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THE GREEN TEA POLYPHENOL, EPIGALLOCATECHIN-3-GALLATE, PROTECTS AGAINST THE OXIDATIVE CELLULAR AND GENOTOXIC DAMAGE OF UVA RADIATION

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A number of biological activities have been ascribed to the major green tea polyphenol epigallocatechin-3-gallate (EGCG) to explain its chemopreventive properties. Its antioxidant properties emerge as a potentially important mode of action. We have examined the effect of EGCG treatment on the damaging oxidative effects of UVA radiation in a human keratinocyte line (HaCaT). Using the ROS-sensitive probes dihydrorhodamine 123 (DHR) and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), we detected a reduction in fluorescence in UVA-irradiated (100 kJ/m^2) cells in the case of the former but not the latter probe after a 24-hr treatment with EGCG (e.g., 14%, [$p < 0.05$] after 10 μM EGCG). In the absence of UVA, however, both DHR and DCFH detected a pro-oxidant effect of EGCG at the highest concentration used of 50 μM. Measurements of DNA damage in UVA-exposed cells using the single cell gel electrophoresis assay (comet assay) also showed the protective effects of EGCG. A concentration of 10 μM EGCG decreased the level of DNA single strand breaks and alkali-labile sites to 62% of the level observed in non-EGCG, irradiated cells ($p < 0.001$) with a 5-fold higher concentration producing little further effect. Correspondingly, EGCG ablated the mutagenic effects of UVA (500 kJ/m^2) reducing an induced hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutant frequency of $(3.39 \pm 0.73) \times 10^{-6}$ to spontaneous levels $(1.09 \pm 0.19) \times 10^{-6}$. Despite having an antiproliferative effect in the absence of UVA, EGCG also served to protect against the cytotoxic effects of UVA radiation. Our data demonstrate the ability of EGCG to modify endpoints directly relevant to the carcinogenic process in skin.

Key words: *epigallocatechin-3-gallate; antioxidant; ultraviolet A*

There has been considerable recent interest in the chemopreventive properties of the polyphenols or catechins of green tea that have been shown to inhibit tumorigenesis in a variety of organs in rodent models.[1–3] The major polyphenol component by mass, (−)-epigallocatechin-3-gallate (EGCG) is an effective protectant against the mutagenicity of a number of chemical carcinogens including benzo[a]pyrene (B[a]P), aflatoxin B1 and 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b]indole.[4–6] EGCG, together with other green tea polyphenols also exhibits growth inhibitory properties in a variety of tumour cell lines.[7–9] Several mechanisms have been proposed by which these compounds exert their anti-tumorigenic action: these include the blockade of growth factors binding to their receptors,[10] phosphorylation (activation) of mitogen-activated protein kinases (MAPKs)[11,12] possibly resulting in the observed induction of Phase II drug-metabolizing enzymes,[13,14] and the generation of oxidative stress leading to apoptosis.[7,9]

In the face of an oxidative challenge, however, it is now well documented that green tea catechins act as antioxidants. For example, EGCG abrogates oxidation by hydrogen peroxide both in a cell free system[15] and in terms of DNA single-strand break damage.[16] In addition, it protects against generation of the mutagenic base 8-hydroxyguanine[17] and genotoxic effects of the superoxide generator paraquat.[18] Although the ability of EGCG to prevent oxidative DNA damage may reflect its direct scavenging of ROS as reported for hydroxyl and superoxide radicals,[19] it has been proposed recently that its mode of action may also involve chemical repair of radicals formed within the DNA structure itself.[20]

Although skin carcinogenesis has long been associated with the UVB portion of the solar spectrum, there is an increasing appreciation of the potential contribution of UVA to tumorigenesis particularly with regard to malignant melanoma.[21,22] Moreover, the increased abundance (90% of UV radiation reaching the earth's surface) and penetration of UVA to the actively dividing basal layer of skin tissue relative to UVB points to a greater relative mutagenic efficacy than that observed in cell monolayers in the laboratory.[23] In our study, we have examined the role of EGCG in protecting against the damaging effects of UVA solar radiation that exerts its effects on the skin via oxidative mechanisms. We and others have shown previously that ablation of the endogenous antioxidant glutathione enhances the level of intracellular ROS[24] and the cytotoxicity generated by UVA irradiation.[25]

Using an immortalized human keratinocyte line (HaCaT), we show that the green tea polyphenol EGCG reduces the production of certain UVA-induced ROS, can decrease UVA cell-killing and protect DNA from the oxidative damage of UVA and its mutagenic consequences. Our findings can be dissociated from any "sunscreen" effect of EGCG because it does not exhibit any absorbance within the UVA wavebands of our radiation sources.

MATERIAL AND METHODS

Chemicals

The green tea polyphenol, EGCG and 6-thioguanine (6-TG) were purchased from Sigma (St. Louis, MO). SYBR Green I stain (10,000 × concentration, Molecular Probes, Eugene, OR) was obtained from Flowgen (Leicestershire, UK). The fluorogenic probes, 2′,7′,-dichlorodihydrofluorescein diacetate (DCFH-DA) and dihydrorhodamine 123 (DHR) (Molecular Probes) were obtained from Cambridge Biosciences (Cambridge, UK).

