Topical application of green and white tea extracts provides protection from solar-simulated ultraviolet light in human skin

Melissa M. Camouse1, Diana Santo Domingo1, Freddie R. Swain1, Edward P. Conrad1, Mary S. Matsui2, Daniel Maes2, Lieve Declercq3, Kevin D. Cooper1,4, Seth R. Stevens1,4 and Elma D. Baron1,4

1Department of Dermatology, University Hospitals Case Medical Center Cleveland, OH, USA; 2Estee Lauder Companies, Melville, NY, USA; 3Estee Lauder Companies, Oevel, Belgium; 4Department of Dermatology, Cleveland Veterans Affairs Medical Center, Cleveland, OH, USA

Correspondence: Elma D. Baron, MD, University Hospitals Case Medical Center, 11100 Euclid Ave., Lakeside 3500, Cleveland, OH 44106, USA, Tel.: 2163684971, Fax: 2163680212, e-mail: elma.baron@uhhospitals.org

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Background: Tea polyphenols have been found to exert beneficial effects on the skin via their antioxidant properties.

Aims: We sought to determine whether topical application of green tea or white tea extracts would prevent simulated solar radiation-induced oxidative damages to DNA and Langerhans cells that may lead to immune suppression and carcinogenesis.

Methods: Skin samples were analysed from volunteers or skin explants treated with white tea or green tea after UV irradiation. In another group of patients, the in vivo immune protective effects of green and white tea were evaluated using contact hypersensitivity to dinitrochlorobenzene.

Results: Topical application of green and white tea offered protection against detrimental effects of UV on cutaneous immunity. Such protection is not because of direct UV absorption or sunscreen effects as both products showed a sun protection factor of 1. There was no significant difference in the levels of protection afforded by the two agents. Hence, both green tea and white tea are potential photoprotective agents that may be used in conjunction with established methods of sun protection.

Key words: contact hypersensitivity – (-)-epigallocatechin-3-gallate – green tea – Langerhans cells – photoprotection – polyphenols – white tea

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Introduction

Tea is one of the most widely consumed beverages in the world and has been heralded for its antioxidant and anti-cancer properties. Green tea has received much interest because of the beneficial role polyphenols play in skin cancer prevention (1,2,3). Its benefits against UV-induced effects were first demonstrated in human skin by Elmets and co-workers (4). White tea is the least processed of the teas and may retain higher levels of polyphenols. In one study, white tea proved more effective than green tea in preventing intestinal tumorigenesis (5). However, there are no published data on white tea for the prevention of detrimental ultraviolet radiation (UVR) effects on the skin. Multiple mechanisms contribute to UVR-induced carcinogenesis, including direct DNA damage and indirect damage secondary to reactive oxygen species (ROS) (6). UVR also induces cutaneous immunosuppression, potentially allowing dysplastic cells to go undetected and progress to neoplasms (7). The overall goal of this study was to compare, in humans, the protective effects of topical white tea or green tea against markers of UVR damage that are associated with immune suppression and carcinogenesis. This was accomplished by performing: (i) immunohistochemical analysis for oxidative DNA damage and for epidermal Langerhans cells (LCs) from biopsies obtained after in vivo irradiation of human skin in the presence or absence of the topical tea formulations; (ii) assessments of in vivo contact hypersensitivity using the contact sensitizer dinitrochlorobenzene (DNCB); and (iii) an analysis of UVR-induced epidermal LC depletion in vitro, using a skin explant model.
Materials and methods

All procedures were approved by the Institutional Review Board of University Hospitals of Cleveland. Volunteers were enrolled after written informed consent.

