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**Original**

# Suppressive Effects of Catechins in UV-Induced Cytotoxicity of Human Corneal Epithelial Cells

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**Abstract**: Photokeratitis is a disease in which the ocular surface is directly affected by oxidative stress caused by exposure to ultraviolet (UV) light and oxygen. It is speculated that the production of free radicals and reactive oxygen species (ROS) is caused by UV-induced cytotoxicity. Recent studies have reported that catechins have antioxidant, antiallergic, antitumor, and antibacterial effects. The aim of our study was to investigate the mechanism of UV-induced cytotoxicity in cultured human corneal epithelial (HCE-T) cells and evaluate the protective effects of the catechins, (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin 3-O-(3-O-methyl) gallate (EGCG3"Me), on apoptosis. HCE-T cells were UV irradiated at 312 nm (4.94 mW/cm$^2$, 296 mJ/cm$^2$). EGCG and EGCG3"Me were dissolved in methanol and adjusted to 5, 10, or 20 μM. Absorption was measured from 250 to 400 nm. EGCG and EGCG3"Me were pre-incubated for 1 hr. After UV irradiation, membrane lipid peroxide, tumor necrosis factor (TNF)-α production, ROS generation, caspase-3 and -8 activities, mitochondrial membrane potential, and cytochrome c levels were measured. Both EGCG and EGCG3"Me had UV absorption, and increased with concentration dependently. The increases in the levels of membrane lipid hyperoxidation, activation of caspase-3 and -8, production of TNF-α and ROS were found, by UV irradiation, to be significant. But these levels were significantly decreased by pretreatment with EGCG and EGCG3"Me. There were no changes in mitochondrial membrane potential and cytoplasmic cytochrome c levels after UV irradiation. Oxidative stress occurs early near the cell membrane in response to UV irradiation. As a result, TNF-α is induced, leading to apoptosis mainly through caspase-8 activation. Conversely, EGCG and EGCG3"Me absorb UV light directly and inhibit lipid peroxidation in the cell membrane. Catechins inhibit the apoptosis cascade by inactivating caspase-3 and caspase-8.

**Key words**: catechins, UV, oxidative stress, cornea, cytotoxicity

## Introduction

Free radicals and reactive oxygen species (ROS) are involved in the etiologies of various dis-

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eases. In keratoconjunctivitis, the ocular surface is affected by oxidative stress caused by direct exposure to ultraviolet (UV) light and oxygen ($O_2$). Ocular surface exposure is more severe than that for internal organs and tissues. Epidemiological data indicate that both pterygium and cataract are caused by UV light[1–3].

Only 6.0% of sunlight reaches the earth's surface, and it is composed of 5.8% UV-A (315–400 nm) and 0.2% UV-B (280–315 nm). Most UV-A and UV-B are absorbed by the cornea and lens, resulting in the production of ROS and free radicals in the cornea. ROS and free radicals then create lipid peroxides by attacking the phospholipid double membrane of cells, resulting in oxidative damage to DNA[4–7]. As a result, UV light causes keratoconjunctivitis with acute pain and corneal injury.

Catechins are polyphenols found in only Japanese green tea, and not in other plants[8]. Catechins have various physiological functions, such as antioxidant[9], antiallergic[10], antitumor[11, 12], anti-inflammatory[13], blood pressure suppression[14], and fat metabolism properties[15]. Catechins naturally occur in 'yabukita', a cultivar of common green tea in Japan, and account for 10%–20% of all green tea flavonoids. These catechins contain 50% (−)-epigallocatechin gallate (EGCG), which has strong physiologic functions[16, 17]. (−)-Epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) is methylated at one of the three hydroxyl groups of EGCG and occurs only in 'Benifuuki', 'Benihomare', and 'Benifuji' green tea cultivars. EGCG3"Me has strong antioxidant and anti-allergic properties, with an anti-allergic strength approximately 2.5-fold greater than that of EGCG[10].

The aim of the present study was to examine the cytoprotective effects of EGCG and EGCG3"Me on UV-induced oxidative stress injury in cultured human corneal epithelial (HCE-T) cells.

## Methods

### *Culture of HCE-T cells*

The SV-40-immortalized HCE-T cell line (RBRC-RCB 2280) was purchased from the Riken Cell Bank (Ibaraki, Japan) 18) and cultured in Dulbecco's Modified Eagle's medium:Ham F12 (1:1) (Sigma-Aldrich, St Louis, MO, USA), containing 5% fetal bovine serum, 5 μg/ml insulin, 10 ng/ml human epidermal growth factor, 0.5% dimethyl sulfoxide, and 0.1 μg/ml penicillin/streptomycin, at 37℃ in 5% $CO_2$.