Cell culture and treatment

HaCaT cells (a kind gift from N.E. Fusenig) were maintained in DMEM, supplemented with 5% (v/v) FCS and penicillin (100 U/ml)/streptomycin (100 μg/ml). For EGCG treatment, cells were plated at 30–40% confluence in 35 mm dishes (Corning, Corning, NY) and, 24 hr later, the culture medium was replaced with fresh

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DMEM containing EGCG (5 mM stock solution in 0.1% (v/v) DMSO) for a further 24-hr period.

Clonogenic survival assays

EGCG-containing medium was removed and replaced with Ca^{++}/Mg^{++}-free Dulbecco PBS. Irradiation was carried out on a cooled plate at 4°C under UVA tubes (Phillips) at a dose rate of 100 W/m.[2] Mylar film (Lee filters) was used to cut out wavelengths in the UVB region of the spectrum. Only 0.052% of total irradiance was below 340 nm. Cells were then harvested and plated at appropriate cloning densities. Seven days after plating, colonies were rinsed in PBS, fixed in 70% (v/v) ethanol and stained with Giemsa (5%,v/v).

Measurements of UVA-induced radical species

Cells were harvested, washed twice in PBS containing 2.1 mM EDTA, 5.2 mM dextrose and 0.1% (v/v) gelatin and resuspended at 2×10^6/ml in Hank's balanced salt solution (HBSS). Each sample was loaded with either DCFH-DA or DHR (2 mM stock solutions in DMSO) to 20 μM for 20 min at 37°C. Because photo oxidation of the fluorescent probes becomes an increasing problem closer to the UVB region of the spectrum, for these experiments, irradiations were carried out using a 360 ± 5 nm interference filter fitted to a 200 W Hg(Xe) arc lamp (Oriel, Stratford, CT). At this wavelength, we have demonstrated that oxidation of the dyes reflects their reaction with free radical species.[24] Immediately after irradiation, samples were run on a FACSCALIBUR flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and geometric means for histograms calculated using CellQuest software.

Single cell gel electrophoresis (comet) assay

Cells were harvested, washed twice in PBS and mixed with 1% low melting point (LMP) agarose in a 1:1 ratio to a density of 2.5×10^4/ml. A 300 μl volume of cells in agarose was applied to slides and coverslips were laid on top to form a thin layer. After 10 min on ice to allow the agarose to solidify, the coverslips were removed and the samples irradiated using UVA tubes and mylar film as for the cell survival experiments. After 1 hr in lysis buffer (60 mM NaOH, 1 M NaCl, 0.5%(w/v) *N*-lauryl sarcosine, >pH 12.5) and a further 1 hr in DNA unwinding solution (40 mM NaOH, 2 mM Na_2 EDTA, pH 13), samples were electrophoresed at 20 V for 25 min. After staining with SYBR Green (0.01% in TBE buffer) for 25 min, comets were analyzed using fluorescence microscopy linked to Optimas software.

HPRT mutation assay

Cells were cultured in HAT medium (100 μM hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine) for at least 14 days to remove existing *HPRT* mutants and plated at 30% confluence in 10 cm dishes. After 24 hr, the HAT-containing medium was replaced with normal medium containing 50 μM EGCG or carrier (0.1% DMSO). After a 24-hr treatment, cells were UVA irradiated (500 kJ/m^2) and cultured for a further 12–14 days to allow expression of *HPRT* mutants before plating in selective medium containing 30 μM 6-thioguanine (3×10^6 cells/T180 cm^2 flask). Cells were also plated in regular medium at cloning density to evaluate plating efficiency for each treatment. Colonies were fixed in 70% ethanol and stained in 5% Giemsa 14 days later.

Annexin V/propidium iodide staining

Cells undergoing early apoptosis or late apoptosis or necrosis were analyzed by flow cytometry after processing using an Apoptosis Detection Kit (Oncogene Research Products, The Netherlands). Briefly, cells were harvested as normal except that 0.5× trypsin was used for cell detachment and cell media, potentially containing dead cells, was retained on ice. Cells and media were spun down (220*g*, 4°C) and resuspended in 1× cold binding buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 4% BSA) at a concentration of 1×10^6/ml. Cells (5×10^5) were incubated with Annexin V-FITC (1 μl, 15 min, room temp), spun down (1,000*g*, 5 min) and resuspended in 0.5 ml binding buffer. Propidium iodide (2.5 μl; 500 μg/ml) was added before flow cytometric analysis to identify late apoptotic or necrotic cells.

Statistical analysis

All data were analyzed with Sigma Stat software (SPSS, Chicago, IL) using Student's *t*-test for pairwise comparisons.

RESULTS

EGCG inhibits UVA-generated ROS detected by DHR

Consistent with previous reports,[44] we confirmed that a solution of EGCG (0.6 mM) in 0.1% DMSO, pH 7.5 produced an absorbance spectrum with a λ_{max} below 300 nm (292 nm) and falling to 1% of this value by 340 nm. The use of UVA tubes and an arc lamp that emitted in the 340–400 nm region and at 365 ± 5 nm, respectively, therefore ensured that the effects of EGCG on UVA irradiated cells were not attributable to UV absorbance by the compound.

The basal and UVA-induced levels of fluorescence for DHR and DCFH-DA loaded HaCaT cells after EGCG treatment are shown in Figures 1 and 2 respectively. In the case of both DHR and DCFH, EGCG treatment has no effect on the levels of fluorescence in unirradiated cells up to 20 μM; however after 50 μM EGCG treatment there are increases of 21% and 29% in the levels of fluorescent rhodamine 123 and DCF respectively indicating that at sufficiently high concentrations, EGCG induces cellular ROS. In the absence of fluorescent probe, 50 μM EGCG-treated cells did not produce any autofluorescent profile on the flow cytometer.

