo* observations suggest that dietary GSPs may prove helpful in ameliorating the harmful effects caused by UV exposure through decreased ROS production. Similar results were obtained when the photoprotective effects of GSPs were examined in terms of UV-induced oxidative stress in normal human epidermal keratinocytes *in vitro* [44]. Treatment of human epidermal keratinocytes with GSPs decreased UV-induced oxidative stress-mediated phosphorylation of the proteins of the MAPK family and activation of the transcription factor, nuclear factor-kappa B (NF-κB). There is evidence that NF-κB regulates a wide variety of genes that encode proteins that are involved in inflammation and carcinogenesis [45–47]. Activation of NF-κB can upregulate the expression of proinflammatory cytokines and inflammatory gene products, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) [46, 47]. Most of these genes have been shown to be upregulated in human cancers, suggesting that inhibition of NF-κB, and subsequently NF-κB-targeted genes, might decrease the development of cancers including skin cancer. The decreasing expression of these NF-κB-targeted genes in UV-exposed mice by GSPs [37]

may explain the antiproliferative, antioxidative, and anti-carcinogenic effects of GSPs.

3.3 UV-induced suppression of immune reactions and possible mechanisms of action

As UV-induced immunosuppression is considered to be a risk factor for the development of skin cancer [24, 27], prevention of UV-induced immunosuppression represents a potential strategy for the management of skin cancer. When the effect of dietary GSPs on UV-induced immunosuppression was examined using a mouse contact hypersensitivity (CHS) model, in which the contact sensitizer, 2,4-dinitrofluorbenzene is applied topically at the UV-exposed skin, the exposure of C3H/HeN mice resulted in suppression of the CHS response. The provision of GSPs in the diet of control mice that were not UVB irradiated did not affect their ability to generate a local CHS response to 2,4-dinitrofluorobenzene [48]. UVB irradiation of mice that did not receive GSPs resulted in a significantly lower (75% suppression, $p < 0.001$) local CHS response than that observed in the mice that did not receive GSPs but were UV irradiated, confirming the immunosuppressive effect of the UVB irradiation. In contrast, the mice that received either 0.5 or 1.0% GSPs w/w in the diet exhibited a significant reduction in UVB-induced suppression of the local CHS response (73 and 89%; $p < 0.001$, respectively). Similar effects of GSPs were also observed in systemic model of CHS where mice were sensitized with 2,4-dinitrofluorobenzene on a skin site distant from the site that is exposed to UV radiation [48]. These data indicate that dietary GSPs are capable of protecting mice from UVB-induced immunosuppression in a local as well as systemic CHS model of immunosuppression. Several mechanisms have been proposed in UV-induced immune suppression. There are studies that implicate the immunoregulatory cytokine, IL-12, in the induction and elicitation of CHS, and CHS is considered to be a Th1-mediated immune response [49]. Langerhans cells, which are critical antigen presenting cells in the induction phase of CHS [50] have been described as an additional source of IL-12. On the other hand, IL-10 possesses immunosuppressive activity and inhibits antigen presentation in *in vitro* and *in vivo* systems [51, 52]. Dietary administration of GSPs to C3H/HeN mice resulted in a reduced level of IL-10 in the UV-irradiated skin, as well as in the draining lymph nodes, as compared to the controls that were not treated with GSPs. These *in vivo* effects of GSPs suggest a possible mechanism by which dietary GSPs decrease UVB-induced immune suppression in mice [48].

IL-12 regulates the growth and functions of T-cells [53] and especially augments the development of Th1 type cells by stimulating the production of IFN-γ [54 – 56]. Intraperitoneal (i.p.) injection of recombinant IL-12 in mice prevents UV-induced immune suppression [57]. The provision of dietary GSPs resulted in higher levels of IL-12 in the skin and draining lymph nodes of UVB-exposed C3H/HeN mice than those observed in UVB-exposed mice that did not receive GSPs [48]. These higher levels of IL-12 may contribute to stimulation of the immune responses. To further confirm the role of IL-12 in GSPs-mediated prevention of UV-induced immunosuppression, C3H/HeN mice were treated i.p. with an anti-IL-12 mAb. In GSPs-treated mice, the i.p. injection of the anti-IL-12 antibody significantly reversed or blocked the preventive effect of GSPs on UV-induced suppression of CHS [48]. These studies provide convincing evidence that prevention of UV-induced suppression of CHS by GSPs is mediated, at least in part, through IL-12 induction, and that the protection from UVB-induced immunosuppression afforded by dietary GSPs may be associated with the protection from UVB-induced photocarcinogenesis in mice.

4 Conclusions

The *in vitro* and *in vivo* experimental data presented indicate a protective effect of GSPs against experimental photocarcinogenesis and suggest possible mechanisms of action. As GSPs are not genotoxic and posses low toxicity as indicated by some *in vitro* tests and *in vivo* animal toxicity studies reviewed elsewhere, the GSPs may be of interest for attenuation of the adverse UV-induced effects on human skin. However, further studies are needed to elucidate the possible efficacy of GSPs in skin cancer prevention.

The work reported from the author's laboratory was supported from the funds from National Cancer Institute/NIH (CA104428) and Veterans Affairs Merit Review Award. The content of this publication does not necessarily reflect the views or policies of the funding sources. Grateful thanks are due to our former and current postdoctoral fellows for their outstanding contributions.

The author has declared no conflict of interest.

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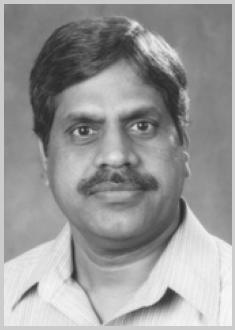

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