### *UV irradiation*

Using a TFX-20MC UV lamp (Vilber Lourmat, Marne-la-Vallée, France), UV light was applied at a central wavelength of 312 nm, an intensity of 4.94 mW/cm$^2$, and a dose of 296 mJ/cm$^2$.

### *Experimental protocol*

Cells were adjusted to appropriate concentrations according to the substance being measured, cultured for 24 to 48 hr in 6- or 96-well plates, and pretreated with normal medium, EGCG (5, 10, or 20 μM), EGCG3"Me (5, 10, or 20 μM), or caspase inhibitors for 1 hr. The cells were

then irradiated with UV light (UV group) or without UV light (non-UV group) and cultured under conditions appropriate for the substance being measured.

*Measurement of UV absorption by catechins*

EGCG and EGCG3"Me concentrations were adjusted to 5, 10, or 20 μM with methanol and their absorptions were measured from 250 to 400 nm using a BioSpec-1600 UV spectrometer (Shimadzu Corporation, Kyoto, Japan).

*Measurement of membrane lipid peroxide activity after UV irradiation*

Cells were adjusted to $5 \times 10^4$ cells/ml, cultured in 96-well plates for 24 hr, pretreated with EGCG (5, 10, or 20 μM) and EGCG3"Me (5, 10, or 20 μM) for 1 hr, and then treated with diphenyl-1-pyrenylphosphine (DPPP), an index of membranous lipid peroxidation, to a final concentration of 0.5 μM. After incubating the cells at 37℃ for 10 min, the medium was replaced with Hank's Balanced Salt Solution (100 μl) and the cells were irradiated with UV light. After incubating for 15 min, DPPP was measured at 352 nm excitation and 380 nm emission using a Twinkle LB 970 Microplate Fluorometer (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany).

*Measurement of tumor necrosis factor alpha (TNF-α) levels*

HCE-T cells were adjusted to $3 \times 10^5$ cells/ml in 6-well plates for 48 hr, then one of the three concentrations of EGCG and EGCG3"Me was added to each well 1 hr before UV irradiation. After irradiation, each cell group was cultured for 30 min and centrifuged at 3,000 ×g for 5 min. TNF-α production was measured in the supernatant using the Human TNF-α Immunoassay ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA) and a microplate reader at an absorbance of 490 nm.

*Measurement of reactive oxygen species (ROS) levels*

Cells were adjusted to $1 \times 10^5$ cells/ml and cultured in 96-well plates for 24 hr. One of the three concentrations of EGCG and EGCG3"Me was added to each group 1 hr before UV irradiation. ROS were measured 1 hr after irradiation using 5-(and-6) chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-$H_2$DCFDA ; Invitrogen Corporation, Carlsbad, CA, USA). CM-$H_2$DCFDA is an active oxygen indicator of the cellular permeability, when it is disintegrated or oxidized by intracellular esterase, it is a pigment which emits fluorescence when acetate is removed. CM-$H_2$DCFDA was dissolved in dimethyl sulfoxide and adjusted to 100 μM, and 7 μl of this solution was added to the 96-well microplates, followed by incubation at 37℃ for 15 min. The medium was changed to 100 μl phosphate-buffered saline, and fluorescence was measured at 488 nm excitation and 525 ± 10 nm emission using a Twinkle LB 970 Microplate Fluorometer (Berthold Technologies GmbH & Co. KG).

*Measurement of mitochondrial membrane potential*

HCE-T cells were adjusted to $3 \times 10^5$ cells/ml in 6-well plates for 48 hr. One of each of three concentrations of EGCG and EGCG3"Me was added to each well 1 hr before UV irradiation. Mitochondrial membrane potential was measured 1 hr after irradiation using the Muse™ Mito Potential Assay Kit (EMD Millipore Corporation, Hayward, CA, USA) with a Muse™ Cell Analyzer (Merck KGaA, Darmstadt, Germany). A dead cell marker was also used as an indicator of cell membrane structural integrity. This marker is excluded from live, healthy cells, as well as from early apoptotic cells.