Immunohistochemical analyses of skin biopsies

Ten healthy volunteers, FST I-III were enrolled and underwent standard minimum erythema dose (MED) testing using solar-simulated ultraviolet radiation (ssUVR) exposure (1000 W xenon arc lamp, 290–400 nm; Oriel Corporation, Stratford, CT, USA). The irradiance of the light source was measured using an IL1700 radiometer (International Light, Newburyport, MA, USA), with a UVA and a UVB sensor. Twenty-four hours later, ssUVR-irradiated sites were analysed via colorimetry (Minolta CR 300 chromometer, Tokyo, Japan) and MED was calculated as the ssUVR dose that results in erythema equivalent to a delta a of 2.5. Three areas on the buttock, each measuring 6 × 8 cm, were then chosen for test product application (one product per skin site), containing either green tea, white tea or vehicle. Tea extracts were formulated in a vehicle containing deionized water, 1,3 butylene glycol, carbopol 980 triethanolamine and methyl paraben. Both investigator and subject were blinded to the identity of the test agents. Each test agent was applied at a dose of 2.5 mg/cm² and allowed to dry for 15 min prior to ssUVR-irradiation with 2× the MED. Repeat application of products was performed immediately after ssUVR exposure. An additional skin area that did not receive any product application was also irradiated with 2× MED of ssUVR to serve as an untreated UV-irradiated control. Seventy-two hours later, a 4-mm punch biopsy was obtained from each of the four irradiated sites. A punch biopsy was also obtained from an untreated, unirradiated site. Tissue samples were embedded in optimal cutting temperature (OCT) and frozen from an untreated, unirradiated site. Tissue samples were then irradiated using an Oriel solar simulator to that used in the in vivo study groups and differences analysed by unpaired t-tests (P < 0.05).

In vivo contact hypersensitivity assay to dinitrochlorobenzene

Ninety healthy volunteers from 18 to 60 years of age, with Fitzpatrick skin types I-III, and MED values between 20 and 50 mJ/cm² were enrolled. This is equivalent to about 2–7 J/cm² of full spectrum UV (i.e. UVA + UVB) delivered by the solar simulator. Excluded were subjects with significant medical and/or dermatological history, abnormal photosensitivity and recent significant sun exposure.

Subjects were randomized into three treatment groups: no treatment, topical green tea and topical white tea. Each treatment group was further subdivided into three groups according to the dose of ssUVR (i.e. 0, 0.75× MED or 2× MED). In vivo sun protection factor (SPF) testing of the two products performed on the first five subjects revealed an average SPF of 1. Contact hypersensitivity assay was performed similar to previous publications (8,9). Briefly, the test product was applied 15 min prior to ssUVR irradiation, as well as immediately after irradiation. Three days later, sensitization was performed on the ssUVR-irradiated site with 40 µg/38 µl of DNCB. Two weeks later, DNCB elicitation was performed on the contralateral arm using five graded concentrations of DNCB (0–0.0625%). Forty-eight hours later, the elicitation sites were evaluated clinically and by measurement of skin fold thickness (SFT) (Mitutuyo micrometer, Tokyo, Japan). For each subject, the contact hypersensitivity (CHS) response was evaluated by the total millimetre increase in SFT. Mean SFT increases were calculated for each of the study groups and differences analysed by unpaired t-tests (P < 0.05).

Analysis of LCs using a skin explant model

The effects of white tea and green tea were examined in vitro using a skin explant model. Discarded abdominal skin tissue from a 31-year-old female who underwent plastic surgery was obtained. The tissue was divided into 51 biopsies (Ø 12 mm) isolated and maintained in basal essential medium (BEM) survival media. These biopsies were then treated at a dose of 2 mg/cm² with test material identical to that used in the in vivo clinical study; either vehicle, white tea or green tea, 4 h prior to UV exposure. The tissues were then irradiated using an Oriel solar simulator with Xenon lamp (900 W/cm²). Using 1.78 J/cm² as the estimated UV dose corresponding to 1 MED for this skin type (FST II), the biopsies were exposed to a range of 0–3.0 MED. The untreated and vehicle-treated tissue samples were exposed to doses of 0, 0.45 (0.25 MED), 0.89 (0.5 MED), 1.78 (1.0 MED), 3.56 (2.0 MED) and 5.34 J/cm² (3.0 MED), while the tea-treated samples were exposed to doses of 0, 0.89, 1.78 and 3.56 J/cm². Twenty-four hours after exposure, the tissue samples were cut and allocated for freezing or paraffin embedding, from which 5-µm serial sections were immunolabelled with anti-CD1a and visualized using DAB or FITC. Frozen tissues were treated with dispose to separate the epidermis for horizontal examination, for better evaluation of LC dendricity. Vertical
sections across the entire epidermis were taken into account for quantification of the number of residual LC.