As reported previously,[24] UVA exposure (100 kJ/m^2) produced an increase in fluorescence in both DHR and DCFH-loaded cells (increases of, on average, 161 and 152% over basal levels, respectively). A prior 24-hr exposure to an EGCG concentration as low as 10 μM, however, resulted in an increase of only 126% ($p < 0.05$) over the corresponding unirradiated level. Moreover, a 50 μM EGCG treatment, despite increasing the unirradiated level of ROS, reduced the UVA-induced fluorescence increase to, on average, 104% over the basal value. In contrast, no such reduction in UVA signal was observed for DCFH-loaded cells. In fact, EGCG treatment resulted in an increase in UVA-induced DCF oxidation of up to 32% ($p < 0.05$, 50 μM EGCG).

EGCG protects against the production of DNA single strand breaks and alkali-labile sites by UVA

In agreement with other studies,[26] UVA (50 kJ/m^2) induced immediate DNA damage in HaCaT cells as measured by the "comet" assay (Fig. 3). Pretreatment with 10 μM EGCG reduced

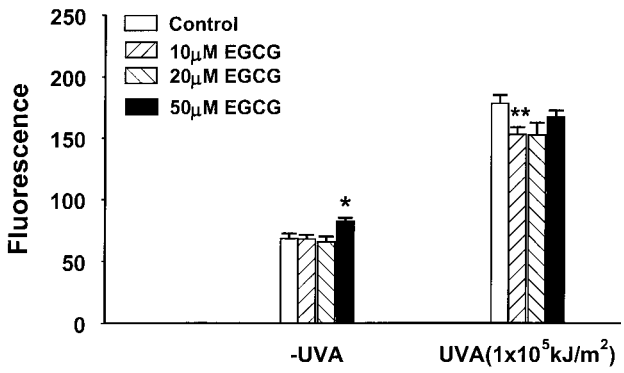

FIGURE 1 – Effect of 24-hr EGCG treatment on levels of DHR (20 μM) fluorescence in unirradiated and UVA-irradiated (100 kJ/m^2) HaCaT cells. Data are presented as mean ± SEM of 3 experiments. *$p < 0.05$, 50 μM EGCG treatment produced significantly greater basal DHR oxidation than other treatments; **$p < 0.05$, 10 μM EGCG significantly reduced UVA-induced fluorescence compared to untreated, irradiated samples.

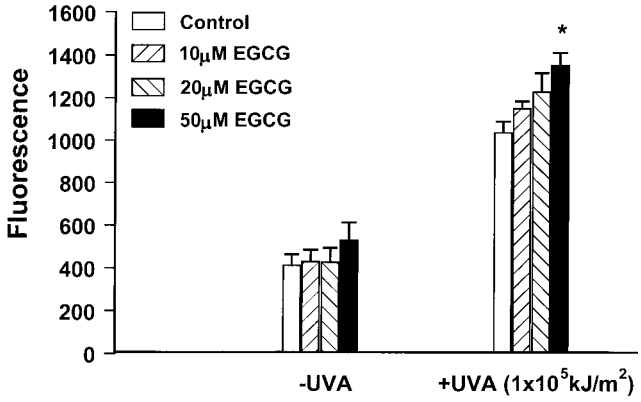

FIGURE 2 – Effect of EGCG treatment on DCFH oxidation in unirradiated and UVA-irradiated (100 kJ/m^2) HaCaT cells. *$p < 0.05$, 50 μM EGCG produced a statistically significant increase in UVA-induced DCF fluorescence. The increase in basal oxidation after 50 μM EGCG was not statistically significant.

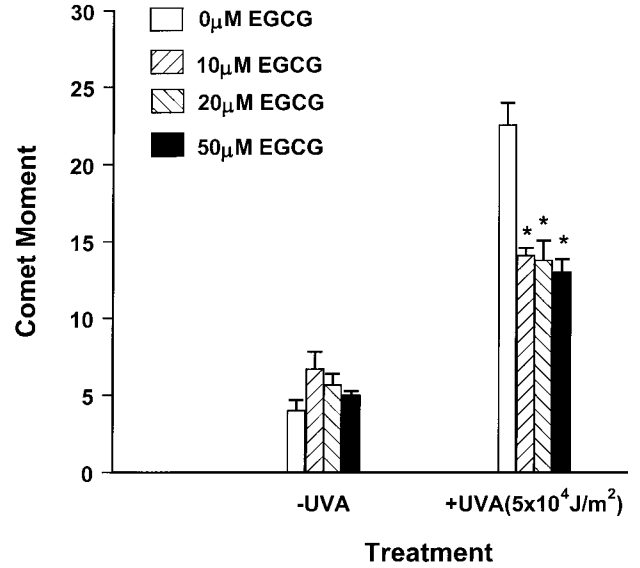

FIGURE 3 – Effect of prior EGCG treatment on basal and UVA-induced DNA damage (single-strand breaks and alkali-labile sites) assayed by the alkaline single cell gel electrophoresis (comet) assay. Data represents mean ± SE of 3 experiments. Each experiment involved measurement of the comet moment of 200 individual cells for each treatment and the mean moment was calculated. The level of UVA DNA damage was significantly different in EGCG–treated cells compared to irradiated controls (*$p < 0.001$).

the level of DNA damage in UVA-irradiated cells to 62% of control levels, on average. Exposing the cells to a 5-fold higher concentration of EGCG *i.e.*, 50 μM did not enhance the level of protection significantly (58% of control). In unirradiated cells, treatment with EGCG produced a small increase in the comet moment compared to untreated cells particularly at 10 μM (comet moment of 6.69 ± 1.1 at 10 μM EGCG *vs.* 4.01 ± 0.67 in control, not statistically significant). The fact that 10 μM EGCG produced a slightly higher proportion of cells in S phase compared to controls (46% *vs.* 41 %) after a 24-hr treatment may have contributed to this increase in damage because there are reports of S-phase cells exhibiting higher basal levels of damage than those in G1 in the comet assay.[27] Treatment with higher concentrations of EGCG up to 100 μM did not result in any changes in the proportion of HaCaT cells in G1, S or G2/M (data not shown).