*Measurement of cytochrome c levels*

HCE-T cells were adjusted to $3 \times 10^5$ cells/ml in 6-well plates for 48 hr. One of each of three concentrations of EGCG and EGCG3"Me was added to each well 1 hr before UV irradiation. The mitochondrial and cytosolic fractions were collected 20 hr after UV irradiation using the Mitochondria/Cytosol Fractionation Kit (BioVision Inc., Milpitas, CA, USA). Cytochrome c concentration (ng/ml) in each fraction of mitochondria and cytoplasm was measured using the Human Cytochrome c Quantikine ELISA Kit (R&D Systems Inc.). The cytochrome c ratio was calculated as cytosolic cytochrome c/total (mitochondrial + cytosolic) cytochrome c.

*Measurement of caspase-3 and caspase-8 activity*

HCE-T cells were adjusted to $3 \times 10^5$ cells/ml in 6-well plates for 48 hr. One of three concentrations of EGCG and EGCG3"Me was added to each well 1 hr before UV irradiation. Three hours after irradiation, the cells were collected in cold phosphate-buffered saline and centrifuged at 1,500 g for 3 min. The resulting pellets were resuspended in a chilled cell lysis buffer (Medical & Biological Laboratories, Co. Ltd., Tokyo, Japan) and incubated on ice for 10 min. Caspase-3 and caspase-8 activity was measured using fluorescence substrates. The changes per hour of isolation fluorescent dye hydrolyzing substrate by specific amino acid sequences at 37°C was measured at 400 nm excitation and 505 nm emission using a Twinkle LB 970 Microplate Fluorometer (Berthold Technologies GmbH & Co. KG).

*Drugs and reagents*

EGCG was purchased from Wako Pure Chemical Industries (Tokyo, Japan) and EGCG3"Me was purchased from Nagara Science Co., Ltd. (Gifu, Japan). The concentrations of EGCG and EGCG3"Me were adjusted to 5, 10, or 20 μM by culture medium. Caspase-3 substrate (N-acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcoumarin) and caspase-8 (N-acetyl-Ile-Glu-Thr-Asp-amido-4-trifluoromethylcoumarin) substrates were purchased from Funakoshi Co., Ltd. (Tokyo, Japan). The concentration of caspase-3 substrate was adjusted to 25 μM and that of caspase-8 substrate was adjusted to 20 μM by culture medium. The specific caspase inhibitors used in this study were z-DEVD-FMK (for caspase-3) and IETD-FMK (for caspase-8) and were purchased from Medical & Biological Laboratories Co., Ltd. (Aichi, Japan). The concentration of the caspase inhibitors were adjusted to 30 μM by culture medium. DPPP was purchased from Dojindo

Laboratories (Kumamoto, Japan) and was adjusted to 0.5 μM.

*Statistical analyses*

Experimental results are shown as the mean ± standard error (n = 5 to 14). The statistical software package Ystat 2010 (Igaku Tosho-Shuppan Ltd., Tokyo, Japan) was used to compare parameters in the EGCG and EGCG3"Me treatment groups by analysis of variance followed by Dunnett's test for repeated measurements; $P < 0.05$ was considered significant.

**Results**

*UV absorption spectrum of EGCG and EGCG3"Me*

Figures 1a and 1b show the results of the UV absorption spectra of EGCG and EGCG3"Me, respectively. For both, maximum absorption was confirmed at 275 nm when measured from 250 to 400 nm. In addition, an increase in concentration-dependent UV absorption was confirmed, with a larger ratio for EGCG than EGCG3"Me.

*UV irradiation-induced membrane lipid peroxide generation*

Figure 2 shows the results of the intensity of fluorescence of DPPP bound to HCE-T cells containing membrane lipid peroxide at 15 min after UV irradiation. The level of DPPP significantly increased in the UV group compared with the non-UV group (n = 7 or 8, $P < 0.01$ vs. UV group) and significantly decreased at each pretreated concentration of EGCG and EGCG3"Me (n = 6 or 9, $P < 0.01$ vs. UV group).

*UV irradiation-induced TNF-α levels*

Figure 3 shows the result of TNF-α generation in HCE-T cells at 30 min after UV irradiation. TNF-α levels were significantly increased by UV irradiation and significantly decreased by pretreatment with both EGCG and EGCG3"Me (n = 5–7, $P < 0.01$ or $P < 0.05$ vs. UV group). In particular, suppression of TNF-α generation was confirmed in the concentration-dependent EGCG group.

*UV irradiation-induced ROS generation*

Figure 4 shows the result of ROS generation in HCE-T cells at 1 hr after UV irradiation. The level of ROS significantly increased in the UV group compared with the non-UV group (n = 6–10, $P < 0.01$ vs. UV group). Pretreatment with EGCG and EGCG3"Me significantly inhibited UV-induced ROS generation (n = 5–10, $P < 0.01$ vs. UV group), and appeared to be stronger with EGCG pretreatment.