Results

Both green tea and white tea partially prevented UV-induced depletion of CD1a+ cells and UV-induced generation of 8-OHdG in healthy subjects irradiated in vivo

Figure 1 shows the mean percentage of CD1a+ per unit area in each experimental condition from a total of 10 volunteers. After receiving 2 MED of ssUVR, untreated skin and vehicle-treated skin showed an almost identical reduction (approximately 57%) in the level of CD1a+ staining compared with unirradiated skin. This indicates that the vehicle alone offered no protection against CD1a depletion. Pretreatment with either white tea or green tea resulted in significantly higher percentages of CD1a+ staining compared with vehicle-protected skin (P = 0.002 and 0.003, respectively). UV-irradiated skin treated with white tea showed a 22% reduction, and UV-exposed green tea-treated skin showed a 35% reduction in CD1a+ staining relative to unirradiated skin. There was no statistical difference between green tea versus white tea in terms of protection against LC depletion (P = 0.09).

Similarly, application of either green tea or white tea showed partial prevention of UV-induced oxidative DNA damage in the form of 8-OHdG (Fig. 2). Exposing skin to 2× MED of ssUVR resulted in 40% increase in 8-OHdG relative to unirradiated skin. Application of vehicle resulted in an even higher (69%) increase in 8-OHdG. However, levels of 8-OHdG in ssUVR-irradiated skin pretreated with white tea and green tea were not significantly different from control unirradiated skin (P = 0.95 nd 0.12, respectively), and were significantly different from vehicle-treated skin (P = 0.002 and 0.0001, respectively). Again, there was no significant difference between green tea and white tea in the level of protection against UV-induced 8-OHdG formation (P = 0.08).

In-vivo CHS assay

A total of 90 subjects, 34 females and 56 males, with age range of 19–58 years (mean 28 years), Fitzpatrick skin types I–III, MED of 20–50 mJ/cm² UVB or 2–7 J/cm² full spectrum UV (i.e. UVA + UVB) completed the CHS portion of the study. In vivo SPF testing in the first five subjects, confirmed that both products had an SPF of 1 (mean white tea SPF = 1.21 and mean green tea SPF = 1.05). Results of the CHS assay are presented in Fig. 3 for the untreated (i.e. no topical test agent) versus the test agent-treated groups across all three doses of ssUVR (0, 0.75 and 2× MED). The unirradiated test agent-treated controls (0 MED groups) had similar CHS responses (untreated = 3.55 ± 0.55, green tea = 3.49 ± 1.26, white tea = 3.6 ± 1.09 mm SFT), indicating that green tea and white tea, by themselves, did not alter DNCB sensitization rates. In the groups not treated with
topical test agents, there was a significant difference in immune response ($P = 0.01$) between unirradiated and irradiated (0.75 MED) subjects, which was not observed in either white or green tea subjects. In contrast, the immune response of subjects who received 0.75 MED but who were pretreated with either green tea or white tea was not significantly different from the immune response of unirradiated controls ($P = 0.31$ and 0.48, respectively). However, within each ssUVR dose group, there was no significant difference between unprotected control versus white tea or unprotected control versus green tea subjects ($P = 0.35$ and 0.30 for white tea; $P = 0.63$ and 0.20 for green tea) although the treated subjects consistently demonstrated higher CHS responses. This may be because of the wide variability of CHS responses when measured by SFT, which may limit the power to detect statistically significant differences with an $n = 10$ per dose group. The trend, however, suggests that tea-treated subjects had greater preservation of their CHS response after ssUVR exposure, relative to untreated subjects.

**Ex vivo skin explant model**

Results are expressed as the residual number of LC per mm$^2$ epidermis as a function of ssUVR dose. The effect of UV exposure on the sites that were not treated by either green or white tea was observed as a dose-dependent decrease in the number of LC per mm$^2$ epidermis (Fig. 4a). Similarly, sites treated with vehicle showed a dose-dependent decrease in LC numbers. In contrast, skin treated with white tea showed no decrease in the number of LC per mm epidermis, up to a dose of 3.56 J/cm$^2$. This dose is roughly equivalent to 2 MED based on the phototype of this skin explant (Fig. 4b). Similar to white tea, green tea also prevented a decrease in the number of LC per mm$^2$ epidermis, up to a dose of 3.56 J/cm$^2$ (Fig. 4c).