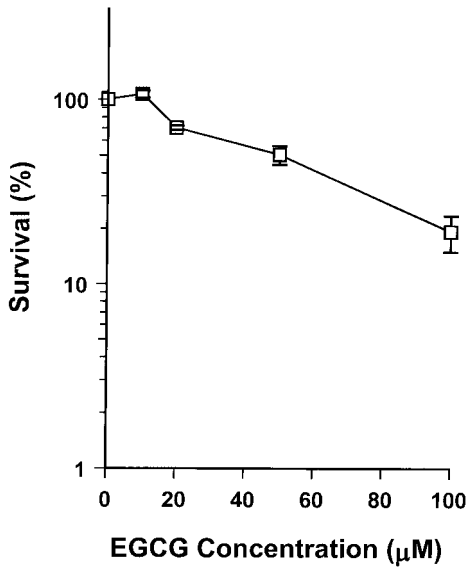

FIGURE 4 – Clonogenic survival of HaCaT cells after 24-hr exposure to various concentrations of EGCG. Within 1 experiment, survival was determined in triplicate for each concentration. Data represent mean ± SEM of 3 experiments.

In the absence of UVA, EGCG is cytotoxic to HaCaT cells

Twenty-four hour EGCG treatment alone decreased cell survival in HaCaT cells (Fig. 4) with a 50 μM exposure reducing it to 50%. In support of a cytotoxic action of EGCG, annexin V/propidium iodide staining of HaCaT cells 4 hr after the end of EGCG treatment indicated an increase in the non-viable annexin+/PI+ fraction of cells from 9% in controls to 15% in cells treated with 50 μM EGCG. At 4 hr post EGCG treatment, there was, however, no increase over the untreated control in the fraction of cells staining for annexin V alone that is indicative of apoptosis.

EGCG modulates cell survival after UVA irradiation

UVA irradiation is cytotoxic and Figure 5*a* shows a reduction in HaCaT cell survival with increasing UVA dose in the absence of EGCG pretreatment. The individual cytotoxic effects of UVA and EGCG, however, are not additive when treatments are combined. As Figure 5*a* indicates, there is an increase in survival from the initial cytotoxic effects of EGCG in the absence of irradiation to overall protection against UVA. For example, 50 μM EGCG pretreatment results in 74% survival after 200 kJ/m^2 in contrast to 50.8% in untreated cells. A similar pattern is observed for 20 μM EGCG (data not shown) with survival at 96% (from an unirradiated level of 66%) after 50 kJ/m^2 UVA, as compared to 89.7% in the absence of EGCG. Expressing the data using the unirradiated survival values with the appropriate level of EGCG as controls (100%) demonstrates the polyphenol protection against UVA cell-killing more clearly (Fig. 5*b*). The survival curves illustrate how increasing concentrations of EGCG effectively shifts the survival curve "shoulder" out to higher doses.

EGCG protects against UVA-induced mutagenesis

In agreement with previous work,[28,29] a UVA dose of less than 500 kJ/m^2 was insufficient to generate mutant levels in mammalian cells above spontaneous levels. Exposing HaCaT cells to a UVA dose of 500 kJ/m^2 produced, on average, a 3.1-fold increase in mutation frequency ($p < 0.05$) (Fig. 6). Twenty-four hour treatment with 50 μM EGCG alone actually increased the frequency of *hprt* mutants ($p = 0.048$) in the absence of UVA but in contrast clearly suppressed UVA-induced mutagenesis ($p < 0.05$) to spontaneous levels.