*UV irradiation-induced mitochondrial membrane potential*

Figure 5 shows the changes in mitochondrial membrane potential in the HCE-T cells at 1 hr after UV irradiation. There was no significant change in mitochondrial membrane potential in the UV and EGCG groups, and the only change was in the 20 μM EGCG3"Me group (n = 4,

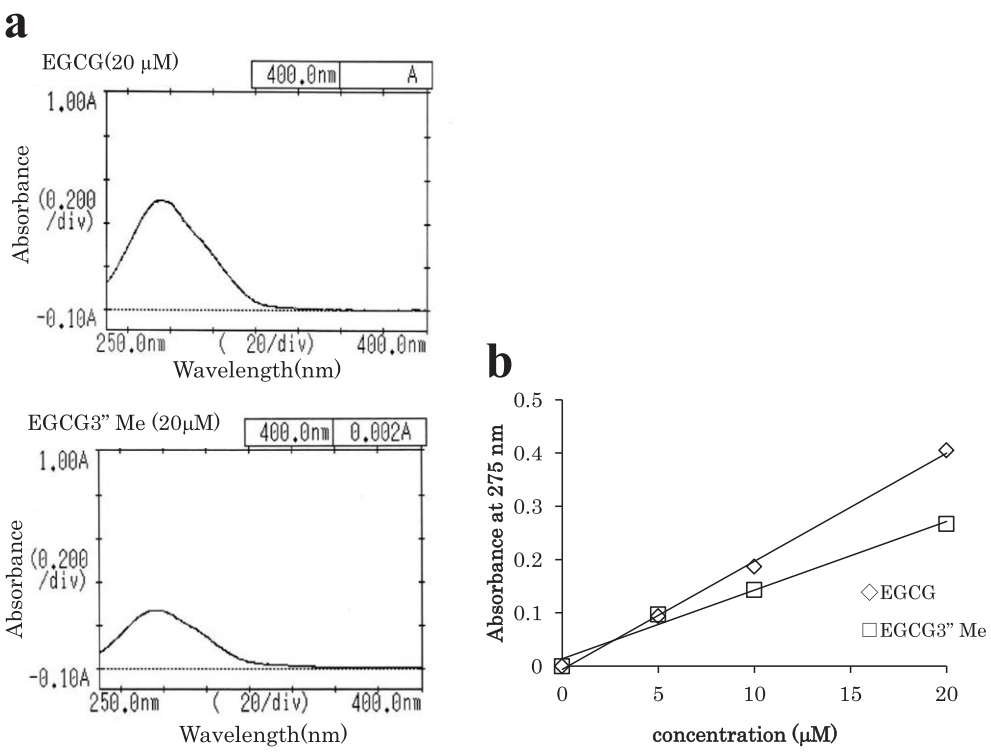


Fig. 1. a : Ultraviolet (UV) absorption spectra of (−)-epigallocatechin gallate (EGCG ; 20 μM) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me ; 20 μM). EGCG and EGCG3"Me concentrations were adjusted with methanol to 5, 10, or 20 μM and the UV absorption spectra were measured from 250 to 400 nm. The forms of all graphs were similar.
b : Correlation between the concentration of EGCG and EGCG3"Me and absorbance at 275 nm. The maximum absorption occurred at 275 nm and correlations between EGCG and EGCG3"Me are shown.

not significant).

*UV irradiation-induced cytochrome c generation*

Figure 6 shows the cytochrome c ratio in the HCE-T cells at 20 hr after UV irradiation. The density of cytochrome c in the mitochondria was predominantly high and most was not released into the cytoplasm, irrespective of UV irradiation ($n = 8$, not significant).

*UV irradiation-induced activation of caspase-3 and caspase-8*

Figures 7a and 7b show the activation of caspase-3 and caspase-8 in HCE-T cells at 3 hr after UV irradiation, respectively. Caspase-3 and -8 activity was significantly increased in the UV-treated group compared to the non-UV group (caspase-3, $n = 10-14$ ; caspase-8, $n = 8-10$ ; $P < 0.01$). In contrast, pretreatment with EGCG and EGCG3"Me significantly inhibited UV-induced activation of caspase-3 ($n = 5-10$, $P < 0.01$ and $P < 0.05$ vs. UV group) and caspase-8 ($n = 5-8$, $P < 0.01$ vs. UV group).