**Discussion**

Despite the increased public awareness of the dangers of solar UV exposure, the incidence of both melanoma and non-melanoma skin cancers continues to rise at an alarming rate (National Cancer Statistics 2005). Whereas UVB is the spectrum that primarily causes direct DNA damage, UVA likewise exerts detrimental effects via the production of ROS (6). As greater than 90% of the UV radiation that reaches the earth’s surface is UVA, the role of UVA in skin carcinogenesis cannot be ignored and has become a major focus of research (10). UVA, as well as UVB, can suppress the immune system, and studies have confirmed that better protection against UVA leads to improved immune protection (9,11–14), again suggesting that UVA-triggered photooxidative mechanisms need to be addressed by sun protection agents. The contact hypersensitivity assay remains a valid tool in the *in vivo* analysis of sun protection agents, as confirmed by a consensus paper by sun protection experts worldwide (8). UV diminishes both epidermal concentration and function of LCs, the dendritic antigen presenting cells responsible for induction of immunity in the skin (15–17). Within hours of UV-exposure, LCs begin to migrate from the epidermis without the functional maturity to produce an effective immune response (15,18). The immature LCs preferentially activate Th2 cells, resulting in an increased generation of T cells with suppressor/regulatory function. Oxidative stress from UV exposure also induces nucleic acid base modifications such as the formation of 8-OHdG, which was also evaluated in this study. Previous studies have shown that even without UV irradiation, there is some amount of 8-OHdG that will be detectable in tissue (19). This amount substantially increases with UVR exposure and via base excision repair pathways, returns to near normal levels by 72–96 h (19). Hence, although we could have obtained more robust increases in 8-OHdG at earlier time-points (e.g. within
24 h of UV irradiation), the levels detected at 72 h may be more relevant in that they reflect residual damage after normal repair mechanisms have taken place.

Chemoprevention, or the use of dietary or pharmacological agents, whether orally or topically, to inhibit or reverse the development of cancer, may be a useful strategy in the overall battle against skin cancer. Excellent reviews on this subject have been published (20,21). Previous studies have shown benefits from the use of tea polyphenols for photoprotection. Using topical GTP (1–6 mg/amaginal), Katiyar et al. demonstrated a dose-dependent protection (25–93%) from UVB-induced immunosuppression in C3H mice via CHS response to 2,4-dinitrofluorobenzene (22). They further demonstrated that topical application of (--)epigallocatechin-3-gallate (EGCG) before UVB exposure of mice prevented depletion of antigen-presenting cells immunohistologically detected as class II MHC+ Ia+ cells (23). In another mouse study, they showed that the topical application of EGCG before UVB exposure reduced the number of CD11b+ monocytes/macrophages and neutrophils that infiltrated the exposed skin, and the concentration of IL-10 in the skin and draining lymph nodes (24). Hsu et al. demonstrated the role of green tea in reducing psoriasiform lesions in the flaky skin mouse model (25). Recently, Puch and co-workers demonstrated enhancement of the skin barrier function in human volunteers after consumption of green tea containing milk (26).

Our study provides further evidence for the protective effects that green tea polyphenols exert on the skin’s immune system. It is also the first demonstration of the prevention of UV immune suppression via topical application of white tea. Overall, the two products performed to a comparable degree. Our results confirm that photoprotective effects of polyphenols involve the prevention of UV-induced LC depletion as was shown after in vivo irradiation of human skin, as well as by an in vitro skin explant model. The fact that both products had an SPF of 1 also confirmed that their protective effects on the skin’s immune system did not result from direct UV absorption or a ‘sunscreen effect’. In light of these observed benefits from topical tea extracts, we recommend that tea polyphenols be considered among compounds that could be developed as photoprotective agents that may be used in conjunction with established sun protection strategies such as sunscreens, protective clothing and sun avoidance. Interestingly, despite differences in the processing of white tea, believed to result in slightly higher polyphenolic content, it did not significantly differ in the ability to provide immune protective benefits from green tea. However given white tea’s lighter colour, it may prove to be more acceptable in topical preparations intended for regular use on areas such as the face.

References