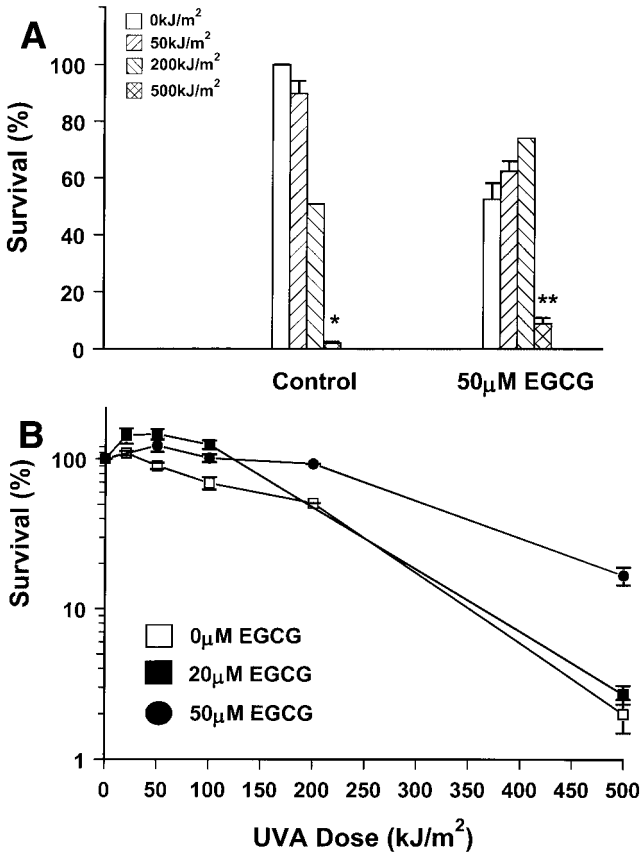

FIGURE 5 – (*a*) Effect of EGCG pre-treatment on HaCaT clonogenic survival after irradiation with various UVA doses (0–500 kJ/m^2). Data with error bars represent mean ± SEM of at least 3 experiments. *$p <$ 0.001, pairwise comparison between survival after 50 kJ/m^2 UVA and survival after 500 kJ/m^2 in absence of EGCG treatment. **$p < 0.05$, 50 μM EGCG significantly protects against the cytotoxicity of 500 kJ/m^2 UVA. (*b*) Modulation of clonogenic survival curves for UVA-irradiated HaCaT cells after a 24-hr pretreatment with 20 μM or 50 μM EGCG. Survival has been set to 100% in all unirradiated samples. Points represent mean and SE of 3 experiments.

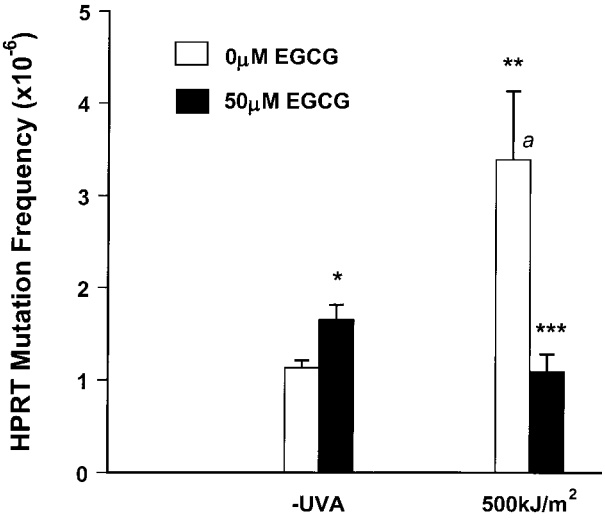

FIGURE 6 – Effect of EGCG on the induction of mutations at the *hprt* locus by UVA radiation. All data represent mean and SE of 3 determinations except bar marked *a* that is mean and range for 2 experiments. *$p < 0.05$, pairwise comparison between −UVA, 0 μM EGCG and −UVA, 50 μM EGCG; **$p < 0.05$, pairwise comparison between −UVA, 0 μM EGCG and +UVA (500 kJ/m^2), 0 μM EGCG; ***$p <$ 0.05, pairwise comparison between +UVA (500 kJ/m^2), 0 μM EGCG and +UVA, 50 μM EGCG.

DISCUSSION

Our study demonstrates that the green tea polyphenol, EGCG, is able to inhibit UVA-induced DNA damage and mutagenesis in human cells and provides evidence, using the ROS-sensitive probe DHR, that it exerts an antioxidant activity by reducing the level of certain UVA-induced ROS. EGCG is also able to modulate UVA cytotoxicity resulting in overall protection at certain doses. Interestingly, inhibition of UVA-induced ROS (DHR oxidation) and DNA damage were achieved at the lowest concentration of EGCG used (10 μM) that is of the same order as concentrations attained in human plasma after diet supplementation with green tea extracts (4.4 μM).[30,31] Although the UVA radical species that determine the endpoints of survival, DNA damage and mutagenesis and against which EGCG acts, are not known, they are clearly of a different makeup to those detected by the probe DCFH because it shows a pro-oxidant effect of EGCG after UVA.

The only previous study examining the effects of EGCG on UVA radiation reported its reduction of UVA-induced AP-1 and NF-KB transcription factor activities,[32] processes known to be affected by redox status[33,34] thus providing additional evidence for EGCG antioxidant action against UVA. Wei et al.[15] also reported the protection afforded by a green tea fraction containing a number of polyphenols against UVA-induced oxidative damage but only in a DNA solution.

There have been a number of studies citing the inhibitory effects of EGCG against UV radiation of shorter wavelengths.[15,35–37] In contrast to UVA, interpreting its protective role against UVC or UVB needs to take into account its strong absorption in the UVC and well into the UVB region (280–320 nm). Nevertheless, by application of EGCG post-irradiation, a few workers have shown that its action against UVB is not merely due to a "sunscreen" effect.[35,38] The UV sources used here emit at wavelengths beyond the absorbance spectrum of EGCG so the observations can be solely ascribed to the biological properties of the compound.