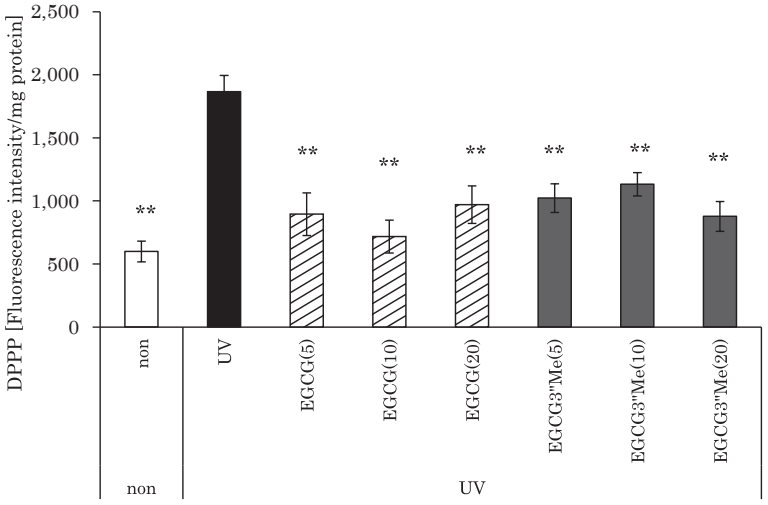


Fig. 2. Effect of (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) on membrane lipid peroxide generation in ultraviolet (UV)-irradiated human corneal epithelial (HCE-T) cells. Membrane hydroperoxide generation in HCE-T cells was evaluated using diphenyl-1-pyrenylphosphine (DPPP). HCE-T cells were pretreated with EGCG or EGCG3"Me for 1 hr, exposed to 296 mJ/cm$^2$ UV irradiation, and incubated with EGCG or EGCG3"Me for 15 min. Values are presented as mean ± standard error, and represent the average fluorescence intensity/well (n = 7 or 8) $^*P < 0.05$, $^{**}P < 0.01$ vs. UV-irradiated HCE-T cells from seven independent experiments.

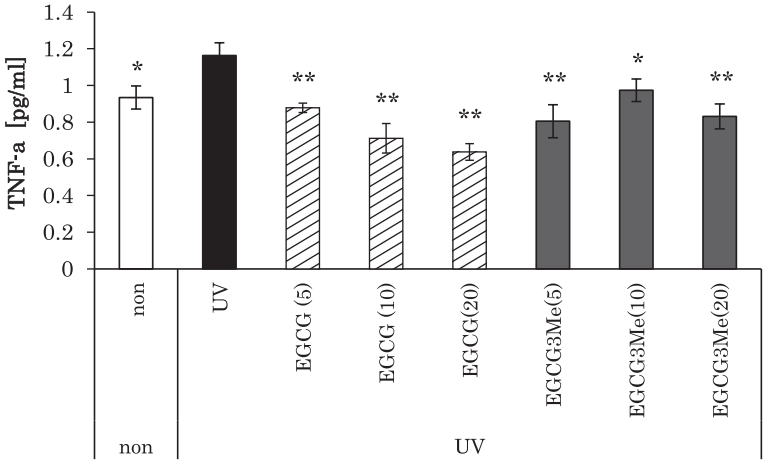


Fig. 3. Effect of (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) on tumor necrosis factor alpha (TNF-$\alpha$) generation in ultraviolet (UV)-irradiated human corneal epithelial (HCE-T) cells. TNF-$\alpha$ generation in HCE-T cells was measured using an ELISA with culture medium from HCE-T cells after UV irradiation. HCE-T cells were pretreated with EGCG or EGCG3"Me for 1 hr, exposed to 296 mJ/cm$^2$ UV irradiation, and incubated with EGCG or EGCG3"Me for 30 min. Values are presented as mean ± standard error (n = 7 or 8). $^*P < 0.05$, $^{**}P < 0.01$ vs. UV-irradiated HCE-T cells from seven independent experiments.

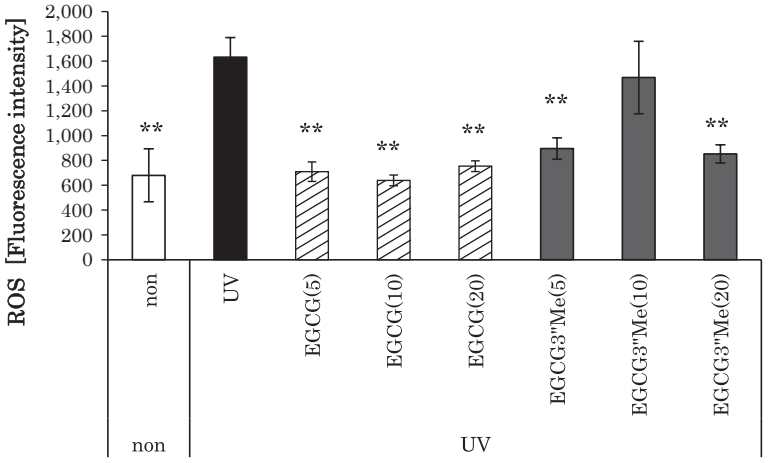


Fig. 4. Effect of (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) on reactive oxygen species (ROS) in ultraviolet (UV)-irradiated human corneal epithelial (HCE-T) cells. ROS generation in HCE-T cells was evaluated using 5-(and-6) chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester. HCE-T cells were pretreated with EGCG or EGCG3"Me for 1 hr, exposed to 296 mJ/cm$^2$ UV irradiation, and incubated with EGCG or EGCG3"Me for 1 hr. Values are presented as mean ± standard error, and represent the average fluorescence intensity/well (n = 5–10). $^{**}P < 0.05$, $^{**}P < 0.01$ vs. UV-irradiated HCE-T cells from seven independent experiments.

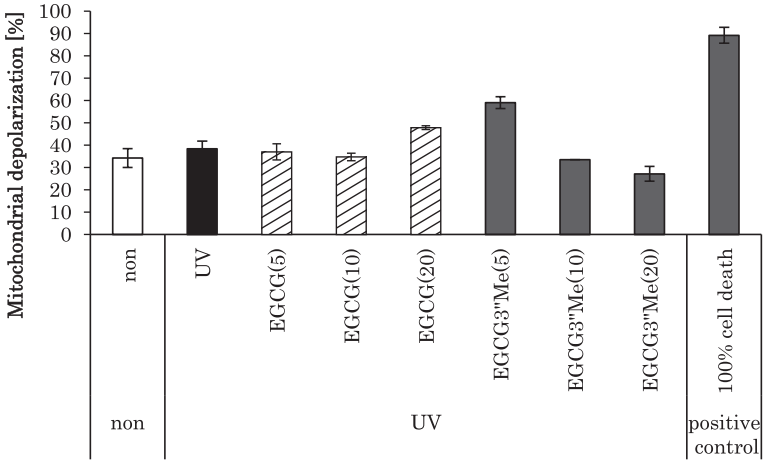


Fig. 5. Effect of (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) on mitochondrial membrane potential in ultraviolet (UV)-irradiated human corneal epithelial (HCE-T) cells. Changes in mitochondrial membrane potential in HCE-T cells 1 hr after UV irradiation were determined using the MuseTM Mito Potential assay kit. HCE-T cells were pretreated with EGCG or EGCG3"Me for 1 hr, exposed to 296 mJ/cm$^2$ UV irradiation, and incubated with EGCG or EGCG3"Me for 1 hr. The positive control was a sample of depolarized and dead cells obtained from sonication. Values are presented as mean ± standard error (n = 3–6). $^{**}P < 0.05$, $^{**}P < 0.01$ vs. UV-irradiated HCE-T cells from seven independent experiments.