Although the data presented indicate that EGCG acts against UVA-induced radical species detected by DHR, the same pattern of inhibition is not seen with DCFH. A difference in the response of the two probes to changes in cellular glutathione status has been reported previously[24] and further suggests that DHR and DCFH possess differing ranges of reactivity. There is an increasing trend to use DCFH or DHR oxidation as evidence of H_2O_2 production[7,37] and although the probes do react with peroxide, they are sensitive to a variety of other free radical species.[39] The fact that there is an increase in DCFH oxidation in UVA-irradiated, EGCG-treated cells suggests that DCFH may react with reactive species generated as a result of the oxidation of EGCG through scavenging UVA ROS or with an EGCG radical itself. Although the scavenging mechanisms for EGCG are still poorly understood, there is recent evidence pointing to the production of superoxide (O^{2-}) by EGCG in the process of scavenging peroxyl radicals.[40] It is of interest that DCFH is readily oxidized by superoxide formation whereas DHR shows little reactivity to this species.[39]

EGCG had an antiproliferative effect on unirradiated HaCaT cells as expressed by a fall in cell survival upon subsequent plating. EGCG has been reported to exert a growth inhibitory effect on a number of tumour cell lines[7,8,41] and on immortalized but non-tumorigenic bronchial epithelial cells.[9] At least part of the inhibition has been ascribed to apoptosis in some of these cell lines.[7,9] It is shown that EGCG is cytotoxic to HaCaT cells as shown by an increase in the fraction of non-viable Annexin V+/Propodium iodide + cells, but there was no increase in the proportion of Annexin only + (*i.e.*, apoptotic) cells within the time frame of our experiments. Ahmad *et al.*[42] reported that HaCaT

cells do undergo apoptosis, as assayed by DNA laddering, after a 48-hr EGCG treatment albeit at a concentration of 175 μM (80 μg/ml). The degree of DNA fragmentation, however, appears to be far less than in a human epidermoid (A431) or prostate carcinoma (DU145) cell line. In agreement with other studies, however, it is shown here that EGCG has a pro-oxidant effect because a 50 μM treatment induces ROS as detected by both the DHR and DCFH probes (Figs. 2,3). Furthermore, the comet and mutation data presented point to EGCG causing an increase in DNA damage. It has been postulated that this ROS generation underlies the cytotoxic action of EGCG.[7,9,43]

Prior treatment of HaCaT cells with EGCG clearly modifies their survival after UVA irradiation. At lower UVA doses, not only does EGCG prevent further cell killing by UVA but there is a clear increase in the level of survival. Interpreting the data in terms of EGCG antioxidant action against UVA-induced ROS, the initial increase in survival is the result of the inactivation of EGCG (and thereby its own growth inhibitory effect) through its scavenging of UVA free radicals. A similar effect has been observed by Cutter *et al.*[44] where oxidative degradation of EGCG via the Fenton reaction by incubation with hydrogen peroxide and $FeSO_4$ abolished the polyphenol inhibitory effect on the growth of HeLa cells. At sufficiently high UVA doses, survival starts to fall as, presumably, the oxidative stress overwhelms the scavenging capacity of EGCG. In practical terms, a 50 μM EGCG treatment offers overall protection to keratinocytes against a UVA dose (500 kJ/m^2) equivalent to 2.5 hr in the summer midday sun at latitude 48′ north.[45] The influence, on survival, of factors that modulate the cell's capacity to combat UVA-induced oxidative stress is well known.[25] The relative contributions of unrepaired DNA damage and membrane perturbations to UVA cytotoxicity remain unclear. EGCG lowers the level of initial DNA ssb after UVA, but the effect on the repair capacity of EGCG-treated cells is not known. Alterations in ssb repair after UVA have been shown to affect cell survival.[46] EGCG may, through its antioxidant action, affect UVA cell signaling events that impinge on cell survival. Indeed, a related green tea polyphenol epigallocatechin (EGC) is reported to prevent UVA induction of cyclooxygenase 2 in keratinocytes.[47] Inhibition of cyclooxygenase is known to reduce UVA stimulated release of arachidonic acid from the cell membrane that seems to be an important cellular signal for halting cell proliferation and inducing cell death.[48,49]

The decrease observed in UVA-induced DNA damage after EGCG treatment is mirrored by data pointing to its protective action against UVA-induced mutagenesis. EGCG has been reported to have an antimutagenic effect against UVAB light (295–400 nm) in *E. coli* although its activity may be related to effects on DNA repair rather than ROS because the reduction is abolished in excision-repair deficient mutants.[50] EGCG reduction of 4-nitroquinoline 1-oxide (4-NQO)-induced mutagenesis in Chinese hamster cells[51] may be based on its antioxidant activity because there is evidence that 4-NQO produces oxidative DNA damage aside from the well established "bulky" quinoline-purine adducts.[52,53] In contrast, there is evidence that spontaneous mutagenesis is not suppressed by green tea polyphenols in a bacterial system.[54] Our data indicate a marginally significant increase in *hprt* spontaneous mutations after EGCG treatment whereas the combination of UVA and EGCG is not significantly different from EGCG treatment alone.

In conclusion, we have demonstrated that EGCG affords protection against the oxidative stress of UVA radiation in terms of biological endpoints relevant to carcinogenesis.