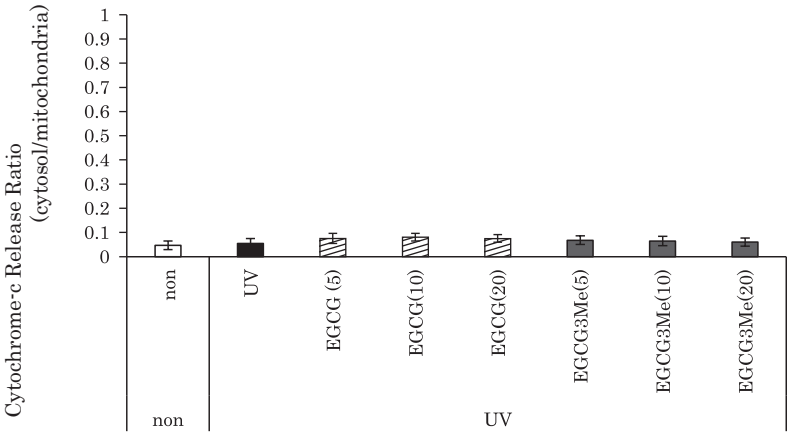


Fig. 6. Effect of (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) on the cytochrome c ratio released from mitochondria to cytosol in ultraviolet (UV)-irradiated human corneal epithelial (HCE-T) cells. HCE-T cells were pretreated with EGCG, EGCG3"Me for 1 hr, and exposed to 296 mJ/cm$^2$ UV irradiation and incubated with catechins for 20 hr at 37°C. The levels of cytochrome c (ng/ml) were measured in each mitochondrial and cytosolic fraction and the cytochrome c ratio was calculated as cytosolic cytochrome c/total (mitochondrial + cytosolic) cytochrome c. Each value represents the mean ± standard error of 8 samples. $^*P < 0.05$, $^{**}P < 0.01$ vs. UV-irradiated HCE-T cells from seven independent experiments.

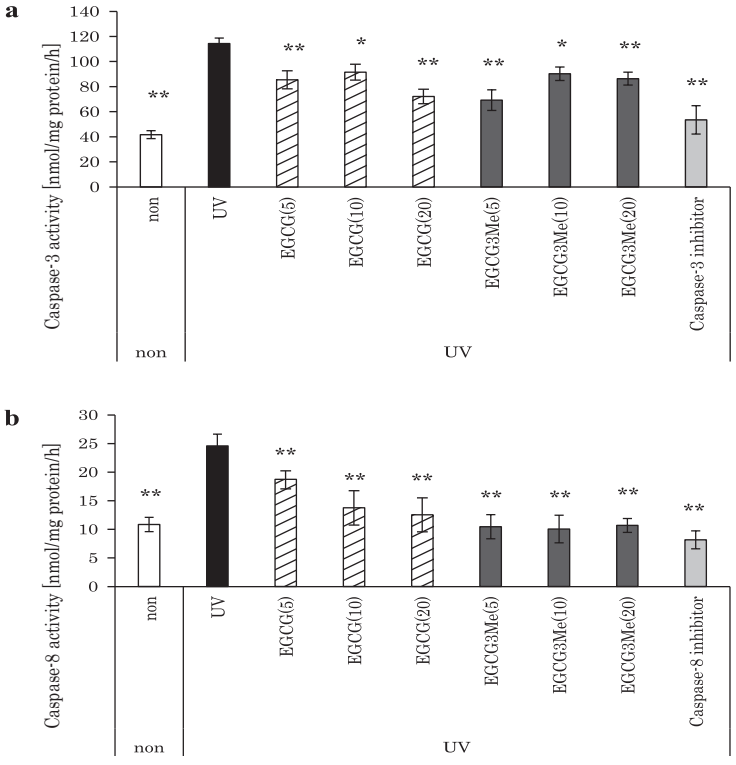


Fig. 7. Effect of (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin-3-O-(3-O-methyl) gallate (EGCG3"Me) on caspase-3 (a) and caspase-8 (b) activity in ultraviolet (UV)-irradiated human corneal epithelial (HCE-T) cells. HCE-T cells were pretreated with EGCG, EGCG3"Me or each caspase-inhibitors for 1 hr, and exposed to 296 mJ/cm$^2$ UV irradiation and incubated with catechins or caspase-inhibitors for 1 hr at 37°C. The activity of caspase-3 and caspase-8 in the cell lysate was determined fluorometrically using the substrate N-acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcoumarin. Each value represents the mean ± standard error of 6–10 samples. $^*P < 0.05$, $^{**}P < 0.01$ vs. UV-irradiated HCE-T cells from seven independent experiments.

## Discussion

UV-induced eye diseases, such as photokeratitis and pterygium, can be prevented by using UV-filtering contact lenses and sunglasses. In addition, sunburn of the eyes is a noted contributor to epidermal pigmentation of the skin. This is due to stimulation of the trigeminal nerve by UV radiation of the eyes, which promotes melanogenesis through the pituitary gland[19]. Studies have has shown that UV-induced ROS production causes damage to various cells, including corneal cells[20]. Ocular surfaces are continuously exposed to photo-oxidation caused by sunlight and $O_2$. Then, atmospheric $O_2$ is activated with photic stimulation resulting in singlet $O_2$, a structure in the strongest ROS, which damages the cell membrane, protein, and nucleic acid. Epithelial cells in the outermost layer of the cornea have a tight intercellular junction that forms a barrier to outside stimulation, such as atmospheric dust and oxidative stress. In addition, many types of radical scavengers, such as superoxide dismutase, catalase, and vitamin C, are found in corneal epithelial cells; however, these defense mechanisms fail when exposed to UV radiation, resulting in cornea injury. The tight intercellular junction is damaged by inflammatory cytokines (e.g., TNF-$\alpha$), anoxia, and ROS overproduction[21, 22].

In this study, cells were irradiated with a central UV wavelength of 312 nm, intensity of 4.94 mW/cm$^2$, and dose of 296 mJ/cm$^2$. As a result, DPPP, which is an index of membranous lipid peroxidation, was significantly increased after only 15 min of UV irradiation. DPPP can specifically detect lipid peroxidation of the cell membrane using the hydroperoxide produced early in cell membrane lipid peroxidation, and it is used as a fluorescence reagent in assays[23]. UV irradiation-induced lipid peroxide was generated in the cell membrane and appeared to trigger a series of damage to the cell. In this study, the generation of TNF-$\alpha$ was significantly increased after 30 min of UV irradiation. TNF-$\alpha$ is an inflammatory cytokine which is induced in the relatively early phase of UV exposure[24] and induces apoptosis. Its binding to TNF receptor 1 promotes apoptosis through activation of an adapter protein (TNF receptor 1–associated death domain protein/Fas-associating protein with death domain/mediator of receptor induced toxicity) and caspase-8. When considering these results, it appears that TNF-$\alpha$ forms an early bond directly to a receptor, and leads to apoptosis directly through activation of caspase-3 by caspase-8 activation. We believe that there is little mitochondrial contribution to this apoptosis pathway. Because mitochondrial membrane potential was measured, UV irradiation after depolarization was not detected. In addition, the release of cytochrome c into the cytoplasm after UV irradiation was not detected. Conventionally, the cornea is small with little mitochondrial distribution. According to the gene profile[25], corneal epithelial cells do not have cytochrome c oxidase expression, which is a functional mitochondrial marker. These results suggest that the contribution to apoptosis through mitochondrial cytochrome c is very low.

In addition, the pentose phosphate pathway (PPP) is more dominant in corneal epithelial cells than aerobic metabolism through the mitochondria[26]. PPP is the production pathway of NADPH (a reducing agent) and D-ribose 5-phosphate (a material of nucleic acid). By UV irradiation, glucose-6-phosphate dehydrogenase (the initial catalyst of PPP) activity increases, and

is reported to protect the cornea from UV-induced oxidative stress[27]. In this study, we cannot deny that the apoptotic pathway goes via the mitochondria, however we think that the contribution of this pathway is very low.

Several antioxidants, such as ascorbic acid, vitamin E, and glutathione, are found in the human cornea and plasma. In a study comparing the antioxidant power (DPPH radical elimination ability) of EGCG with vitamin E, it was reported that EGCG is 10 times stronger that vitamin E. In Japan, Japanese green tea is regularly consumed. Catechins account for 10%–20% of the substances in Japanese green tea and one-half of the catechins comprise EGCG[28]; therefore, the inhibitory effects of catechins on UV-induced injury was examined because even the strongest corneal epithelial cells when exposed to excessive UV irradiation undergo oxidative stress. High-performance liquid chromatography using a UV absorbance detector is commonly used for catechin analysis[29]. The results of this analysis confirmed that maximum UV absorption occurs at 275 nm for both EGCG and EGCG3"Me. Benzene rings also absorb UV radiation, which is believed to increase with the presence of a gallate group. These results suggest that EGCG and EGCG3"Me play roles in UV absorption and inhibit peroxidative stress to the cell membrane to protect HCE-T cells from UV irradiation. The results of an *in vivo* study using EGCG eye drops in mice with a UV-induced corneal injury, and mice with dry eye, showed that EGCG contains antioxidants[30].

In this study, we showed that EGCG absorbs UV light and helps to suppress lipid peroxidation. After having examined the absorption dynamics of catechins after ingesting Benifuuki green tea, it was shown that EGCG3"Me was absorbed at levels approximately 6.5-fold greater than EGCG and was slowly metabolized[10]. Because tears are produced in the capillaries of the lacrimal sac, it is expected that ingesting green tea containing catechins will protect the cornea through tear production.

**Conflict of interest disclosure**

Because both EGCG and EGCG3"Me absorb UV light, they reduce the generation of membrane lipid peroxide and inhibit the apoptotic pathway through caspase-8. These results suggest that drinking green tea containing EGCG and EGCG3"Me, or using catechin-containing eye drops, can protect the ocular surface from UV irradiation.

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