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REFERENCES

1. Xu Y, Ho CT, Amin SG, Han C, Chung FL. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. Cancer Res 1992;52: 3875–9.
2. Katiyar SK, Agarwal R, Mukhtar H. Protective effects of green tea polyphenols administered by oral intubation against chemical carcinogen-induced forestomach and pulmonary neoplasia in A/J mice. Cancer Lett 1993;73:167–72.
3. Cao J, Xu Y, Chen J, Klaunig JE. Chemopreventive effects of green and black tea on pulmonary and hepatic carcinogenesis. Fund Appl Toxicol 1996;29:244–50.
4. Wang ZY, Cheng SJ, Zhou ZC, Athar M, Khan WA, Bickers DR, Mukhtar H. Antimutagenic activity of green tea polyphenols. Mutat Res 1989;223:273–85.
5. Muto S, Yokoi T, Gondo Y, Katsuki M, Shioyama Y, Fujita K, Kamataki T. Inhibition of benzo[a]pyrene-induced mutagenesis by (−)-epigallocatechin gallate in the lung of rpsL transgenic mice. Carcinogenesis 1999;20:421–4.
6. Hayatsu H, Inada N, Kakutani T, Arimoto S, Negishi T, Mori K, Okuda T, Sakata I. Suppression of genotoxicity of carcinogens by (−)-epigallocatechin gallate. Prev Med 1992;21:370–6.
7. Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. Carcinogenesis 1998;19:611–6.
8. Liang YC, Chen YC, Lin YL, Lin-Shiau SY, Ho CT, Lin JK. Suppression of extracellular signals and cell proliferation by the black tea polyphenol, theaflavin-3,3′-digallate. Carcinogenesis 1999;20:733–6.
9. Yang GY, Liao J, Li C, Chung J, Yurkow EJ, Ho CT, Yang CS. Effect of black and green tea polyphenols on c-jun phosphorylation and H(2)O(2) production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction. Carcinogenesis 2000;21:2035–9.
10. Liang YC, Lin-Shiau SY, Chen CF, Lin JK. Suppression of extracellular signals and cell proliferation through EGF receptor binding by (−)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. J Cell Biochem 1997;67:55–65.
11. Chen C, Yu R, Owuor ED, Kong AN. Activation of antioxidant response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch Pharm Res 2000;23:605–12.
12. Kong AN, Yu R, Chen C, Mandlekar S, Primiano T. Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. Arch Pharm Res 2000;23:1–16.
13. Khan SG, Katiyar SK, Agarwal R, Mukhtar H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. Cancer Res 1992;52:4050–2.
14. Maliakal PP, Coville PF, Wanwimolruk S. Tea consumption modulates hepatic drug metabolizing enzymes in Wistar rats. J Pharm Pharmacol 2001;53:569–77.
15. Wei H, Zhang X, Zhao JF, Wang ZY, Bickers D, Lebwohl M. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. Free Radic Biol Med 1999;26:1427–35.
16. Johnson MK, Loo G. Effects of epigallocatechin gallate and quercetin on oxidative damage to cellular DNA. Mutat Res 2000;459:211–8.
17. Fiala ES, Sodum RS, Bhattacharya M, Li H. (−)-Epigallocatechin gallate, a polyphenolic tea antioxidant, inhibits peroxynitrite-mediated formation of 8-oxodeoxyguanosine and 3-nitrotyrosine. Experientia 1996;52:922–6.
18. Tanaka R. Protective effects of (−)-epigallocatechin gallate and (+)-catechin on paraquat-induced genotoxicity in cultured cells. J Toxicol Sci 2000;25:199–204.
19. Shi X, Ye J, Leonard SS, Ding M, Vallyathan V, Castranova V, Rojanasakul Y, Dong Z. Antioxidant properties of (−)-epicatechin-3-gallate and its inhibition of Cr(VI)-induced DNA damage and Cr(IV)- or TPA-stimulated NF-kappaB activation. Mol Cell Biochem 2000;206:125–32.
20. Anderson RF, Fisher LJ, Hara Y, Harris T, Mak WB, Melton LD, Packer JE. Green tea catechins partially protect DNA from (ṡ)OH radical-induced strand breaks and base damage through fast chemical repair of DNA radicals. Carcinogenesis 2001;22:1189–93.
21. Moan J, Dahlback A, Setlow RB. Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation. Photochem Photobiol 1999;70:243–7.
22. Setlow RB, Grist E, Thompson K, Woodhead AD. Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci USA 1993;90:6666–70.
23. Robert C, Muel A, Benoit L, Dubertret A, Sarasin A, Stary A. Cell

survival and shuttle vector mutagenesis induced by ultraviolet A and ultraviolet B radiation in a human cell line. J Invest Dermatol 1996; 106:721–8.
24. Tobi S, Paul N, McMillan TJ. Glutathione modulates the level of free radicals produced in UVA-irradiated cells. J Photochem Photobiol B 2000;57:102–12.
25. Tyrrell RM, Pidoux M. Endogenous glutathione protects human fibroblasts against the cytotoxic action of UVB, UVA and near-visible radiations. Photochem Photobiol 1986;44:561–4.
26. Lehmann J, Pollet D, Peker S, Steinkraus V, Hoppe U. Kinetics of DNA strand breaks and protection by antioxidants in UVA- or UVB-irradiated HaCaT keratinocytes using the single cell gel electrophoresis assay. Mutat Res 1998;407:97–108.
27. Olive PL, Banath JP. Induction and rejoining of radiation-induced DNA single-strand breaks: "tail moment" as a function of position in the cell cycle. Mutat Res 1993;294:275–83.
28. Applegate LA, Lautier D, Frenk E, Tyrrell RM. Endogenous glutathione levels modulate the frequency of both spontaneous and long wavelength ultraviolet-induced mutations in human cells. Carcinogenesis 1992;13:1557–60.
29. Drobetsky EA, Turcotte J, Chateauneuf A. A role for ultraviolet A in solar mutagenesis. Proc Natl Acad Sci USA 1995;92:2350–4.
30. Nakagawa K, Okuda S, Miyazawa T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3- gallate and (−)-epigallocatechin, into human plasma. Biosci Biotechnol Biochem 1997;61:1981–5.
31. Miyazawa T. Absorption, metabolism and antioxidative effects of tea catechin in humans. Biofactors 2000;13:55–9.
32. Kim J, Hwang J-S, Cho Y-K, Han Y, Jeon Y-J, Yang K-H. Protective effects of (−)-epigallocatechin-3-gallate on UVA- and UVB-induced skin damage. Skin Pharmacol Appl Skin Physiol 2001;14:11–9.
33. Soriani M, Hejmadi V, Tyrrell RM. Modulation of c-jun and c-fos transcription by UVB and UVA radiations in human dermal fibroblasts and KB cells. Photochem Photobiol 2000;71:551–8.
34. Vile GF, Tanew-Ilitschew A, Tyrrell RM. Activation of NF-κB in human skin fibroblasts by the oxidative stress generated by UVA radiation. Photochem Photobiol 1995;62:463–8.
35. Katiyar SK, Elmets CA, Agarwal R, Mukhtar H. Protection against ultraviolet-B radiation-induced local and systemic suppression of contact hypersensitivity and edema responses in C_3H/HeN mice by green tea polyphenols. Photochem Photobiol 1995;62:855–61.
36. Katiyar SK, Matsui MS, Elmets CA, Mukhtar H. Polyphenolic antioxidant (−)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. Photochem Photobiol 1999;69:148–53.
37. Katiyar SK, Afaq F, Perez A, Mukhtar H. Green tea polyphenol (−)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. Carcinogenesis 2001;22: 287–94.
38. Barthelman M, Bair WB III, Stickland KK, Chen W, Timmermann BN, Valcic S, Dong Z, Bowden GT. (−)-Epigallocatechin-3-gallate inhibition of ultraviolet B-induced AP-1 activity. Carcinogenesis 1998;19:2201–4.
39. Hempel SL, Buettner GR, O'Malley YQ, Wessels DA, Flaherty DM. Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2′-7′-dichlorodihydrofluorescein diacetate, 5(and 6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate and dihydrorhodamine 123. Free Radical Biol Med 1999;27:146–59.
40. Kondo K, Kurihara M, Miyata N, Suzuki T, Toyoda M. Scavenging mechanisms of (−)-epigallocatechin gallate and (−)-epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action. Free Radic Biol Med 1999;27:855–63.
41. Gupta S, Ahmad N, Nieminen AL, Mukhtar H. Growth inhibition, cell-cycle dysregulation and induction of apoptosis by green tea constituent (−)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol Appl Pharmacol 2000;164:82–90.
42. Ahmad N, Feyes DK, Nieminen A-L, Agarwal R, Mukhtar H Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997;89:1881–6.
43. Chung LY, Cheung TC, Kong SK, Fung KP, Choy YM, Chan ZY, Kwok TT. Induction of apoptosis by green tea catechins in human prostate cancer DU145 cells. Life Sci 2001;68:1207–14.
44. Cutter H, Wu LY, Kim C, Morre DJ, Morre DM Is the cancer protective effect correlated with growth inhibitions by green tea (−)-epigallocatechin gallate mediated through an antioxidant mechanism? Cancer Lett 2001;162:149–54.
45. Jeanmougin M, Civatte J. Dosimetry of solar ultraviolet radiation. Daily and monthly changes in Paris. Ann Dermatol Venereol 1987; 114:671–6.
46. Churchill ME, Peak JG, Peak MJ. Correlation between cell survival and DNA single-strand break repair proficiency in the Chinese hamster ovary cell lines AA8 and EM9 irradiated with 365-nm ultraviolet-A radiation. Photochem Photobiol 1991;53:229–36.
47. Soriani M, Rice-Evans C, Tyrrell RM. Modulation of the UVA activation of haem oxygenase, collagenase and cyclooxygenase gene expression by epigallocatechin in human skin cells. FEBS Lett 1998; 439:253–7.
48. Hanson DL, DeLeo VA. Long wave ultraviolet radiation stimulates arachidonic acid release and cyclooxygenase activity in mammalian cells in culture. Photochem Photobiol 1989;49:423–30.
49. Surette ME, Fonteh AN, Bernatchez C, Chilton FH. Perturbations in the control of cellular arachidonic acid levels block cell growth and induce apoptosis in HL-60 cells. Carcinogenesis 1999;20:757–63.
50. Shimoi K, Nakamura Y, Tomita I, Hara Y, Kada T. The pyrogallol related compounds reduce UV-induced mutations in *Escherichia coli* B/r WP2. Mutat Res 1986;173:239–44.
51. Kuroda Y. Bio-antimutagenic activity of green tea catechins in cultured Chinese hamster V79 cells. Mutat Res 1996;361:179–86.
52. Yamamoto K, Inoue S, Kawanishi S. Site-specific DNA damage and 8-hydroxydeoxyguanosine formation by hydroxylamine and 4-hydroxyaminoquinoline 1-oxide in the presence of Cu(II): role of active oxygen species. Carcinogenesis 1993;14:1397–401.
53. Ruiz-Laguna J, Ariza RR, Prieto-Alamo MJ, Boiteux S, Pueyo C. Fpg protein protects *Escherichia coli* K-12 from mutation induction by the carcinogen 4-nitroquinoline 1-oxide. Carcinogenesis 1994;15:425–9.
54. Yonezawa Y, Kawamura S, Yamato M, Nishioka H. Mut-Test to detect substances suppressing spontaneous mutation due to oxidative damage. Mutat Res 2001;490:21–6.